Nutritional effect of including egg yolk in the weaning diet of breast-fed and formula-fed infants: a randomized controlled trial[1–3]

Maria Makrides, Joanna S Hawkes, Mark A Neumann, and Robert A Gibson

ABSTRACT

Background: Egg yolks can be a source of docosahexaenoic acid (DHA) and iron but are often associated with adverse consequences on plasma cholesterol.

Objective: Our goal was to investigate the effect of consumption of 4 egg yolks/wk on infant DHA status and hemoglobin, ferritin, and plasma cholesterol concentrations. Secondary outcomes included plasma iron, transferrin, and transferrin saturation.

Design: This was a randomized controlled trial comparing no dietary intervention, consumption of 4 regular egg yolks/wk, and consumption of 4 n−3 fatty acid–enriched egg yolks/wk in breast-fed and formula-fed infants from 6 to 12 mo of age. Erythrocyte DHA concentrations, cholesterol, and iron status were assessed at 6 and 12 mo of age.

Results: Of the 82 breast-fed infants recruited, 23 of 28 (no intervention), 23 of 27 (regular eggs), and 24 of 27 (n−3 eggs) completed the trial. Of the 79 formula-fed infants enrolled, 23 of 27 (no intervention), 24 of 26 (regular eggs), and 20 of 26 (n−3 eggs) completed the trial. Erythrocyte DHA concentrations were 30–40% higher after the n−3 egg intervention than after treatment with regular eggs or no eggs in both breast-fed and formula-fed infants. Egg treatment had no significant effect on plasma cholesterol, hemoglobin, ferritin, and transferrin but did result in improvements in plasma iron and transferrin saturation compared with no egg treatment.

Conclusions: n−3 Fatty acid–enriched eggs may provide a means of increasing dietary DHA during the second 6 mo of life. Egg yolks may also be a useful source of iron during the weaning period and can be safely included in the weaning diet with no perturbations in plasma cholesterol. *Am J Clin Nutr* 2002; 75:1084–92.

KEY WORDS Weaning diet, infants, randomized controlled trial, docosahexaenoic acid, iron, cholesterol, egg

INTRODUCTION

Weaning of both breast-fed and formula-fed infants is usually recommended from 4 to 6 mo of age (1). Although it is common to introduce iron-fortified cereals as a first food, this contrasts with more traditional weaning practices in which egg yolks and brains were used as first foods (2). Iron stores in breast-fed infants become depleted by ≈6 mo. Because breast milk is not a good source of iron, iron-rich weaning foods are considered important to avoid iron deficiency (3). Like meat, egg yolks contain both heme and nonheme iron. Heme iron refers to the iron in hemoglobin, myoglobin, and heme-containing enzymes; nonheme iron includes all other forms of iron. Heme iron is absorbed more efficiently than is nonheme iron and its absorption is not significantly influenced by iron status or other constituents of the diet (3, 4). Conversely, the absorption of nonheme iron, mainly from iron salts, can be modified by different dietary factors, and studies investigating the addition of whole eggs to meals containing nonheme iron show a reduction in iron absorption (reviewed in reference 5). These observations are supported by other studies showing that egg white inhibits nonheme iron absorption (6). However, iron absorption from egg yolk alone has not been investigated. Given that egg yolks contain some heme iron, are small in volume, and have a soft texture suitable for weaning infants, an investigation of whether egg yolk in the weaning diet can influence iron status is warranted.

The n−3 long-chain polyunsaturated fatty acid (LCPUFA) docosahexaenoic acid (DHA; 22:6n−3) is an integral component of breast milk and until recently was not added to infant formulas. Randomized controlled trials of infant formula supplemented with DHA compared with formula containing only precursor fatty acids have consistently shown short-term improvements in visual and neural development of preterm infants (7). Trials involving term infants have reported neutral or positive outcomes (7). Although the potential long-term benefits of DHA are still being explored, biochemical data indicate that breast-fed infants accumulate DHA in the brain until ≥12 mo of age and at a greater rate than do infants fed formula without DHA (8, 9). To our knowledge,

[1] From the Child Nutrition Research Centre, Child Health Research Institute, Women's & Children's Hospital, North Adelaide, Australia (MM and RAG), and the Department of Paediatrics & Child Health, Flinders University of South Australia and Flinders Medical Centre, Bedford Park (Adelaide), Australia (JSH and MAN).

