Eggs as a source of essential Docosahexaenoic Acid (DHA)

In The Diets Of Weaning Infants

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NOTE: This is the full report of a RIRDC short report no. 37 titled 'Egg yolks – nature's wonder food for babies'. You may view this short report on RIRDC's website or contact the Corporation on 02 6272 4819 for free printed copies.

Foreword

Egg yolks are known to be a nutritious source of protein, iron, vitamins and polyunsaturated fats. Recently their nutritional value has been increased by enrichment with omega-3 long chain polyunsaturates, such as docosahexaenoic acid (DHA).

The possible nutritional benefits and perceived risks of including four egg yolks per week in the weaning diet were tested in over 130 breast and formula fed infants aged 6-12 months.

The study established that regardless of whether infants are breast or formula fed, mothers normally include very few eggs in their baby's diet - on average only one egg yolk per week.

Increasing infant egg yolk consumption to four egg yolks per week did not raise cholesterol levels any more than breast feeding alone and did not show any effect on plasma measures of allergy or clinical markers of iron status.

Consuming four omega-3 egg yolks per week resulted in an improvement in DHA status of formula fed infants to a level seen in control breast fed infants.

Egg yolks are confirmed as a safe and nutritious weaning food.

Peter Core
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Contents

Abbreviations

°	Degrees
μ	Micro
AA	Arachidonic Acid
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
C	Celsius
CI	Confidence Interval
cm	Centimetre
DHA	Docosahexaenoic Acid
EDTA	Ethylenediaminetetraacetic Acid
FMC	Flinders Medical Centre
g	Gram
h	Hour
H_2SO_4	Sulphuric Acid
Hb	Haemoglobin
i.d.	Internal Diameter
ID	Iron Deficiency
IDA	Iron Deficiency Anaemia
IgE	Immunoglobulin E
IU	International Units
L	Litre
LCPUFA	Long Chain Polyunsaturated Fatty Acids
m	Milli
MDI	Mental Developmental Index
min	Minute
Mg	Milligram
mol	Molar
n	Nano
ns	Not Significant
PDI	Psychomotor Developmental Index
RAST	Radio-allergosorbent Test
RDI	Recommended dietary intake
s	Second
sd	Standard Deviation
sem	Standard Error of the Mean
WHO	World Health Organisation

Executive Summary

EGGS AS A SOURCE OF ESSENTIAL DOCOSAHEXAENOIC ACID (DHA) IN THE DIETS OF WEANING INFANTS

Objectives
The primary objective of this study was to determine the nutritional value of including egg yolks in the weaning diet of healthy breast and formula fed infants and to investigate some of the perceived risks associated with consuming eggs.

Specifically, to investigate the effect of four egg yolks per week on:
- Infant long chain polyunsaturated fatty acid status - in particular DHA
- Plasma iron status
- Plasma cholesterol levels
- Plasma markers of allergy
- Growth and developmental quotient at 12 months of age.

Background
Egg yolks have often been described as nature's 'wonder food' because they pack such an array of nutrients into such a small volume, making them ideal for babies and children. Probably the two biggest nutritional risks for normal infants in our society are deficiencies in DHA and iron, and both can be supplied by egg yolk. However, over the last decade eggs have become less used as a suitable food for babies partly because of the fear of cholesterol and allergies, even though current guidelines recommend the introduction of egg yolk into the infant diet from 6 months of age.

Research
The study was a partially blinded, randomised, outpatient study conducted in healthy 6-12 month old infants. Following consent, breast or formula fed infants were randomised to receive either no dietary intervention, four regular egg yolks per week or four omega-3 enriched (NewStart, high in DHA, an omega-3 long chain polyunsaturated fatty acid) egg yolks per week according to a central, computer-generated, randomisation schedule. Dietary intervention with omega-3 eggs was designed to match the level of DHA an infant would normally receive if she/he were fully breast fed, i.e. ≈100mg per day. A total of 70 breastfed and 69 formula fed infants completed the study.

Outcomes
General conclusion:
Egg yolks are a safe and nutritious weaning food. Our study demonstrated that including four egg yolks per week in the diet of infants can have nutritional benefits and are not associated with health risks such as raised cholesterol or an increased incidence of allergy.

Specific findings:

- Between 6 and 12 months mothers offer infants very few eggs - on average only one egg yolk per week.
- When mothers were given advice and appropriate recipes, they easily incorporated four egg yolks per week into their baby's diet and the egg yolks were well tolerated.
- Increase in egg yolk consumption was not as a result of other weaning foods being replaced by egg yolks - meats and cereals were consumed to the same degree by all infants regardless of the number of egg yolks in the diet.
- Increasing the consumption of regular egg yolks in the diet had little effect on DHA status of the infants. However, the effect of eating four omega-3 egg yolks per week increased the level of DHA in the blood of formula fed infants up to levels seen in control breast-fed infants.
- Consuming omega-3 egg yolks was equally effective in breast and formula fed infants - levels of DHA increased approximately 30% in all infants.
- Consumption of four egg yolks per week did not result in cholesterol levels of formula fed infants exceeding levels seen in control breast fed infants, nor did they affect the cholesterol levels of breast fed infants.
- In this well nourished group of infants, consumption of egg yolks had no effect on the clinical markers of iron status.
- The study could not detect an effect on indices of allergy as a result of increasing the number of egg yolks included in infant diets.
- In this socially advantaged and healthy population there was no evidence of an effect of omega-3 egg consumption on mental or physical development.

Implications
Health professionals can safely recommend that the weaning diet of 6-12 month old infants can include up to four egg yolks per week. The use of omega-3 egg yolks provides additional DHA to the growing infant.

Publications Nil to date

1 INTRODUCTION

The weaning diet is important from both a developmental and nutritional point of view. The introduction of solid foods at 4-6 months should coincide with the loss of the primitive sucking reflex where the tongue is thrust forward. With the introduction of finely pureed foods the baby learns to take control of the tongue and to take food from a spoon and swallow solids. By the end of the eighth month the baby has learnt to chew and bite and is eating foods with soft lumps. By 12 months the child has usually developed some independence and wants to feed her/him self. An appropriate weaning diet, with a variety of foods, aids this developmental process as well as providing the extra nutrition (such as energy and iron) a baby requires in the second 6 months of life.

Recent infant weaning practices have resulted in a trend towards the use of inappropriate foods. For example, adult cereals are generally low in iron and protein. Canned baby foods are also generally low in iron, have a very smooth texture and are virtually devoid of long chain polyunsaturated fatty acids (LCPUFA) (1). The increasing use of cow's milk, fruit juices and cordials are also a problem. Cow's milk has a high solute load, low iron and has been associated with occult gastro-intestinal bleeding (2), while the high carbohydrate load of fruit juices has been associated with an increased occurrence of adverse reaction to fruit juices (3). Traditional weaning foods such as egg yolk and brains, which are excellent sources of energy, protein, iron and LCPUFA, are becoming increasingly rare in the diets of infants (4). *In this study we sought to investigate the benefits of egg yolk as a source of nutrition in the diets of weaning infants.*

Eggs have often been described as nature's 'wonder food' because they pack such an array of nutrients into such a small volume, making them ideal for babies and children. Probably the two biggest nutritional risks for normal infants in our society are deficiencies in iron and LCPUFA, and both can be supplied by egg yolk. However, over the last decade eggs have become less used as a suitable food for babies partly because of the fear of cholesterol and allergies, although current guidelines recommend the introduction of egg yolk into the infant diet from 6 months of age with the introduction of egg white delayed until 12 months of age.

1.1 Eggs As A Source Of LCPUFA

Breast milk is an excellent source of docosahexaenoic acid (DHA), an omega-3 LCPUFA and arachidonic acid (AA), an omega-6 LCPUFA. One of the unique attributes of eggs is that they may be an alternative source of LCPUFA for infants from 6 months of age. Most Australian eggs contain some omega-6 LCPUFA but only trace amounts of omega-3 LCPUFA. However, newer varieties of eggs (e.g. NewStart) are now enriched in omega-3 LCPUFA, with a single yolk supplying double the daily amount of DHA consumed by a fully breast fed infant (Table 1.1). These eggs more closely match the polyunsaturated fatty acids composition of true free range eggs (5).

***Table 1.1**: DHA and cholesterol content of eggs and breast milk*

	Omega-3 Eggs	Regular Eggs	Breast Milk
Percentage fat as DHA	3.6	0.9	0.25
mg DHA*	200	52	100
mg of cholesterol*	170	170	150 (range 80-200)

*Based on consumption of one egg yolk containing ≈6g fat or one day of full breast feeding.

New studies are showing that DHA may be a conditionally essential nutrient in early infancy for the optimal development of visual and cognitive processes (6-9). Further, it may be prudent that preformed DHA be available in the diets of infants for the whole of the first year. For example, the DHA content of the cerebral cortex is higher in breast fed (receiving DHA) than formula fed infants; DHA accumulation is dependent on the supply of DHA through breast milk and continues to accumulate for at least 10 months (10).

The marked decline in the incidence of breast feeding beyond 6 months often implies the removal of the infant's major source of DHA because some infant formula and most weaning foods are poor sources of DHA (1,11). Eggs rich in omega-3 fats may be an ideal source of these important LCPUFA for the growing infant. *The effect of omega-3-rich egg yolks on the biochemistry and development of Australian infants was determined in this study.*

1.2 Eggs As A Source Of Iron

The recommended dietary intake (RDI) of iron for 6-12 month old infants is set at 9mg per day. However, the estimated requirement of iron actually absorbed from the diet is 0.93mg per day for 6-12 month old infants (12). The major sources of dietary iron in the second half of infancy are iron fortified baby cereals (7.5mg iron per 15g serve) and infant formula (7-12mg iron per L). Both baby cereals and formula are fortified with inorganic forms of iron that are poorly absorbed (≤10%) and this explains why the RDI is set 10-fold higher than the actual estimated requirement (9mg vs 0.93mg).

Foods such as egg yolk (0.7mg iron per yolk) and red meat (0.6mg iron per 20g serve) contain natural sources of iron as haem iron, which are better absorbed than iron from cereals or formula. However, such foods are not generally introduced into the infant's diet until after 8 months (4), although there is no reason for this delay. Infant weaning practices in the Middle East and some European countries advocate the introduction of egg yolk as a first food (5). Indeed, such a practice appears sensible, as egg yolk is a better source of iron, protein and energy than baby cereals.

Iron deficiency is a nutritional risk in the second half of infancy, particularly amongst infants who have been fully breast-fed. A number of factors can contribute to this situation. For example, the concentration of iron in breast milk is low (0.3-0.5mg per L), although the bioavailability is high (50%) (13), and recent infant weaning practices demonstrate that it is difficult to meet estimated iron dietary requirements (4). Our estimates suggest that a 6-12 month old breast fed infant receives less than it's estimated iron requirement. However, despite this a recent Adelaide survey showed that only 7% of Caucasian infants/children were anaemic due to iron deficiency (14). It is possible that iron requirements have been overestimated and/or the iron contribution of individual foods underestimated. Modest and realistic dietary interventions to improve iron availability in the weaning diet may make a significant contribution to improving the iron status of infants. *In this study we investigated the effectiveness of egg yolk as a source of iron for both breast and formula fed infants.*

1.3 What About Cholesterol And Allergies?

The cholesterol content of a single egg yolk is approximately 170mg and this is comparable with the amount of cholesterol a fully breast-fed infant would receive from just breast milk in one day (15). Further, there is no evidence in the scientific literature to suggest cholesterol intake in infancy is related to the development of cardiovascular disease in adult life. Public health messages about cholesterol and heart disease have been translated by the public to mean that cholesterol is bad and foods containing cholesterol should be avoided. It is, however, high saturated fat diets, not cholesterol intake *per se,* that increase plasma cholesterol levels. Indeed, a recent study demonstrated that cholesterol intake from either regular or omega-3 eggs is not related to increased blood lipids in adults when four eggs per week were consumed (16). *The total cholesterol level in the plasma of infants enrolled in this study was determined to verify this finding in infants.*

The main reason for delaying the introduction of egg white is to avoid sensitising infants to egg proteins and hence the development of egg-related allergies. A prospective study assessing complaints of adverse food reactions in the first 3 years of life in 480 children reported only 3 (0.6%) probable or confirmed reactions to eggs (3). In all these cases, the whole egg was introduced (yolk + white) and re-challenges within 3-6 months produced no reaction, suggesting that even in sensitive individuals eggs can be re-introduced into the diet. *In this study, the concentrations of total immunoglobulin E (IgE), circulating egg white- and egg yolk-specific IgE antibodies in the blood of infants were determined as a measure of allergic response.*

2 OBJECTIVES

The primary objective of this study was to determine the nutritional value of including egg yolks in the weaning diet of healthy infants. In addition, the study was designed to investigate some of the perceived risks associated with consuming eggs.

Specifically:

(i) To investigate the efficacy of dietary egg yolk as a source of LCPUFA as assessed by erythrocyte fatty acid status.

(ii) To investigate the impact of introducing four egg yolks per week in addition to a normal weaning diet on plasma iron status (haemoglobin, ferritin, iron, transferrin and transferrin saturation).

(iii) To investigate the impact of introducing egg yolk on plasma cholesterol levels.

(iv) To investigate the impact of introducing egg yolk on circulating total IgE, egg yolk-specific IgE and egg white-specific IgE levels in plasma.

(v) To investigate whether dietary DHA in the second 6 months of life is associated with developmental quotient at 12 months of age in breast and formula fed infants. In addition, to assess the correlation between iron status at 6 and 12 months of age and developmental quotient at 12 months of age.

3 METHODOLOGY

3.1 Subjects

The study was a partially blinded, randomised, outpatient study conducted in Adelaide, South Australia. Healthy 6 month old infants, who were born at term, were recruited from immunisation clinics in the vicinity of Flinders Medical Centre (FMC). All infants were appropriate weight for age and had no known protein intolerances or allergies. Infants were eligible for entry into the breast fed group if they received less than 120mL formula (or cow's milk) per week and were eligible for entry into the formula fed group if they were receiving all their nutrition as formula feeds by 4 weeks after birth and subsequently formula fed. *All formula consumed by participants in this study was without LCPUFA supplementation*. Written informed consent was obtained from all participating parents in adherence to the guidelines of the FMC Committee on Clinical Investigation (Ethics) and the Research and Ethics Committee of the Women's and Children's Hospital prior to commencing the study.

3.2 Dietary Intervention

Following consent, infants who had been breast fed exclusively for 6 months were randomised to receive either no dietary intervention, regular eggs or omega-3 enriched (NewStart) eggs according to a central, computer-generated, randomisation schedule. Similarly, formula fed infants were also randomised to receive either no dietary intervention, regular eggs or omega-3 eggs. Participants in the study and research personnel were unaware of the type of eggs provided.

Dietary intervention was between 6 and 12 months of age. Mothers of infants assigned to the egg groups were asked to offer their infants four egg yolks per week. All mothers received advice regarding preparation as well as a recipe booklet compiled by a dietician.

Eggs (60-65gm) were purchased from South Coast Eggs, Myponga, South Australia and were supplied in cartons coded "A" or "B". Compliance was encouraged by providing the family with two dozen eggs per fortnight, delivered by courier to the home of each participant randomised to an egg group. Dietary intervention with omega-3 eggs was designed to match the level of DHA an infant would normally receive if she/he was fully breast fed, i.e. ≈100mg per day. The fatty acid composition of the eggs is shown in Table 3.1. The code was disclosed following completion of the trial.

Table 3.1: Fatty acid composition of eggs (mean±sd)

Fatty Acid		mg/100g egg (Average egg weight 62g)		% Total fatty acids	
Common name	Abbrev	Omega-3 Eggs	Regular Eggs	Omega-3 Eggs	Regular Eggs
Palmitic	16:0	2151±132	2030±215	24.3±0.1	23.7±0.4
Stearic	18:0	712±66	670±66	8.0±0.2	7.8±0.1
Arachidic	20:0	11±5	13±1	0.1±0.1	0.2±0.1
Total Saturates		2951±204	2772±284	33.4±0.2	32.4±0.4

Fatty Acid		mg/100g egg (Average egg weight 62g)		% Total fatty acids	
Palmitoleic	16:1ω7	275±1	253±14	3.1±0.2	3.0±0.1
Oleic	18:1ω9	3594±343	3687±230	40.6±1.3	43.1±1.2
	18:1ω7	181±13	194±18	2.1±0.1	2.3±0.1
Eicosaenoic	20:1ω9	33±3	23±3	0.4±0.1	0.3±0.1
Total Mono's		4194±367	4258±272	47.4±1.2	49.8±1.3
Linoleic	18:2ω6	889±35	1141±168	10.1±0.2	13.3±0.8
Arachidonic	20:4ω6	68±6	170±21	0.8±0.1	2.0±0.1
	22:4ω6	6±1	16±3	0.1±0.1	0.2±0.1
	22:5ω6	-	31±10	-	0.4±0.1
Total omega-6		983±41	1385±206	11.1±0.2	16.1±1.0
α Linolenic	18:3ω3	317±66	41±2	3.6±1.0	0.5±0.1
Eicosapentaenoic	20:5ω3	26±3	-	0.3±0.1	-
Docosapentaenoic	22:5ω3	44±1	11±1	0.5±0.1	0.1±0.1
Docosahexaenoic	22:6ω3	315±16	73±10	3.6±0.1	0.9±0.1
Total omega-3		701±54	126±14	8.0±1.1	1.5±0.1

3.3 Assessments

The study assessments are shown in Table 3.2. Infants participating in the study attended an appointment at 6, 9 and 12 months of age. At each appointment the weight, length and head circumference of each infant was measured and a brief dietary questionnaire completed. Further information regarding the infant's diet was collected by phone between 6 and 9 months of age and between the 9 and 12 month visits. At 6 and 12 months a blood sample was taken. Infant development was assessed at the 12 month appointment.

Table 3.2: Study Assessments

Age (weeks)	Diet Questionnaire (Compliance check)	Anthropometrics	Blood Sample*	Developmental Assessment
26 ± 2	✓	✓	✓	
32 ± 2	✓			
39 ± 2	✓	✓		
45 ± 2	✓			
52 ± 2	✓	✓	✓	✓

* Fatty acids, iron status, cholesterol, IgE

3.3.1 Anthropometrics

Weight, length and head circumference were assessed at 6, 9, and 12 months. Bare weights were obtained from each infant on a Seca Baby Balance (Model 727), length was determined by two individuals with the infant in the supine position using an infant measuring mat. Head circumference was measured at the largest occipito-frontal circumference with a non-stretch tape.

3.3.2 Diet

Diet was monitored by completion of a questionnaire for iron and LCPUFA intake in infancy at 26, 32, 39, 45 and 52 weeks (interview in person at 26, 39 and 52 weeks and by telephone at 32 and 45 weeks). Particular attention was paid to the mode of feeding (quantity of breast milk and formula feeds) and intake of eggs as well as other foods in the weaning diet that also contain iron and LCPUFA such as fish and meat. The questionnaire thus enabled us to determine whether the dietary intervention (4 egg yolks/week) replaced other solid meals that may contribute significantly to the iron or LCPUFA intake of infants. The questionnaire is reproduced as Appendix A.

3.3.3 Blood samples

Blood samples (2mL) were taken by venepuncture when infants were 6 and 12 months of age. A portion of the sample (500µL) was placed in Ethylenediaminetetraacetic Acid (EDTA) for haemoglobin (Hb) determination and the remainder was stored in containers with lithium heparin. Plasma was used to determine ferritin, iron, transferrin, transferrin saturation and total cholesterol. The remaining plasma sample was stored at -80°C until analysis of total circulating IgE and allergen-specific IgE. Packed red blood cells were used for analysis of erythrocyte fatty acid phospholipids.

(i) Fatty acids

The cellular component of the blood sample was washed three times with isotonic saline and the lipids were extracted with chloroform:propanol (17). The phospholipid fraction of the lipid extract was separated by thin-layer chromatography and evaporated to dryness under nitrogen. The samples were methylated in 1% sulphuric acid (H_2SO_4) in methanol at 70°C for 3h. When cooled, the resulting methyl esters were extracted into n-heptane and transferred into vials containing anhydrous sodium sulfate (Na_2SO_4) as the dehydrating agent. Fatty acid methyl esters were separated and quantified using a Hewlett-Packard 6890 gas chromatograph with a 50m capillary column (0.33mm internal diameter [i.d.]) coated with BPX-70 (0.25µm film thickness, SGE Pty Ltd, Victoria). The injector temperature was set at 250°C and the detector (flame ionisation) temperature at 300°C. The initial oven temperature was 140°C and was programmed to rise at 5°C/min to 220°C. Helium was used as the carrier gas at a velocity of 35cm/s. Fatty acid methyl esters were identified based on the retention time of authentic lipid standards (Nuchek Prep Inc., Elysian, MN). Results are expressed as percentage (%) of total phospholipid fatty acids.