[2] Supported by Rural Industries Research and Development Corporation (project no. 98/17), a National Health & Medical Research Council Applied Health Sciences Fellowship (to MM), and the MS McLeod Research Trust (partial support to RAG).

[3] Reprints not available. Address correspondence to RA Gibson, Child Nutrition Research Centre, Flinders Medical Centre, Bedford Park, SA 5046, Australia. E-mail: rgibson@flinders.edu.au.

Received November 28, 2000.

Accepted for publication July 2, 2001.

TABLE 1
Nutrient composition of whole eggs and egg yolks[1]

Nutrient	Whole egg	Egg yolk
Energy (kJ)	594	1311
Protein (g)	12.7	15.6
Fat (g)	10.1	28.2
Cholesterol (mg)	375	1050
Iron (mg)	1.6	4.0

[1] Composition is per 100 g whole egg or per 100 g egg yolk. Average egg weight = 62 g; average egg yolk weight = 20 g. Data are from the Australian food-composition tables (10).

no trials have addressed whether weaning foods high in n−3 fatty acids, such as n−3 fatty acid–enriched egg yolks, can improve infant DHA status during late infancy.

The most commonly cited reason for delaying the introduction of whole eggs to infants is to avoid sensitizing infants to egg white proteins and hence the development of egg-related allergies (1). Furthermore, although dietary recommendations for adults to reduce their intake of eggs to limit the cholesterol content of the diet have little relevance to young children, such guidelines may influence a parent's food choices for his or her child. The purpose of our trial was to investigate the nutritional role of egg yolks in the weaning diets of breast-fed and formula-fed infants. Primary outcome measures included erythrocyte DHA concentrations, infant iron status, and plasma cholesterol concentrations. Secondary outcomes included growth and plasma indexes of allergy.

SUBJECTS AND METHODS

Protocol

Healthy 6-mo-old infants born at term (>37 wk gestation) with birth weights >2500 g were eligible for the trial. Infants were excluded if they had known protein intolerances or allergies. Infants were eligible for entry into the breast-fed cohort if they received <120 mL formula (or cow milk)/wk and were eligible for entry into the formula-fed cohort if they were receiving all their nutrition as formula feeds by 4 wk after birth and were subsequently formula fed. All formulas consumed by infants did not contain LCPUFAs and were fortified with iron.

A research nurse attended immunization clinics in the Adelaide metropolitan area and screened mother-infant pairs for eligibility. The research nurse explained the trial, provided an information sheet to mothers with eligible infants, and invited their participation. Written, informed consent was obtained in adherence to the protocol approved by the Committee on Clinical Investigation (Ethics), Flinders Medical Centre (FMC), and the Research and Ethics Committee, Women's and Children's Hospital.

Mother-infant pairs attended an initial appointment at the FMC. Each appointment was made according to the date the infant was expected to turn 6 mo of age (26 ± 1 wk). Just before this appointment, the clinical trials staff randomly allocated infants to receive either no dietary intervention, regular eggs, or n−3 fatty acid–enriched eggs (n−3 eggs) according to a computer-generated randomization schedule. Breast-fed and formula-fed infants were allocated by using separate schedules. The investigator who generated these randomization schedules (MAN) was not involved in the day-to-day management of the trial.

Dietary intervention

The aim of the dietary intervention was to include 4 egg yolks/wk in the diet of weaning infants between 6 and 12 mo of age without significantly altering intake from other foods. The dietary intervention with n−3 eggs was designed to match the amount of DHA a breast-fed infant would normally receive, ie, ≈100 mg/d. The fatty acid and nutrient compositions of the eggs are shown in **Tables 1** and **2**. The eggs were enriched with DHA by feeding hens diets rich in n−3 fatty acids, including DHA (11). Eggs (weighing 60–65 g each) were purchased from South Coast Eggs (Myponga, South Australia) and were supplied in plain cartons coded A or B. The regular and n−3 eggs were similar in appearance, taste, and smell. Both the study participants and the research personnel were unaware of the type of eggs provided.

Compliance was encouraged by providing the family with 2 dozen eggs per fortnight, delivered by courier to the home of each infant allocated to an egg group. All mothers received advice regarding preparation, received instruction about separating the egg yolk from the egg white, and were supplied with plastic egg separators and a recipe booklet compiled by a dietitian.