(ii) Iron studies

All iron studies were conducted by SouthPath SA at FMC. SouthPath staff were blinded to each infant’s dietary allocation. Haemoglobin concentration was measured spectrophotometrically using a Coulter STKS analyser. Plasma ferritin and transferrin were determined by immunoturbidimetric assays (Boehringer Mannheim Systems) on a BM/Hitachi 917 Automatic Analyser. Plasma iron was measured on the same analyser using the BM/Hitachi 917 Systems Pack. Precision values (expressed as a coefficient of variation) for each of these parameters were Hb 1.5%, ferritin 5%, iron 2.3%, and transferrin 2.1%.

The reference ranges used in this study for the iron status of infants (6 months-2 years) are as follows: Hb 105-135g/L, ferritin 10-250μg/L, iron 8-30μmol/L, transferrin 2.0-3.6g/L, and transferrin saturation 10-60%. Infants whose plasma iron levels fell within these limits were considered *iron sufficient*. All infants with a Hb concentration <105g/L were classified as having *anaemia*. Infants who had plasma ferritin ≤10μg/mL were classified as having *iron deficiency* (ID). Infants with ID who also had a Hb level <105g/L were classified as having *iron deficiency anaemia* (IDA).

For ethical reasons, infants who had anaemia at 6 months were withdrawn from the study and referred for treatment. If an infant was ID at 6 months, the infant-mother pair were invited back for a repeat blood sample in 6-8 weeks. If IDA developed in that time, the infant was withdrawn and referred for treatment.

(iii) Cholesterol

Total cholesterol analyses of non-fasting blood samples were also performed by SouthPath SA, FMC. Plasma cholesterol was determined by the Cholesterol CHOD-PAP-method enzymatic colourimetric test (Boehringer Mannheim Systems) using a BM/Hitachi 917 analyser.

(iv) Immunoglobulin E

The total IgE levels in plasma were determined by Microparticle Enzyme Immunoassay using commercially available kits (Imx Total IgE assay, Abbott Laboratories, Abbott Park, IL, USA). The Imx Total IgE Calibrators are referenced against the WHO-IgE standards and results are expressed as IU/mL (1 IU = 2.4ng). In this laboratory (SouthPath SA, FMC) the measured imprecision levels were 2% at the appropriate level of detection. The reference range for total IgE in infants < 3 years is < 60 IU/mL.

The concentration of circulating allergen specific IgE antibodies in plasma was measured using the Pharmacia CAP System Radio-allergosorbent Test (RAST) Radioimmunoassay (Kabi Pharmacia Diagnostics, Sweden). World Health Organisation (WHO) IgE standard based calibrators are used for the determination of specific IgE antibodies and values are expressed in kU_A/L, where A represents Allergen specific antibodies. The measuring range for undiluted plasma is 0.35-100kU_A/L and results can be reported either in kU_A/mL or as RAST classes (Table 3.3).

Table 3.3: Evaluation as RAST classes

RAST Class	Range (kU_A/L)	Level of allergen specific IgE
0	<0.35	Absent or undetectable
1	0.35 - <0.7	Low
2	0.7 - <3.5	Moderate
3	3.5 - <17.5	High
4	17.5 - <50	Very High
5	50 - <100	Very High
6	≥100	Very High

The family history of allergic disease was obtained from each mother at the first appointment.

3.3.4 Developmental assessment

A developmental assessment was conducted at 12 months of age using the Bayley's Scales of Infant Development. The Bayley's Scales are universally accepted as a good global assessment of development at 12 months of age (18). Mental (MDI) and Psychomotor (PDI) Developmental Indices were standardised for age based on established reference norms. A home environment questionnaire assessing the infant's home environment was administered (19) and information regarding other social variables known to influence development were collected.

3.4 Sample Size And Statistics

The target sample size for each group (three breast fed groups and three formula fed groups) was 20 infants to complete. Analyses were completed using SPSS for Windows 6.0 (SPSS Inc., Chicago). Data are expressed as mean ± sd unless otherwise stated.

3.4.1 Growth

Comparisons of weight, length and head circumference between breast fed infants allocated to different weaning diets were made by analysis of covariance (ANCOVA). Gender and gestational age were considered as co-variants. Similarly, ANCOVA was used to compare growth parameters of formula fed infants in each of the three weaning groups. As there were no growth differences within the breast and formula fed cohorts, data were combined and the growth of breast and formula fed infants were compared. Gender and gestational age were considered as co-variants in these analyses.

3.4.2 Fatty acids

Within the breast and formula fed cohorts, the effect of dietary grouping on the various red blood cell fatty acids was investigated using analysis of variance (ANOVA).

3.4.3 Iron status

Within the breast and formula fed cohorts, the effect of dietary grouping on 12 month Hb, ferritin, transferrin, iron and transferrin saturation was investigated using ANCOVA. Measures of iron status at 6 months were included in the model to adjust for iron status before intervention. Rates of ID, anaemia and IDA were compared with cross tabulations and chi square analysis. As there were no differences in iron status between dietary groups within the breast and formula fed cohorts, all data were combined and measures of iron status compared according to whether infants were in egg intervention or no intervention groups. These analyses were also by ANCOVA. Six month measures of iron status and whether infants were breast or formula fed were included as covariants in the analysis. Ferritin data were log transformed for all analyses, as the raw data were not normally distributed.

3.4.4 Cholesterol

Independent samples T test was used to compare breast and formula fed infants level of plasma cholesterol. Within the breast and formula fed cohorts, the effect of dietary grouping on plasma cholesterol was investigated using ANCOVA. Six month cholesterol values and mode of feeding were used as covariants.

3.4.5 Allergy markers

The incidence of allergic disorders amongst family members was compared between breast and formula fed groups with cross tabulations and chi square analysis. Total IgE data were log transformed and compared between dietary groups by ANOVA. Rates of positive egg white- and yolk-specific IgE levels were compared with cross tabulations and chi square analysis. This data was arranged into two groups, the level of allergen specific IgE being (1) absent or undetectable (% with RAST class < 1) and (2) IgE present (% with RAST class ≥ 1).

3.4.6 Bayley's Scales of Infant Development

All possible environmental and nutritional variables that may impact on development were considered as possible independent variables for the regression models. Models were constructed using the independent variables that were associated ($p<0.2$) with the dependent variable. Independent variables were removed from a given model if the independent variable's presence or absence did not influence the model. No independent variables in the final regression models were found to be collinear.

4 RESULTS

4.1 Subject Enrolment

A total of 251 eligible infants were approached during the period of enrolment. Written, informed consent to participate in the study was obtained from the parents of 161 infants (82 breast fed and 79 formula fed). Characteristics of the subjects in the study and their parents are shown in Table 4.1. The parents of all infants were of middle socioeconomic status, however mothers who were still successfully breast feeding their baby at 26 weeks had on average attained a higher level of education than those who were feeding formula (i.e. breast fed for less than 4 weeks). The average length of breast feeding for the formula fed group was 1.9 weeks.

Table 4.1: *Characteristics of subjects and their parents*

Variable	Breast Fed Infants	Formula Fed Infants
n	**80**	**70**
Gender (M:F)	41:39	30:40
Gestational Age (weeks, mean±sd)	39.7 ± 1.1	39.8 ± 1.1
Apgar at 5 min (mean±sd)	9.0 ± 0.9 (n=79)	9.0 ± 0.7 (n=69)
Mother's Level of Education[a]	3.0±1.0*	2.5±1.1
Spouse's Level of Education	4.0±1.1* (n=74)	3.0±1.0 (n=67)
Mother's Socioeconomic Status[b]	4.9 ± 1.0	5.4 ± 0.9
Spouse's Socioeconomic Status	4.5 ± 1.2 (n=74)	5.0 ± 1.0 (n=67)

* $p<0.005$

[a] Education was ranked using a 7 point scale where 0 = no formal education, 1 = primary school level, 2 = mid secondary school level. 3 = completion of secondary school level, 4 = certificate or diploma, 5 = tertiary degree and 6 = higher degree. Results expressed as median ± sd.

[b] The highest rank [1] was assigned to professionals and academic occupations and the lowest rank [6] to manual unskilled occupations (20). Results expressed as median ± sd.

4.2 Subject Follow-Up

The distribution of all eligible infants approached for the study is given in Figure 4.1.

Of the 82 *breast* fed infants enrolled, 24/27 (omega-3 eggs), 23/27 (regular eggs) and 23/28 (control diet) infants completed the study. Twelve breast fed infants were withdrawn from the study for the following reasons:

(i) 8 infants were found to have anaemia
(ii) 2 infants failed to attend the initial appointment
(iii) 1 infant ceased to be breast fed
(iv) 1 infant appeared to be "unsettled" on eggs and was withdrawn at the parent's request.

Of the 79 *formula* fed infants enrolled, 20/26 (omega-3 eggs), 24/26 (regular eggs) and 23/27 (control diet) infants completed the study. Twelve formula fed infants were withdrawn from the study for the following reasons:

(i) 9 infants failed to attend the initial appointment
(ii) 1 infant was lost to follow up
(iii) 1 infant was withdrawn at the parents request
(iv) 1 infant appeared to be "unsettled" on eggs and was withdrawn at the parent's request.