Assessments

At 6 (26 ± 1 wk), 9 (39 ± 1 wk), and 12 (52 ± 1 wk) mo of age, infant weight, length, and head circumference were measured and a brief feeding questionnaire was completed by the infants' mothers. Particular attention was paid to the mode of feeding (quantity of breast milk and formula feeds) and intake of eggs and other foods in the weaning diet that also contain iron and LCPUFAs, such as fish and meat. The questionnaire thus enabled us to determine whether the dietary intervention (4 egg

TABLE 2
Fatty acid composition of regular and n−3 fatty acid–enriched eggs[1]

Fatty acid composition	Regular egg	n−3 Egg
Total saturates		
(mg)	2772 ± 284	2951 ± 204
(% of total fatty acids)	32.4 ± 0.4	33.4 ± 0.2
Total monoenes		
(mg)	4258 ± 272	4194 ± 367
(% of total fatty acids)	49.8 ± 1.3	47.4 ± 1.2
Linoleic acid		
(mg)	1141 ± 168	889 ± 35
(% of total fatty acids)	13.3 ± 0.8	10.1 ± 0.2
Arachidonic acid		
(mg)	170 ± 21	68 ± 6
(% of total fatty acids)	2.0 ± 0.1	0.8 ± 0.1
Total n−6		
(mg)	1385 ± 206	983 ± 41
(% of total fatty acids)	16.1 ± 1.0	11.1 ± 0.2
α-Linolenic acid		
(mg)	41 ± 2	317 ± 66
(% of total fatty acids)	0.5 ± 0.1	3.6 ± 1.0
Eicosapentaenoic acid		
(mg)	ND	26 ± 3
(% of total fatty acids)	ND	0.3 ± 0.1
Docosahexaenoic acid		
(mg)	73 ± 10	315 ± 16
(% of total fatty acids)	0.9 ± 0.1	3.6 ± 0.1
Total n−3		
(mg)	126 ± 14	701 ± 54
(% of total fatty acids)	1.5 ± 0.1	8.0 ± 1.1

[1] $\bar{x}$ ± SD. Composition is per 100 g whole egg; average egg weight = 62 g.

yolks/wk) replaced other solid meals that may contribute significantly to the iron or LCPUFA intake of infants. At 6 mo, information regarding parental socioeconomic status and education was also collected (12).

At 6 and 12 mo, a blood sample (2 mL) was taken by venipuncture for the assessment of LCPUFA status, iron status, plasma cholesterol, and plasma indexes of allergy. A portion of the sample (500 μL) was placed in EDTA for hemoglobin determination and the remainder was stored in containers with lithium heparin. Plasma was used to measure ferritin, iron, transferrin, transferrin saturation, and total cholesterol. The remaining plasma sample was stored at −80°C until analyzed for allergen-specific immunoglobulin E (IgE). Packed red blood cells were used to analyze erythrocyte phospholipid fatty acids.

Fatty acid analysis

Plasma and erythrocytes were separated by centrifugation (700 × *g* for 5 min at room temperature). The erythrocytes were washed 3 times with isotonic saline, and the lipids were extracted with chloroform:propanol (13). The total phospholipid fraction of the lipid extract was separated by thin-layer chromatography and methylated. Fatty acid methyl esters were separated and quantified by capillary gas chromatography as previously reported (14). Results are expressed as percentages of total phospholipid fatty acids.

Iron studies

All iron studies were conducted by the SouthPath SA laboratory at FMC. SouthPath staff were blinded to each infant's dietary allocation. Hemoglobin concentrations were measured spectrophotometrically by using a Coulter STKS analyzer (Beckman Coulter, Hialeah, FL). Plasma ferritin and transferrin were determined by immunoturbidimetric assays (Boehringer Mannheim Systems, New South Wales, Australia) on a BM/Hitachi 917 Automatic Analyzer. Plasma iron was measured on the same analyzer by using the BM/Hitachi 917 Systems Pack. Precision values for the SouthPath SA laboratory (expressed as CVs) for each of these indexes were as follows: hemoglobin, 1.5%; ferritin, 5%; iron, 2.3%; and transferrin, 2.1%.