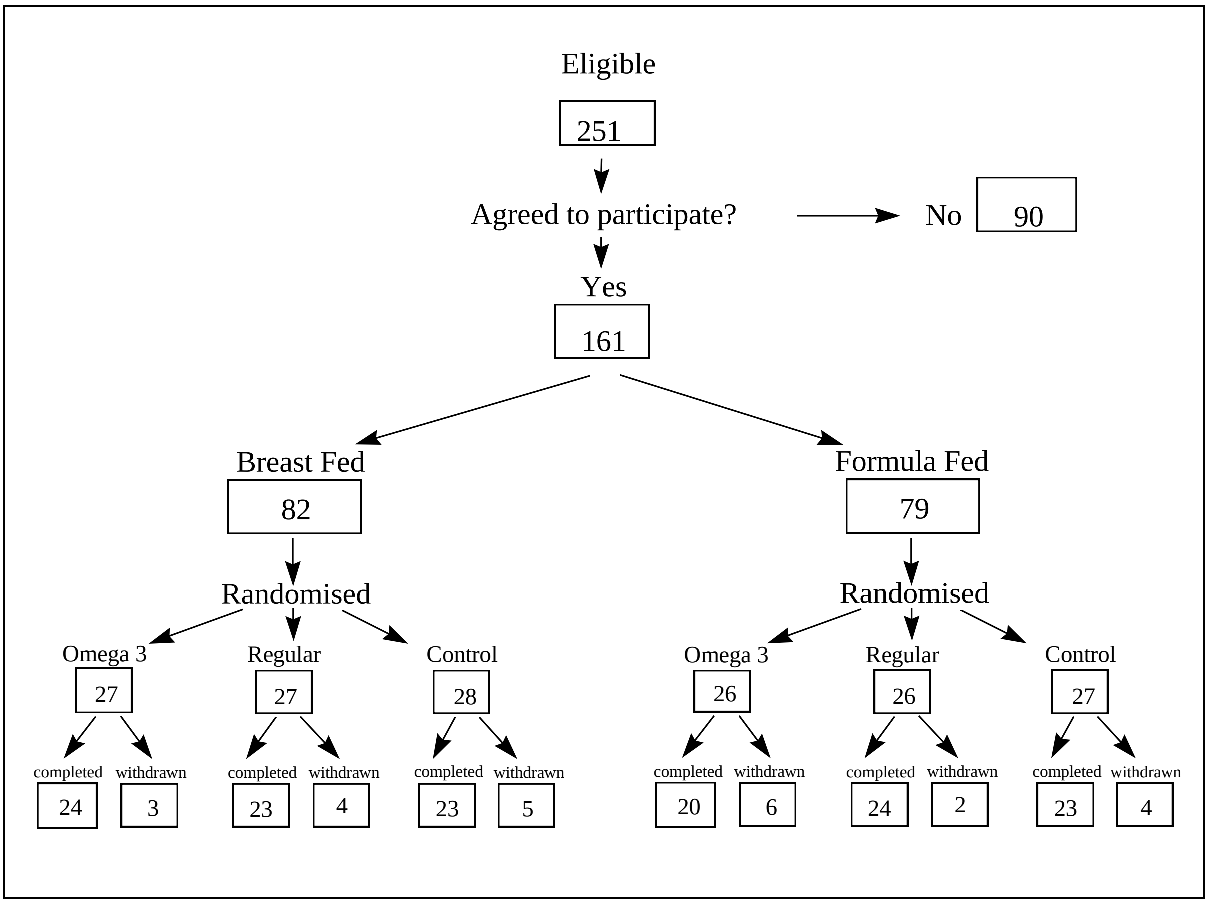

***Figure 4.1**: Distribution of subjects approached to participate in the study*

The number of infants attending each appointment is shown in Table 4.2.

***Table 4.2**: Attendance at each appointment*

	Breast Fed Infants			Formula Fed Infants		
Visit	**Omega-3 Eggs**	**Regular Eggs**	**Control Diet**	**Omega-3 Eggs**	**Regular Eggs**	**Control Diet**
Randomised	27	27	28	26	26	27
6 months	26	27	27	23	24	23
9 months	24	23	24	21	24	23
12 months	24	23	23	20	24	23

4.3 Compliance And Dietary Intake

Analysis of diet questionnaires completed throughout the study enabled an assessment of the level of compliance with the dietary intervention. The average egg yolk consumption by all groups randomised to receive eggs was 3.8 egg yolks per week (Table 4.3). Infants in the control group consumed an average of 1.1 egg yolks per week throughout the study. Few infants were consuming eggs prior to the commencement of the study (8/150). The average age at which whole eggs were introduced was 48 weeks.

Table 4.3: *Egg yolk consumption*

		Omega-3 Eggs (number of egg yolks/week)		Regular Eggs (number of egg yolks/week)		Control Diet (number of egg yolks/week)	
		mean±sd	**n**	**mean±sd**	**n**	**mean±sd**	**n**
BREAST FED							
	33wks	3.4±0.9	24	3.7±0.9	23	0.9±1.1	23
	39wks	4.0±0.7	23	4.0±0.8	23	0.9±1.0	23
	46wks	4.1±1.1	24	4.0±0.8	23	1.3±1.1	23
	52wks	3.9±1.6	24	3.9±0.9	23	1.6±1.4	23
	Average	3.8±0.8	24	3.9±0.6	23	1.2±0.9	23
FORMULA FED							
	33wks	3.5±0.9	19	3.8±0.9	24	0.5±0.9	23
	39wks	3.6±1.0	19	3.9±1.2	24	0.8±1.1	23
	46wks	3.8±1.4	19	4.0±0.8	24	1.0±1.1	23
	52wks	3.5±1.9	19	4.0±1.3	24	1.2±0.8	23
	Average	3.6±1.0	19	3.9±0.8	24	1.0±0.8	23

The intake of solid meals of meat, chicken or fish increased from 1-2 per week at 6 months of age to 7 per week at 12 months in all feeding groups. The use of baby cereal was at it's highest at 6 months with an average of 5 meals per week across all groups and this decreased to approximately 1 meal per week by 12 months of age. The reduction in consumption of baby cereal was compensated for by the use of adult breakfast cereals, which increased from approximately 1 meal per week at 6 months to 5 meals per week at 12 months. These data clearly indicate that assigning infants to receive 4 egg yolks per week did not limit the variety of other solid foods eaten or influence their eating pattern of non-egg containing solid foods (Table 4.4).

Table 4.4: *The intake of solid meals of meat, chicken or fish (home cooked or commercial), adult cereal and baby cereal by infants randomised to receive eggs (dietary intervention) or consuming their usual weaning diet (control diet) at 6 and 12 months of age. Number of meals per week, mean±sd.*

	Dietary Intervention				Control Diet			
	6 months	**n**	**12 months**	**n**	**6 months**	**n**	**12 months**	**n**
Breast Fed								
Meat/Chicken/Fish	1.4±2.6	53	6.5±2.0	48	1.4±2.6	27	6.9±2.7	23
Adult Cereal	0.6±1.8	53	4.7±3.0	48	0.4±1.5	27	4.1±3.3	23
Baby Cereal	5.0±2.9	53	1.4±2.6	48	4.9±3.2	27	2.8±3.3	23
Formula Fed								
Meat/Chicken/Fish	2.4±2.8	46	7.8±2.8	44	1.9±2.8	23	8.1±3.3	23
Adult Cereal	0.5±1.6	46	5.3±3.1	44	1.5±2.8	23	5.7±2.0	23
Baby Cereal	5.1±3.1	46	0.9±2.4	44	4.2±3.1	23	1.0±2.1	23

4.4 Anthropometrics

Within the breast and formula fed cohorts, there was no difference in weight, length or head circumference between dietary groups, even after adjusting for gender and gestational age (Table 4.5).

***Table 4.5**: Anthropometric measurements of infants. Actual age at assessment, weeks (95% CI); weight, g; length, cm; head circumference, cm (mean ± sd).*

	Omega-3 Eggs	n	Regular Eggs	n	Control Diet	n
BREAST						
6 months Actual age	26.1(25.7-26.4)		26.2(25.8-26.5)		26.2(26.0-26.5)	
Weight	7488±738	26	7679±894	27	7379±1021	27
Length	66.8±2.9	26	67.3±1.8	27	66.5±2.9	27
Head circumf.	43.4±1.5	26	43.5±1.0	27	43.7±1.3	27
12 months Actual age	52.7(52.2-53.1)		52.2(51.9-52.5)		52.7(52.4-53.0)	
Weight	9661±1073	24	9661±1089	23	9554±1069	23
Length	74.9±2.8	24	74.5±2.2	23	75.1±2.5	23
Head circumf	46.2±1.2	24	46.6±1.1	23	46.7±1.4	23
FORMULA						
6 months Actual age	26.1(25.7-26.4)		25.9(25.5-26.4)		26.3(25.8-26.8)	
Weight	7900±868	23	7883±1142	24	7940±852	23
Length	67.7±2.2	23	67.4±2.5	24	67.7±2.3	23
Head circumf	43.4±1.5	23	43.4±1.3	24	43.8±1.3	23
12 months Actual age	52.5(52.1-53.0)		52.2(51.9-52.5)		52.3(51.8-52.8)	
Weight	10261±1058	20	10076±1274	24	10047±1069	23
Length	75.8±2.6	20	75.8±3.0	24	75.8±3.5	23
Head circumf	46.6±1.2	20	46.4±1.4	24	46.6±1.2	23

When all growth data for breast and formula fed infants were combined, differences in weight and length were evident. Breast fed infants weighed less than formula fed infants at both 6 (95% CI -713g, -152g) and 12 (95% CI -896g, -200g) months of age. Similarly, breast fed infants were also shorter than formula fed infants at 6 (95% CI -1.52cm, -0.12cm) and 12 (95% CI -1.98cm, -0.22cm) months of age. There were no differences in head circumference between breast and formula fed infants. The magnitude of weight and length differences between breast and formula fed infants reported here are similar to other Australian (21) and overseas data (22) from infants with similar socio-demographic characteristics.

4.5 Fatty Acid Data

The effect of egg yolk on erythrocyte (red blood cell) membranes was a major outcome measure of the study. Changes in the fatty acid pattern in erythrocyte membranes are seen as indicative of changes likely to be seen in other tissues in the body. The fatty acid composition of cell membrane phospholipids are shown in full in Tables 4.6 and 4.7. The main fatty acid of interest was DHA since this is the fatty acid thought to be associated with neural development in infants, is present in breast milk but not many formulas and is present in higher levels in omega-3 eggs (300mg/100gm) than regular eggs (70mg/100gm).