The normal reference ranges used in this study for the iron status of infants (6 mo to 2 y) are as follows: hemoglobin, 105–135 g/L; ferritin, 10–250 μg/L; iron, 8–30 μmol/L; transferrin, 2.0–3.6 g/L; and transferrin saturation, 10–60% (4). Infants whose hemoglobin and plasma iron markers fell within these limits were considered iron sufficient. All infants with a hemoglobin concentration <105 g/L were classified as having anemia (15). Infants who had plasma ferritin concentrations ≤10 mg/L were classified as having iron deficiency (15). Infants with iron deficiency who also had hemoglobin concentrations < 105 g/L were classified as having iron deficiency anemia (15).

For ethical reasons, infants who had anemia at 6 mo were withdrawn from the study and referred for treatment. If an infant was iron deficient at 6 mo, the infant-mother pair was invited back for a repeat blood sample in 6–8 wk. If iron deficiency anemia developed in that time, the infant was withdrawn and referred for treatment.

Plasma cholesterol analysis

Total cholesterol analyses of nonfasting blood samples were also performed by SouthPath SA at the FMC. Plasma cholesterol was determined by the cholesterol CHOD-PAP method enzymatic colorimetric test (Boehringer Mannheim Systems) with a BM/Hitachi 917 analyzer.

Immunoglobulin E analysis

The concentration of circulating allergen-specific IgE antibodies in plasma was measured by using the Pharmacia CAP System RAST (radioallergosorbent test) Radioimmunoassay (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden). World Health Organization IgE standard-based calibrators (Kabi Pharmacia Diagnostics AB) were used to measure specific IgE antibodies, and values are expressed in kU_A/L, where A is allergen-specific antibodies. The measurement range for undiluted plasma is 0.35–100 kU_A/L, and the results are reported as the percentage with a RAST class ≥ 1 (where RAST = 1 is defined as 0.35 to <0.7 kU_A/L).

Sample size and statistics

For erythrocyte DHA and plasma cholesterol values, an α of 0.01 was used in estimates of sample size and power to allow for multiple comparisons. A sample size of 20 infants per group allowed us to detect a mean (±SD) difference of 1 ± 0.80% in phospholipid erythrocyte fatty acids between dietary groups with 89% power. Similarly, a sample size of 20 infants per group allowed us to detect a change of 1 ± 0.80 mmol/L in mean plasma cholesterol between dietary groups with 89% power. Estimates of sample size and power were undertaken for hemoglobin and ferritin values because these are the primary measures of iron status. Sample sizes of 40 infants per egg treatment group and 20 infants per control group allowed us to detect a difference of 8 ± 10 g/L in hemoglobin (α = 0.05) and a difference of 20 ± 25 μg/L in ferritin (α = 0.05) between groups with 82% power. When egg treatment groups were combined, statistical comparisons were conducted across 4 groups (egg treatment groups combined within each cohort) and cross-checked across all 6 groups to ensure that there were no inconsistencies in the outcomes of the statistical analyses.

Differences in baseline characteristics (before intervention) among dietary groups were tested by analysis of variance (ANOVA) and adjusted for multiple comparisons. Differences in baseline characteristics between breast-fed and formula-fed infants were also assessed. The effects of the dietary intervention, mode of feeding (breast-fed or formula-fed), and time were evaluated by using three-factor repeated-measures ANOVA for the outcomes of reported number of egg yolks consumed; reported consumption of meat, chicken, and fish; reported consumption of baby cereal; reported consumption of adult cereal; erythrocyte DHA and AA; plasma cholesterol; and indexes of iron status. Analysis of plasma ferritin values was performed by using log-transformed data. Post hoc tests were adjusted for multiple comparisons. Comparisons of weight, length, and head circumference were also by three-factor repeated-measures ANOVA in which dietary grouping, mode, and time were treated as the main effects. All analyses were conducted with SPSS for WINDOWS (version 10.0; SPSS Inc, Chicago). All data are expressed as means ± SDs unless otherwise stated.

RESULTS

Study sample

A total of 251 eligible mother-infant pairs were approached to enter the trial, and 161 gave written, informed consent to participate

FIGURE 1. Enrollment, random assignment, and study outcome of breast-fed and formula-fed infants.