***Table 4.6**: Breast fed infant erythrocyte phospholipids at 6 and 12 months of age (fatty acids expressed as a % of total fatty acids ± sd)*

		Omega-3 Eggs			**Regular Eggs**			**Control Diet**			
Erythrocyte	**Age**	**mean**	**sd**	**n**	**mean**	**sd**	**n**	**mean**	**sd**	**n**	**p<**
Fatty Acids	**Mths**										
Total Sats	6	42.9	0.8	26	42.9	0.7	27	43.2	1.2	27	ns
	12	43.8	0.7	24	43.4	0.7	23	43.5	1.0	23	ns
18:1□9	6	12.3	0.7	26	12.3	0.6	27	12.1	0.6	27	ns
	12	12.4	1.1	24	12.3	0.7	23	12.3	0.7	23	ns
Total Monos	6	16.7	1.0	26	17.0	0.9	27	16.6	1.1	27	ns
	12	16.5	1.0	24	16.2	0.6	23	16.3	0.9	23	ns
18:2□6	6	9.0	1.0	26	8.8	0.9	27	9.1	1.3	27	ns
	12	9.6	0.9	24	9.6	1.0	23	9.4	1.1	23	ns
20:3□6	6	1.6[a]	0.2	26	1.6[a]	0.3	27	1.4[b]	0.2	27	0.01
	12	1.5	0.3	24	1.5	0.3	23	1.5	0.2	23	ns
20:4□6, AA	6	15.8	0.7	26	15.7	1.1	27	15.9	0.9	27	ns
	12	13.9[a]	0.9	24	15.3[b]	0.8	23	15.3[b]	0.7	23	0.001
22:4□6	6	3.3	0.5	26	3.6	0.5	27	3.5	0.5	27	ns
	12	3.0[a]	0.5	24	3.6[b]	0.4	23	3.7[b]	0.5	23	0.001
22:5□6	6	0.88	0.23	26	0.94	0.23	27	0.96	0.25	27	ns
	12	0.67[a]	0.25	23	1.07[b]	0.26	23	1.03[b]	0.36	23	0.001
Total □6	6	30.8	1.4	26	30.9	1.2	27	31.2	1.9	27	ns
	12	28.9[a]	1.8	24	31.4[b]	1.3	23	31.2[b]	2.0	23	0.001
20:5□3	6	0.44	0.13	26	0.40	0.10	27	0.38	0.11	27	ns
	12	0.66[a]	0.19	24	0.45[b]	0.12	23	0.52[b]	0.20	23	0.001
22:5□3	6	2.4	0.2	26	2.3	0.2	27	2.3	0.3	27	ns
	12	2.3[a]	0.2	24	2.3[a]	0.3	23	2.6[b]	0.3	23	0.001
22:6□3, DHA	6	5.5	0.9	26	5.3	0.7	27	5.3	0.8	27	ns
	12	6.7[a]	1.3	24	5.1[b]	0.9	23	4.8[b]	0.8	23	0.001
Total □3	6	8.4	1.0	26	8.1	0.8	27	8.0	1.0	27	ns
	12	9.8[a]	1.4	24	7.9[b]	1.0	23	7.9[b]	1.0	23	0.001

Values with different superscripts indicate significant differences between feeding groups

Table 4.7*: Formula fed infant erythrocyte phospholipids at 6 and 12 months of age (fatty acids expressed as a % of total fatty acids ± sd)*

		Omega-3 Eggs			**Regular Eggs**			**Control Diet**			
Erythrocyte	**Age**	**mean**	**sd**	**n**	**mean**	**sd**	**n**	**mean**	**sd**	**n**	**p<**
Fatty Acids	**Mths**										
Total Sats	6	42.3	1.7	22	42.1	2.1	24	42.5	1.7	23	ns
	12	43.2	0.9	20	42.9	1.0	24	43.1	0.6	23	ns
18:1□9	6	13.2	0.9	22	13.1	1.3	24	13.2	1.2	23	ns
	12	12.8	0.6	20	13.0	0.8	24	13.1	0.8	23	ns
Total Monos	6	17.3	1.3	22	17.2	2.0	24	17.3	1.9	23	ns
	12	16.4	0.9	20	16.9	1.0	24	16.9	1.1	23	ns
18:2□6	6	12.4	1.3	22	12.6	1.0	24	12.1	1.3	23	ns
	12	10.8	1.2	20	10.1	1.2	24	9.9	1.2	23	0.1
20:3□6	6	1.8	0.3	22	1.8	0.4	24	1.9	0.5	23	ns
	12	1.9	0.3	20	1.8	0.4	24	2.0	0.5	23	ns
20:4□6, AA	6	14.3	0.8	22	14.2	0.9	24	14.1	0.8	23	ns
	12	14.5^{a}	1.0	20	15.1^{b}	0.7	24	15.0^{ab}	0.8	23	0.05
22:4□6	6	4.0	0.7	22	4.1	0.5	24	3.9	1.2	23	ns
	12	3.6	0.6	20	3.9	0.5	24	3.9	0.7	23	ns
22:5□6	6	1.0	0.3	22	1.1	0.4	24	1.0	0.4	23	ns
	12	0.8^{a}	0.3	20	1.1^{b}	0.3	24	1.0^{b}	0.3	23	0.05
Total □6	6	34.0	1.7	22	34.1	1.3	24	33.4	1.7	23	ns
	12	31.9	1.4	20	32.3	1.2	24	32.1	1.6	23	ns
20:5□3	6	0.30	0.12	22	0.31	0.13	24	0.32	0.13	23	ns
	12	0.58	0.16	20	0.54	0.20	24	0.64	0.20	23	ns
22:5□3	6	2.2	0.4	22	2.2	0.6	24	2.3	0.6	23	ns
	12	2.6^{a}	0.5	20	2.7^{ab}	0.5	24	3.0^{b}	0.5	23	0.05
22:6□3, DHA	6	2.6	0.5	22	2.6	0.5	24	2.7	0.6	23	ns
	12	4.2^{a}	1.2	20	3.3^{b}	0.7	24	3.0^{b}	0.5	23	0.001
Total □3	6	5.2	0.9	22	5.2	1.0	24	5.4	1.1	23	ns
	12	7.5^{a}	1.3	20	6.8^{b}	0.8	24	6.8^{ab}	0.9	23	0.05

Values with different superscripts indicate significant differences between feeding groups

4.5.1 Dietary intake

Infants supplemented with eggs consumed four egg yolks per week while the unsupplemented infants consumed on average one egg yolk per week. Analysis of the dietary questionnaires indicated that the dietary intake of LCPUFA from other sources such as fish and meats was insignificant and that there was no difference between the groups (data not shown). Assuming that a breast fed infant receives ≈100mg DHA/day from breast milk, it is possible to estimate the amount of DHA in the diet of the infants in the various dietary groups (Table 4.8).

Table 4.8*: Estimated daily intake (mg) of DHA from eggs* and milk by infants in the study*

	Omega-3 Eggs (4 egg yolks/week)	**Regular Eggs (4 egg yolks/week)**	**Control Diet (1 egg yolk/week)**
Breast Fed	200	125	105
Formula Fed	100	25	5

* Calculations based on one egg yolk from a 60g egg

Likewise, assuming that a breast fed infant receives ≈200mg AA/day from breast milk, it is possible to estimate the amount of AA in the diet of the infants in the various dietary groups (Table 4.9).

Table 4.9: *Estimated daily intake (mg) of AA from eggs* and milk by infants in the study*

	Omega-3 Eggs (4 egg yolks/week)	**Regular Eggs (4 egg yolks/week)**	**Control Diet (1 egg yolk/week)**
Breast Fed	225	260	215
Formula Fed	25	60	15

* Calculations based on one egg yolk from a 60g egg

4.5.2 Effect of egg yolk consumption on DHA status of the infant

The DHA status of the infants at the end of the 6 month dietary intervention (12 months of age) is shown in Figure 4.2 and Table 4.10. Results are expressed as the percentage of the total fatty acids in the erythrocyte membranes.

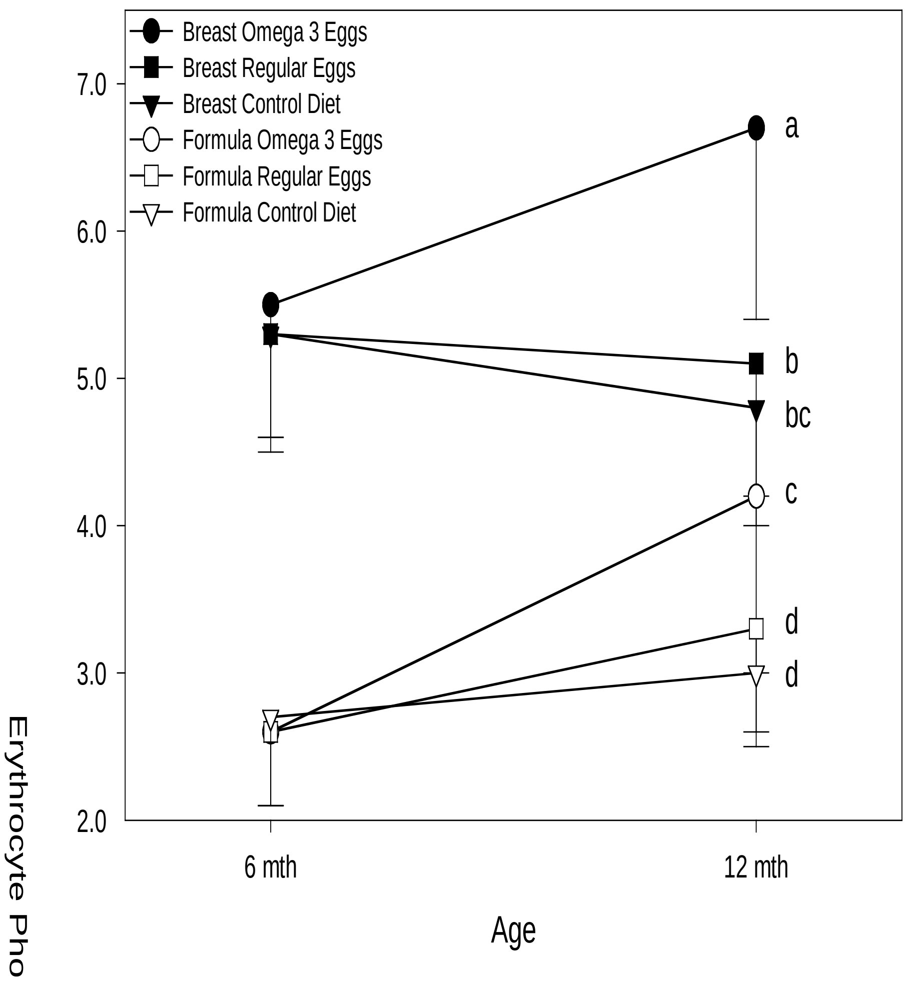

Figure 4.2 *Erythrocyte phospholipid DHA in breast and formula fed infants at 6 and 12 months of age (mean - 1sd). At 12 months, values with different superscripts indicate significant differences between feeding groups*

Table 4.10: *DHA content of erythrocyte fatty acids at 12 months of age*

	Omega-3 Eggs		**Regular Eggs**		**Control Diet**		**p<**
	mean%±sd	n	mean%±sd	n	mean%±sd	n	
Breast Fed	6.7±1.3[a]	24	5.1±0.9[b]	23	4.8±0.8[b]	23	0.001
Formula Fed	4.2±1.2[a]	20	3.3±0.7[b]	24	3.0±0.5[b]	23	0.001

Values with different superscripts indicate significant differences between feeding groups

The level of DHA was lowest in the erythrocytes of infants fed formula (no DHA) and only one regular egg yolk in the diet (control). Increasing the consumption of regular egg yolks to 4 per week had no effect on the DHA status of formula fed infants. However, when the egg yolks consumed were enriched with DHA (omega-3 eggs) there was an increase in the level of erythrocyte DHA. Four omega-3 egg yolks per week resulted in a 27% increase in the DHA content of cell membranes compared with the level following the consumption of 4 regular egg yolks per week and a 40% increase compared with the control weaning diet which includes 1 regular egg yolk per week. The level of DHA attained in the red blood cells of formula fed infants in the omega-3 egg group (95% CI 3.6%, 4.8%) was the same as that observed in the breast fed control group (95% CI 4.4%, 5.1%).