(**Figure 1**). Of these, 82 were breast-fed and 79 were formula-fed. Of the 82 breast-fed infants recruited, 23 of 28 (control group), 23 of 27 (regular egg group), and 24 of 27 (n−3 egg group) completed the trial. Twelve breast-fed infants were withdrawn for the following reasons: anemia at trial entry ($n = 8$), failure to attend the initial appointment ($n = 2$), ceased breast-feeding before 9 mo ($n = 1$), and perceived adverse reaction to eggs by the parents ($n = 1$). Of the 79 formula-fed infants recruited, 23 of 27 (control group), 24 of 26 (regular egg group), and 20 of 26 (n−3 egg group) completed the trial. Twelve formula-fed infants were withdrawn for the following reasons: failure to attend the initial appointment ($n = 9$), loss to follow-up ($n = 1$), perceived adverse reaction to eggs by the parents ($n = 1$), and unknown reason ($n = 1$).

The demographic characteristics of the infants who completed the trial are shown in **Table 3**. There were no significant differences between the dietary groups within the breast-fed and formula-fed cohorts. However, compared with the formula-fed infants, the breast-fed infants had mothers who were less likely

TABLE 3
Characteristics of the infants who completed the trial and their parents[1]

	Breast-fed cohort			Formula-fed cohort		
	Control (n = 13 M, 10 F)	Regular egg (n = 11 M, 12 F)	n−3 Egg (n = 11 M, 13 F)	Control (n = 12 M, 11 F)	Regular egg (n = 8 M, 16 F)	n−3 Egg (n = 9 M, 11 F)
Gestational age (wk)	39.9 ± 1.1[2]	40.0 ± 1.1	39.4 ± 0.9	39.9 ± 1.2	39.9 ± 1.0	39.5 ± 1.0
Birth weight (g)	3640 ± 492	3722 ± 516	3540 ± 445	3453 ± 438	3541 ± 491	3463 ± 484
Birth length (cm)	51.2 ± 2.1	51.7 ± 2.2	50.7 ± 2.0	50.4 ± 1.9	51.1 ± 2.3	50.9 ± 1.9
Birth head circumference (cm)	35.4 ± 1.3	35.4 ± 1.1	34.8 ± 1.1	34.9 ± 1.3	34.9 ± 1.5	34.9 ± 1.3
Apgar score at 5 min	9.3 ± 0.7	9.2 ± 0.8	8.9 ± 0.6	9.1 ± 0.4	9.0 ± 1.0	9.0 ± 0.6
Mother's education score[3]	3.4 ± 0.9	3.2 ± 1.1	3.5 ± 1.1	2.6 ± 0.8	3.1 ± 1.3	2.9 ± 1.2
Mother's social score[4]	4.7 ± 0.8	5.0 ± 1.0	4.6 ± 1.0	5.1 ± 0.9	5.0 ± 1.0	5.1 ± 0.8
Mother smokes (% yes)	8.7	0.0	4.2	47.8	33.3	40.0
Male caregiver's education score[3]	3.4 ± 1.1 [23][5]	3.4 ± 1.0 [20]	3.8 ± 1.1	2.9 ± 1.0	2.9 ± 0.9 [23]	3.2 ± 1.0 [19]
Male caregiver's social score[4]	4.7 ± 0.9 [23]	4.6 ± 1.1 [20]	4.5 ± 1.4	4.9 ± 0.9	5.3 ± 1.0 [23]	4.6 ± 1.1 [19]
Male caregiver smokes (% yes)	39.0 [23]	25.0 [20]	8.3	43.5	34.8 [23]	37.0 [19]

[1] Statistical comparisons were as follows. Mother's education, breast-fed cohort compared with fomula-fed cohort: mean rank 78.8, median 3.0 ± 1.0, $n = 70$, compared with mean rank 58.8, median 2.5 ± 1.1, $n = 67$, $P < 0.005$. Male caregiver's education, breast-fed cohort compared with formula-fed cohort: mean rank 75.3, median 4.0 ± 1.1, $n = 67$, compared with mean rank 57.5, median 3.0 ± 1.0, $n = 65$, $P < 0.005$. Mother smokes, breast-fed cohort compared with formula-fed cohort: 4.3%, $n = 70$, compared with 40.3%, $n = 67$, $P < 0.001$.

[2] $\bar{x}$ ± SD.

[3] Education was ranked by using a 7-point scale on which 0 was for no formal education, 1 was for completion of primary school, 2 was for completion of midsecondary school, 3 was for completion of secondary school, 4 was for completion of a certificate or diploma, 5 was for a tertiary degree, and 6 was for a higher degree.

[4] The highest rank (1) was assigned to professional and academic professions and the lowest rank (6) to unskilled occupations (12).

[5] n in brackets.