Breast fed infants at 12 months had higher levels of DHA in their erythrocytes than formula fed infants on each of the 3 diets. Simply increasing the consumption of regular egg yolks from 1 to 4 per week had no effect on the DHA status of breast fed infants. However, the use of omega-3 egg yolks resulted in a 31% increase in DHA levels compared with that achieved with 4 regular egg yolks per week and 39% over 1 regular egg yolk per week.

In summary, the inclusion of four omega-3 egg yolks in the diet of healthy 6-12 month old babies resulted an approximate 30% increase in DHA status of the infants regardless of whether they were breast or formula fed.

4.5.3 Effect of egg yolk consumption on AA status of the infant

There was no effect on the AA content of cell membranes of consuming either one or four regular egg yolks per week in either breast or formula fed infants (Table 4.11). Consumption of omega-3 egg yolks resulted in a larger drop in cell membrane AA in the breast fed infants (9%) compared with formula fed infants (3%).

Table 4.11*: AA content of erythrocyte fatty acids at 12 months of age*

	Omega-3 Eggs		Regular Eggs		Control Diet		p<
	mean%±sd	n	mean%±sd	n	mean%±sd	n	
Breast Fed	13.9±0.9[a]	24	15.3±0.8[b]	23	15.3±0.7[b]	23	0.001
Formula Fed	14.5±1.0[a]	20	15.1±0.7[b]	24	15.0±0.8[ab]	23	0.05

Values with different superscripts indicate significant differences between feeding groups

4.6 Iron Status

Of 80 breast fed infants assessed at 6 months of age, 8 (10%) had an Hb <105g/L. Six were classified as anaemic and two had IDA (Hb <105g/L and ferritin ≤10μg/L). These 8 infants were withdrawn from the trial and referred for treatment. There was no anaemia or IDA amongst the formula fed infants assessed at 6 months (0/69). The rate of ID (ferritin ≤10μg/L) at 6 months amongst the breast fed infants was approximately 6% (5/80) and approximately 1% (1/69) amongst the formula fed infants.

Iron parameters and classification of iron status at the end of dietary intervention (12 months of age) are shown in Table 4.12. The rates of ID, IDA and anaemia were also similar between the intervention groups amongst breast or formula fed infants (Table 4.12). However regardless of intervention, breast fed infants tended to have a higher rate of ID and more anaemia compared with formula fed infants.

***Table 4.12**: Measures of iron status at the end of dietary intervention (12 months of age)*

	Omega-3 Eggs	**Regular Eggs**	**Control Diet**
BREAST (n)	24	23*	23
Hb (g/L, mean±sd)	119±9	121±10	120±7
Ferritin (μg/L, mean±sd)	24±12	30±24	21±11
Iron (μmol/L, mean±sd)	10±4	11±5	8±3
Transferrin (g/L, mean±sd)	2.9±0.3	3.1±0.5	3.0±0.4
Saturation (%, mean±sd)	14±7	15±7	11±5
ID (n)	4	4	4
Anaemia (n)	1	2	0
IDA (n)	0	1	0
FORMULA (n)	20*	24	23
Hb (g/L, mean±sd)	123±9	123±8	121±6
Ferritin (μg/L, mean±sd)	36±21	37±37	34±17
Iron (μmol/L, mean±sd)	10±5	11±5	8±3
Transferrin (g/L, mean±sd)	2.9±0.4	2.9±0.4	2.9±0.4
Saturation (%, mean±sd)	14±7	16±6	12±5
ID (n)	1	2	2
Anaemia (n)	0	0	0
IDA (n)	0	0	0

*One Hb value was unavailable because the blood sample was either haemolysed or clotted

At 12 months, there was no significant difference in Hb, ferritin, iron, transferrin or transferrin saturation between the dietary groups for both the breast and formula fed cohorts. However, when all data were combined and measures of iron status were compared between the egg intervention groups and non-intervention groups, plasma iron (95% CI 0.4, 3.3) and transferrin saturation (95% CI 0.5, 4.7) were higher in infants allocated to the egg groups compared with the non-intervention or control groups (Table 4.13). It is important to note that plasma iron and transferrin saturation are relatively poor markers of iron status on their own. This expanded analysis clearly demonstrated that the major indices of iron status (Hb and ferritin) were not affected by additional egg yolks to the weaning diets of non-anaemic infants.

Table 4.13*: Measures of infant iron status at 12 months of age according to dietary intervention*

Parameter	Egg intervention groups		Non intervention groups		p<*
	mean±sd	n	mean±sd	n	
Hb (g/L)	121±9	89	121±7	46	
Ferritin (µg/L)	32±25	91	27±16	46	
Iron (µmol/L)	11±5	91	8±3	46	0.05
Transferrin (g/L)	3.0±0.4	91	3.0±0.4	46	
Saturation (%)	15±7	91	11±5	46	0.05

*following adjustment for 6 month iron measurement and breast or formula feeding.

4.7 Cholesterol

The changes in total plasma cholesterol concentrations over the period of the study are summarised in Figure 4.3 and Tables 4.14 and 4.15. There was no difference in plasma cholesterol levels at 12 months between the two egg intervention groups, whether breast or formula fed (Table 4.14).

Table 4.14*: Effect of intervention on plasma cholesterol at 6 months and 12 months of age*

	Breast Fed				Formula Fed			
	6 months	**n**	**12 months**	**n**	**6 months**	**n**	**12 months**	**n**
Omega-3 Eggs	4.3 ±0.7	26	4.4±0.7	24	3.8±0.7	22	4.2±0.7	20
Regular Eggs	4.3±0.8	27	4.2±0.8	24	3.8±0.7	24	4.2±0.6	24
Control Diet	4.3±0.9	27	4.1±0.9	23	3.8±0.7	23	3.9±0.8	23

The total cholesterol level of 6 month old breast fed infants was higher than that of age matched formula fed infants (4.3±0.8 v 3.8±0.7, $p<0.0005$), which is consistent with the literature (23). However, this difference had disappeared by 12 months of age (4.3±0.7 v 4.1±0.7). The inclusion of up to four egg yolks per week in the weaning diet between 6 and 12 months of age did not significantly change the total cholesterol levels in either the breast fed or formula fed infants compared with infants consuming 1 egg yolk per week only as part of their normal weaning diet. At 12 months, when data from all infants were combined and adjusted for 6 month values and mode of feeding, there was still no statistically significant effect of egg intervention on cholesterol levels.

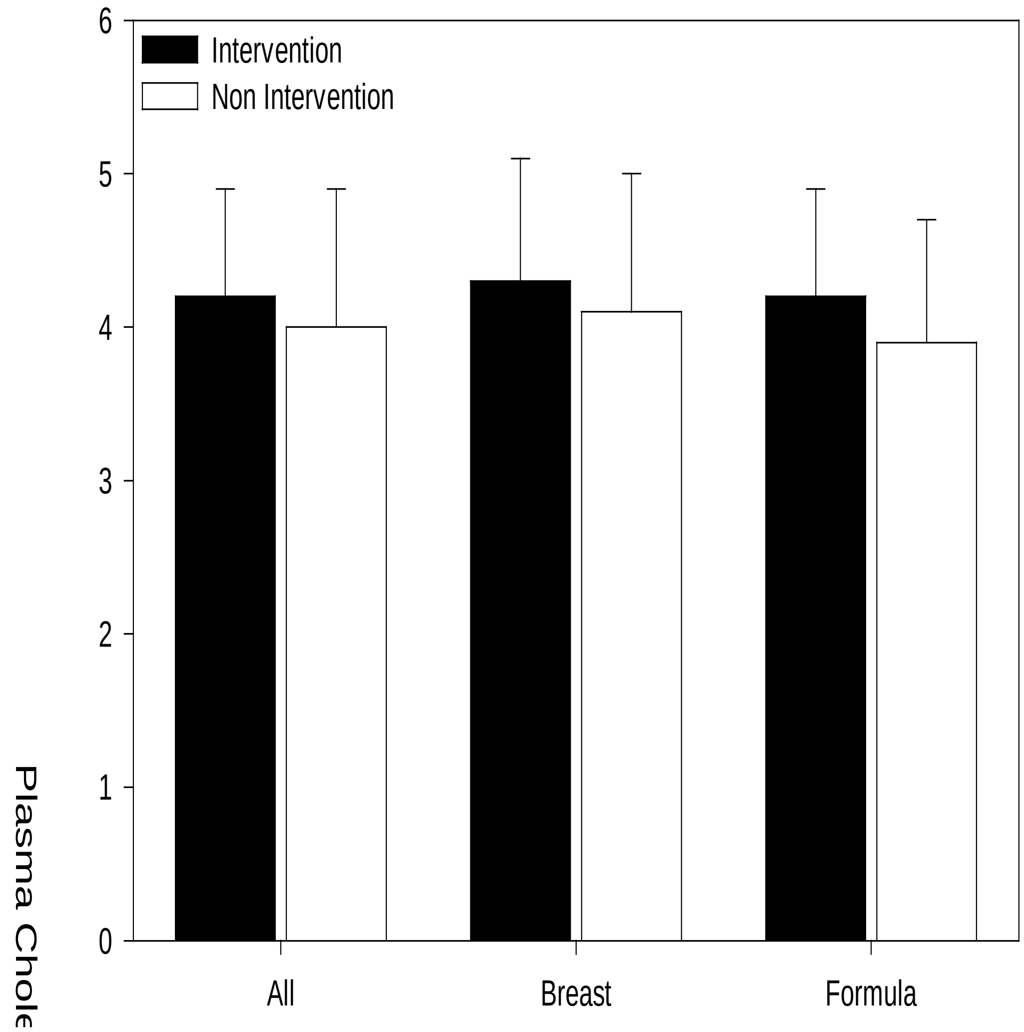

Figure 4.3 *Plasma cholesterol levels (mean±sd) at 12 months in all, breast and formula fed infants randomised to either dietary intervention (4 egg yolks per week) or no dietary intervention (1 egg yolk per week)*

Table 4.15*: Effect of breast or formula feeding and dietary intervention on plasma cholesterol levels (mmol/L ± sd) at 6 and 12 months of age*

	Breast Fed	**n**	**Formula Fed**	**n**	**All Infants**	**n**
Age 6 months	4.3±0.8*	80	3.8±0.7	69	-	
12 months	4.3±0.7	70	4.1±0.7	67	-	
Effect at 12 months						
no intervention	4.1±0.9	23	3.9±0.8	23	4.0±0.9	46
4 egg yolks/week	4.3±0.7	47	4.2±0.7	44	4.2±0.7	91

* $p<0.0005$

In summary, the cholesterol intake from consuming up to four egg yolks per week (either regular eggs or omega-3 enriched eggs) from 6 to 12 months of age is not associated with an increase in total plasma cholesterol.

4.8 Immunoglobulin E

Table 4.16 shows the family history of allergic disorders as given by the parents of breast fed and formula fed infants. The incidence of asthma and hay fever was greater in mothers who were breast feeding their infants than in those who chose to feed formula but there was no difference between groups in the incidence of any allergic symptoms of fathers or siblings of infants in the study.

The total plasma IgE was greater in breast fed infants than formula fed infants at both 6 and 12 months of age (Table 4.17). Statistical analysis was performed on log transformed data as described in Sample Size and Statistics 3.4.5 and data are presented as mean ± sem. Although a difference between breast and formula fed infants was detected, it is important to note that 97% of all plasma samples tested fell within the reference range for infants in this age group.

There was no difference in the incidence of positive values for egg white- and egg yolk-specific antibodies with regards to mode of feeding (breast or formula) (Table 4.17).

Table 4.16*: Family history of allergic disorders*

Family History		**Breast Fed**		**Formula Fed**		
		%	**n**	**%**	**n**	**p<**
Mother	Asthma	21.3	80	8.6	70	0.05
	Hay Fever	25.0	80	10.0	70	0.05
	Eczema	8.8	80	10.0	70	
Father	Asthma	6.8	74	11.9	67	
	Hay Fever	21.6	74	23.8	67	
	Eczema	1.4	74	4.5	67	
Siblings	Asthma	15.1	53	25.6	39	
	Hay Fever	1.9	53	2.6	39	
	Eczema	17.0	53	7.7	39	

Table 4.17*: Total IgE, egg white-specific and egg yolk-specific antibodies (% positive) in the plasma of breast fed and formula fed infants at 6 and 12 months of age*

		Breast Fed	**n**	**Formula Fed**	**n**	**p<**
Total IgE, IU/mL	6 months	9.7 ± 1.5	69	5.9 ± 1.1	61	0.05
(mean±sem)	12 months	21.2 ± 5.1	63	9.5± 2.0	62	0.05
Egg White Ab	6 months	6	79	6	69	
(% with RAST Class ≥ 1)	12 months	13	70	8	67	
Egg Yolk Ab	6 months	6	79	1	69	
(% with RAST Class ≥ 1)	12 months	6	70	2	67	

At *6 months* of age only one infant out of 130 tested (0.8%) had a total circulating IgE value greater than 60 IU/mL (62 IU/mL). Following randomisation into a dietary intervention group, this breast fed infant was found to have eczema and some respiratory problems all of which suggests an increased susceptibility to atopy. At 12 months of age, the total IgE was 83 IU/mL and this infant had developed antibodies to egg white (and continued to test positive for egg yolk specific antibodies).

At *12 months* of age 7 infants out of 126 infants tested (6%) had total IgE levels greater than 60 IU/mL (range 64-261). Of these infants, 6 had been in dietary intervention groups (including the infant described above) and 1 was in a control group. Apart from the infant described above, none of these infants tested positive for egg white-specific antibodies and only 1 tested positive for egg yolk-specific antibodies. These data suggest that the high levels of total circulating IgE detected in these few infants at 12 months of age were not associated with the increased consumption of egg yolk in the weaning diet but may possibly indicate a susceptibility to allergic response to another antigen (i.e. other foods, house dust mite, animal dander or pollens and grasses).

Prior to the dietary intervention, 9 infants out of 148 tested for egg white-specific antibodies and 6 infants out of 147 tested for egg yolk-specific antibodies had detectable levels by RAST radioimmunoassay (range 1-3). By 12 months of age 14 infants were positive for egg white-specific antibodies and 5 infants were positive for egg yolk-specific antibodies. Ten infants who had undetectable levels of egg white-specific antibodies at 6 months developed some egg white specific-antibody activity by 12 months of age. Of these 10 infants, 4 were in egg intervention groups and 6 were consuming their usual weaning diet. These data suggest that the specific introduction of four egg yolks per week to the diet of healthy infants with no known susceptibility to allergy does not increase the risk of developing antibodies specific to egg components.

In Table 4.18 the total plasma IgE and specific IgE antibodies to egg white and egg yolk in 12 month old infants who were randomised to receive egg yolks are compared with those who consumed their normal weaning diet (including one egg yolk per week only). There were no differences in these indices of allergy between infants randomised to receive egg yolks and those in the control groups.

Table 4.18*: Total IgE, egg white-specific and egg yolk-specific antibodies (% positive) in the plasma of breast fed and formula fed infants consuming either four egg yolks per week (+Egg) or one egg yolk only per week (Cont) measured at 12 months of age*

	Total IgE (IU/mL, mean±sem)				**Egg White Antibodies (% RAST Class ≥ 1)**				**Egg Yolk Antibodies (% RAST Class ≥ 1)**			
	+Egg	**n**	**Cont**	**n**	**+Egg**	**n**	**Cont**	**n**	**+Egg**	**n**	**Cont**	**n**
Breast	19±5	41	25±11	22	11	47	18	23	6	47	4	23
Formula	10±3	42	8±2	20	5	44	13	23	2	44	0	23
All	15±2	83	17±6	42	8	91	15	46	4	91	2	46

4.9 Bayley's Scales of Infant Development

Regression models for Bayley's MDI and PDI indicated that dietary intervention with egg yolks during the second half of infancy did not influence either index of developmental outcome at one year of age (Table 4.19). Nutritional and environmental variables accounted for 17% of the variance in MDI. Nutritional variables did not account for any of the variance in PDI, while environmental factors contributed 8%. Although these models may have had limited power to detect subtle nutritional influences on developmental indices, it would appear that dietary intervention of increased egg yolk consumption during the second 6 months of infancy was without an effect of developmental outcome in this healthy and socially advantaged population.

***Table 4.19**: Multiple linear regression results for independent factors predicting mental (MDI) and psychomotor (PDI) developmental indices*

Dependent Variable	**Independent Variables**	**B**	**β**	**cr^2**	**p<**
MDI (n=136)	Apgar at 5 min 6 month plasma iron spouse smoker birth order	-3.8 -0.9 -5.0 -2.4	-0.23 -0.27 -0.20 -0.18	0.06 0.11 0.14 0.17	0.005 0.001 0.01 0.05
PDI (n=136)	mother smoker spouse education	9.3 -2.6	0.23 -0.16	0.07 0.08	0.05 0.1

B, unstandardised regression coefficient
β, standardised regression coefficient
cr^2, cumulative r^2

5 DISCUSSION

Our trial was a unique, systematic study of the nutritional value of including egg yolk in the weaning diet of breast and formula fed infants. With the separate randomisation of breast and formula fed infants, the trial was ideally poised to address current nutritional issues pertinent to all infants. For example, although formula fed infants are generally iron sufficient, the issue of improving their LCPUFA status remains topical. Conversely, breast fed infants have an ample supply of dietary LCPUFA but have been reported to be at risk of depleted iron stores during late infancy. Our trial addressed whether egg yolk, which can be a good source of both LCPUFA and iron, is beneficial for all infants regardless of whether they are breast or formula fed.

Of 161 infants recruited, 137 successfully completed the trial representing an 85% follow-up of all infants enrolled. An equal number of breast and formula fed infants were withdrawn and these infants were evenly distributed amongst the three dietary groups. A high degree of confidence can be placed in the trial results because of the high completion rate.

5.1 Dietary Intervention

Our specific intervention was to encourage mothers to feed their infants four egg yolks per week from 6 months of age. Mothers were supplied with the eggs and given specific instructions about their preparation and were encouraged to incorporate them into custards, vegetables and foods the child was already consuming in order not to affect the rest of the weaning diet. The success of this intervention is highlighted by the fact that infants assigned to eggs did in fact consume an average of four egg yolks per week consistently throughout the study period, without significantly altering their intake of other solid foods that may contribute to either LCPUFA or iron intake.

Infants in the control (non-intervention) groups consumed on average one egg yolk per week. Although this reported consumption is low, it may still be an overestimation of the average egg consumption by healthy Australian infants. All mothers who agreed to participate in this trial were aware that the aim of our trial was to investigate the nutritional impact of egg yolk in the weaning diet of infants and that they had an equal chance of being recruited to one of two egg groups or a control group. It is therefore possible that mothers with infants allocated to a control group unconsciously increased the number of egg yolks offered to their infant. However, even if this assertion were false, our trial has highlighted the relatively low rate of egg yolk consumption by healthy Australian infants.

5.2 Specific Nutritional Benefits

A major finding of the current study was that formula fed infants who consumed four omega-3 egg yolks per week had DHA levels in their blood cells similar to control breast fed infants. Until recently there were no formulas that contained omega-3 LCPUFA available for term infants and even today the formulas supplemented with LCPUFA are designed for the first 3 months of life. Because of the absence of LCPUFA in most formulas, it has long been known that infants fed formula have lower levels of omega-3 LCPUFA, particularly DHA, in their blood (plasma and red blood cells) than those who are breast fed. Furthermore, we have previously shown (1) that weaning diets are very low in LCPUFA so that formula fed infants have little chance in the normal course of events to achieve LCPUFA levels similar to those seen in breast fed infants. In that earlier study (1) we calculated that it could take up to 14 regular egg yolks per week to provide an infant with the same amount of DHA that breast fed infants receive. The current study demonstrates the value of as few as four omega-3 egg yolks per week in the weaning diet of babies fed formula, raising their levels of DHA to levels similar to those in breast fed infants. In other words, enriching eggs with omega-3 fatty acids makes them more nutritionally valuable as a weaning food for infants.