TABLE 4
Frequency of solid food intake reported by mothers of infants who completed the trial[1]

	Breast-fed cohort			Formula-fed cohort		
	Control (n = 23)	Regular egg (n = 23)	n−3 Egg (n = 24)	Control (n = 23)	Regular egg (n = 24)	n−3 Egg (n = 20)
	no. of servings/wk			*no. of servings/wk*		
Egg yolks						
6 mo (baseline)	0.1 ± 0.4[a,2]	0.0 ± 0.0[a,2]	0.0 ± 0.2[a,2] [23]	0.1 ± 0.5[a,2]	0.1 ± 0.4[a,2]	0.0 ± 0.0[a,2]
9 mo	0.9 ± 1.0[a,2]	4.0 ± 0.8[b,3]	4.0 ± 0.7[b,3] [23]	0.8 ± 1.1[a,2]	3.9 ± 1.2[b,3]	3.6 ± 1.0[b,3]
12 mo	1.6 ± 1.4[a,2]	3.9 ± 0.9[b,3]	3.9 ± 1.6[b,3] [23]	1.2 ± 0.8[a,2]	4.0 ± 1.3[b,3]	3.4 ± 1.9[b,3]
Meat, chicken, and fish						
6 mo (baseline)	1.7 ± 2.8[2]	1.8 ± 3.3[2]	0.9 ± 1.6[2]	1.9 ± 2.8[2]	2.8 ± 3.0[2]	2.3 ± 2.7[2]
9 mo	6.0 ± 2.8[3]	4.6 ± 3.5[2]	5.7 ± 3.3[3]	5.9 ± 3.3[3]	6.0 ± 3.6[2]	7.0 ± 2.6[3]
12 mo	6.9 ± 2.7[3]	6.5 ± 2.1[3]	6.6 ± 2.1[3]	8.1 ± 3.3[4]	7.7 ± 3.0[3]	7.9 ± 2.6[3]
Baby cereal						
6 mo (baseline)	5.0 ± 3.1[2]	4.8 ± 3.2[2]	5.4 ± 2.5[2]	4.2 ± 3.1[2]	5.6 ± 3.3[2]	5.0 ± 2.8[2]
9 mo	5.0 ± 3.2[2]	4.9 ± 3.0[2]	4.1 ± 3.2[3]	2.9 ± 3.1[3]	2.9 ± 3.4[3]	3.0 ± 3.0[3]
12 mo	2.8 ± 3.3[3]	1.8 ± 2.9[3]	1.1 ± 2.3[4]	1.0 ± 2.1[3]	1.1 ± 2.6[3]	0.7 ± 2.2[3]
Adult cereal						
6 mo (baseline)	0.0 ± 0.2[2]	0.4 ± 1.6[2]	0.7 ± 1.8[2]	1.5 ± 2.8[2]	0[2]	1.0 ± 2.4[2]
9 mo	2.3 ± 3.3[3]	2.3 ± 3.0[3]	2.0 ± 2.8[2]	3.5 ± 3.0[2]	3.4 ± 4.0[3]	3.2 ± 3.0[3]
12 mo	4.1 ± 3.3[3]	4.3 ± 3.3[3]	5.3 ± 2.5[3]	5.7 ± 2.0[3]	4.8 ± 3.2[3]	5.8 ± 3.1[4]

[1] $\bar{x}$ ± SD; n in brackets. Values with different superscript letters indicate significant differences between dietary groups, $P < 0.05$ (three-factor repeated-measures ANOVA). Egg yolks: dietary group, $P < 0.001$; mode, NS; dietary group × mode, NS; time, $P < 0.001$; time × dietary group, $P < 0.001$; time × mode, NS; time × dietary group × mode, NS. Meat, chicken, and fish: dietary group, NS; mode, $P < 0.01$; dietary group × mode, NS; time, $P < 0.001$; time × dietary group, NS; time × mode, NS; time × dietary group × mode, NS. Baby cereal: dietary group, NS; mode, $P < 0.01$; dietary group × mode, NS; time, $P < 0.001$; time × dietary group, NS; time × mode, $P < 0.05$; time × dietary group × mode, NS. Adult cereal: dietary group, NS; mode, $P < 0.01$; dietary group × mode, NS; time, $P < 0.001$; time × dietary group, NS; time × mode, NS; time × dietary group × mode, NS.