The study did not demonstrate any major effect on the iron status of these well-nourished 6-12 month old infants. This was not surprising since the amount of iron received from four additional egg yolks per week to the infant diet would contribute approximately 0.5mg of iron per day or 6% of the iron RDI for 6–12 month old infants. Our data suggest that such a modest increase in dietary iron does not significantly improve the iron status of healthy, non-anaemic infants, despite the fact that egg yolk is a haem source of iron. This may be because the increase in dietary iron was not large enough to influence iron status in this population. Indeed, we have demonstrated in a separate trial that 6 month old infants randomly allocated to a high iron weaning diet (8.2 $\pm$ 2.9mg/d, n=36) or a control group (5.2 $\pm$ 3.4mg/d, n=26) had similar iron status at 12 months of age (24). The infants in this earlier trial were also all non-anaemic but the intervention group received 3mg of extra iron per day compared with 0.5mg extra iron in the current trial. Taken together, these data indicate that iron intake from the current weaning diet of healthy infants may be adequate and further increases in iron intake do not significantly improve iron status. This is biologically plausible since the human intestine has the ability to regulate iron absorption based on need (25). It is possible that our results would have been quite different if we had studied a population of iron deficient infants who may have a greater capacity to absorb dietary iron.

5.3 Perceived Risks Of Eggs In Weaning Diets

There are two areas where eggs in the diet of infants older than 6 months are perceived to constitute a risk, namely, cholesterol and allergy. While egg yolks contain cholesterol so does breast milk. The cholesterol intake by a formula fed infant consuming four egg yolks per week is about the same as that of a typical breast fed infant. Furthermore, the cholesterol levels of those infants in the study who received four egg yolks per week was not statistically higher than those receiving one egg yolk regardless of the mode of feeding. Given that there have been calls for cholesterol to be included in the diets of infants in the first year of life to provide substrate for brain lipids we can see little evidence for risk in regard to hypercholesterolemia in infants who get up to four egg yolks per week.

The allergic condition (atopy) reflects an increased propensity for the production of allergen-specific IgE antibodies, which leads to clinical symptoms such as asthma, rhinitis and eczema. One of the most significant risk factors for the development of atopy is family atopic history. Other risk factors that have been shown to be important in this process are increased total serum/plasma IgE in infancy and childhood and significant specific IgE (26). Under normal conditions, plasma total

IgE concentrations are low, but may become raised in the presence of allergic or parasitic disease.

This study was too small to examine the clinical evidence for an increase the incidence or degree of atopy in infants whose diets included four egg yolks per week - that would require hundreds of infants. However, we explored the effect of our dietary intervention on plasma markers of allergic response by examining total IgE, as well as egg yolk- and egg white-specific IgE at 12 months. The only difference detected in this study was in the total circulating IgE concentration of breast fed infants compared with formula fed infants regardless of the inclusion of egg yolks in the diet. It is likely that this is related to the increased incidence of atopy amongst mothers of breast fed infants compared with mothers of formula fed infants rather than any dietary effect. We could not detect an effect on total plasma IgE concentration or the incidence of specific antibodies to egg components following the inclusion of egg yolk in the weaning diet regardless of whether the infant was breast or formula fed. The combined data for plasma IgE's would suggest that there is no support for the contention that the inclusion of egg yolks in the diets of healthy 6-12 month old infants places them at increased risk of an allergic response.

In summary, there is no evidence in this study of health risks associated with the inclusion of egg yolks in the diets of babies 6-12 months of age.

5.4 Growth And Development

Growth has been reported to be inhibited in preterm infants who received formula supplemented with omega-3 LCPUFA in the first months of life and it has been hypothesised that it could be due to reductions in AA levels induced by the diets (27,28). Although we detected a small reduction in erythrocyte AA levels due to consumption of four omega-3 egg yolks per week, we could not detect an effect on weight, length or head circumference. Our study was capable of detecting the well-known differences in weight and length between breast and formula fed infants and the observed differences are in line with results we have seen in other studies (21,22). There is no evidence that the inclusion of omega-3 eggs in the diet had any effect on growth of either breast or formula fed infants.

DHA has been reported to be limiting in the diets of preterm and term infants as indicated by improvements of visual and developmental outcome measures following supplementation of the early infant diet with DHA (7-9,29,30). In the current study we measured the effect dietary intervention on the Bayley's Scales of Infant Development at 12 months of age. The current study was not sufficiently powered to detect differences between dietary groups but we hypothesised that if present, we could detect an association between DHA status of all infants in a feeding mode (breast/formula) and Bayley Mental Developmental Index. However, while regression analysis revealed expected associations between socio-economic and environmental factors and Bayley scores it failed to detect any influence of fatty acid status. It may well be that the period of dietary treatment between 6 and 12 months was too late to induce changes in development or that DHA was not limiting in these well nourished and socially advantaged infants.

6 IMPLICATIONS AND RECOMMENDATIONS

This study was designed to test the nutritional efficacy of egg yolks as a source of DHA and iron in the diets of Australian infants and to examine issues of safety with regard to plasma cholesterol and IgE levels. Eggs have a poor public image largely because they are perceived to be associated with the risk of elevated cholesterol and egg-induced allergy. The focus of concern in relation to cholesterol is egg yolks while the major concern in relation to allergy is egg white. The concerns persist about the use of eggs in the weaning diet despite the known nutritional value and digestibility of egg yolks and the fact that egg yolks appear in the list of recommended foods for infants for the second 6 months of life. Our study has confirmed the fact that egg yolks are a minor part of infant weaning diets between the ages of 6 and 12 months of age.

Most Australian babies start out being breast-fed but by 6 months of age over half have been changed to infant formula. It was therefore important to conduct our study in both breast and formula fed infants. This was certainly relevant in relation to allergy since it could be argued that breast and formula fed infants could have differential sensitivity to eggs because of the potential for early exposure to egg antigens through breast milk. There were also separate nutritional issues pertaining to the two groups. For example, although formula fed infants are generally iron sufficient, the issue of improving their LCPUFA status was considered relevant since at the time the study commenced there were no formulas on the market that contained DHA. Conversely, breast fed infants have an ample supply of dietary LCPUFA but have been reported to be at risk of depleted iron stores during the second 6 months of life.

Our trial addressed whether egg yolk, which can be a good source of both DHA and iron, is beneficial and safe for all infants regardless of whether they are breast or formula fed.

6.1 Are egg yolks a good source of DHA and iron?

The results of the study are very clear on the subject of DHA. Even four regular egg yolks per week have little effect on that group of infants for whom DHA is not a regular dietary component, that is, those fed infant formulas. On the other hand, four omega-3 egg yolks per week resulted in the red blood cell levels of DHA being elevated to that of control breast-fed infants. The effect of four omega-3 egg yolks per week on breast fed infants was equally effective but did not push the values outside the range seen in infants who consume breast milk from mothers who include fish (≈100g can of salmon per week) in their diets.

The data are also clear with regard to iron. Including egg yolks in weaning diets had no effect on the two major indicators of iron status, namely Hb and ferritin. This was almost certainly due to the good nutritional status of the infants in the study and that four egg yolks per week provide only 0.5mg of iron per day. Even if this iron is very well absorbed (the iron in eggs is in the haem form) this amount constitutes only about 6% of the RDI of iron for babies in this age group. Infants who are less well nourished may benefit from such an intake since the absorption of iron is related to the infant's iron status.

6.2 Are egg yolks safe for infants 6-12 months?

A chief finding of the study was that up to four egg yolks per week in the diet of babies do not place them at increased risk from hypercholesterolemia or allergy. There were non-significant increases in the cholesterol levels of both breast and formula fed infants in response to four, compared with one, egg yolk per week but these small changes were unlikely to be clinically relevant.

We explored the effect of our intervention on plasma markers of allergic response by examining total IgE, as well as egg yolk-, and egg white-specific IgE at 12 months. We could not detect an effect of increased egg intake on any of the IgE levels regardless of whether the infant was breast or formula fed. The combined data for plasma IgE's would suggest that there is no support for the contention that the inclusion of egg yolks in the diets of healthy 6-12 month old infants places them at increased risk of an allergic response to eggs.

6.3 Should we recommend that all infants eat more egg yolks?

There is little doubt that based on the data obtained in this study, babies older than 6 months can have up to four egg yolks included in their total diet without risk of hypercholesterolemia or allergic response. However this is a separate question to asking whether infants *should* eat four egg yolks per week. The study established that including four omega-3 egg yolks in the weekly diet can improve the DHA status of the baby but no functional outcome was associated with this improved status. It may require a separate study to target health outcomes that are likely to benefit from elevated DHA intake in the second 6 months of life.

The purpose of the weaning diet is to act as a transition between breast milk or infant formula and the diet of the older child. In addition to providing supplementary nutrition, weaning diets are designed to provide a variety of tastes and textures that are important in this transition. In this context there are many foods that are included in recommended diets for 6-12 month old infants that provide good nutrition without linking them to specific health benefits. Foods such as green and yellow vegetables, meat and iron-enriched cereals fall into this category and the inclusion of egg yolks in the weaning diet should be seen in this overall context.

The results of our study suggest that it is safe for health professionals to recommend up to four egg yolks per week for 6-12 month old infants. Given the range of vitamins, minerals (including haem iron), proteins and fats (including DHA-rich fats in omega-3 egg yolks) egg yolks should be seen as a vital part of a well balanced diet for Australian infants and toddlers.

APPENDIX A

Iron/PUFA Questionnaire

Date: .... Name of Infant: .... DOB: ....

Milks

☐ Breastmilk No of feeds/24 hours ....

☐ Formula Type ....
No. of bottles/24 hours ....

☐ Cow's milk No. of bottles/24 hours ....
Do you add anything to bottle? ....

Solids

How many times a week does your baby have:-

	Home Cooked	Commercial Baby Food i.e. tins/jars
red meat (beef/lamb/veal)	....	....
white meat (chicken/turkey)	....	....
fish fresh or canned (e.g. salmon/tuna) sardines	....	....
liver/kidney/paté	....	....
brains	....	....

Egg yolks No. per week ....

What type? please circle one
carton A
carton B
normal
high omega-3

Have you introduced whole eggs? Y/N
How many whole eggs/week? ....

Cereal

adult type
e.g. weetbix/vitabrits no. of serves/week ....
dry infant cereal
e.g. Heinz/Farex baby rice no. of serves/week ....
Any vitamins/minerals/other nutritional supplement
e.g. Kindivite/pentavite ....

LIST OF TABLES AND FIGURES

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