[2–4] Values with different superscript numbers indicate significant differences by time within each dietary group, $P < 0.05$ (three-factor repeated-measures ANOVA).

to smoke and had mothers and male caregivers with higher education scores. Infants in the formula-fed cohort were breast-fed for an average of 1.9 ± 1.6 wk.

The dietary intervention resulted in mothers reporting increased use of egg yolks in both the breast-fed and formula-fed cohorts (**Table 4**). Reported consumption of ≈4 egg yolks/wk did not affect the frequency of consumption of other foods, such as meats and cereals, that are likely to contribute iron or LCPUFAs to infant diets. The formula-fed infants consumed more adult cereal, meat, chicken, and fish than did the breast-fed infants, whereas the breast-fed infants consumed more baby cereal.

Compliance with the dietary intervention is highlighted by the fact that erythrocyte DHA concentrations were 30% higher after treatment with n−3 eggs than after treatment with regular eggs in both the breast-fed and formula-fed infants at 12 mo of age (**Table 5**). Note that adding ≈4 n−3 egg yolks/wk to the diets of infants fed formula without LCPUFAs resulted in erythrocyte DHA concentrations that were not significantly different from those in the breast-fed infants in the control group at 12 mo of age. Erythrocyte AA concentrations were <10% lower after treatment with n−3 eggs than after the control or treatment with regular eggs in both the breast-fed and formula-fed cohorts at 12 mo of age.

Increasing egg yolk consumption from ≈1 to 4/wk did not alter mean plasma cholesterol concentrations in either the breast-fed or formula-fed infants (Table 5). The breast-fed infants did, however, have higher cholesterol concentrations than the formula-fed infants at 6 mo of age [4.3 ± 0.8 (n = 70) compared with 3.8 ± 0.7 (n = 67) mmol/L; $P < 0.001$]. This difference disappeared by 12 mo of age [4.3 ± 0.7 (n = 70) compared with 4.1 ± 0.7 (n = 67) mmol/L], when all infants were consuming a greater variety of foods.

Hemoglobin, ferritin, and transferrin values did not differ significantly between the combined egg treatment groups and the control group within both the breast-fed and formula-fed cohorts (**Table 6**). At 12 mo, however, plasma iron and transferrin saturation were higher in the egg-treated breast-fed and formula-fed infants than in the respective control groups. Only one breast-fed infant receiving regular eggs had iron deficiency anemia at 12 mo of age. Iron status did not differ significantly between the breast-fed and formula-fed infants, except that plasma ferritin concentrations tended to be lower in the breast-fed infants (P = 0.06). At 12 mo of age, more breast-fed infants (17.1%, 12 of 70) than formula-fed infants (7.5%, 5 of 67) were iron deficient (NS). The frequency of positive values for antibodies specific to egg yolk and egg white did not differ significantly between the combined egg treatment group and the control group within both cohorts or between the breast-fed and formula-fed cohorts (**Table 7**).

Weight, length, and head circumferences were not significantly different between the dietary groups nor were there any significant interactions between diet and time (data not shown). However, the breast-fed infants were consistently lighter [at 6 mo, 7575 ± 892 (breast-fed, n = 70) compared with 7942 ± 943 (formula-fed, n = 67) g; at 9 mo, 8584 ± 999 compared with 9081 ± 1043 g; and at 12 mo, 9626 ± 1060 compared with 10121 ± 1130 g; $P < 0.01$] and shorter [at 6 mo, 67.1 ± 2.6 (breast-fed, n = 70) compared with 67.6 ± 2.3 (formula-fed, n = 67) cm; at 9 mo, 70.9 ± 2.4 compared with 72.1 ± 2.5 cm; and at 12 mo, 75.1 ± 2.5 compared with 75.9 ± 3.5 cm; $P < 0.05$] than the formula-fed infants. There was no significant difference in head circumference between the breast-fed and formula-fed infants.

TABLE 5
Erythrocyte fatty acid status and plasma cholesterol concentrations of infants who completed the trial[1]

	Breast-fed cohort			Formula-fed cohort		
	Control (n = 23)	Regular egg (n = 23)	n−3 Egg (n = 24)	Control (n = 23)	Regular egg (n = 24)	n−3 Egg (n = 20)
DHA (% of total phospholipid fatty acids)						
6 mo	5.2 ± 0.7[a,2]	5.4 ± 0.7[a,2]	5.6 ± 0.9[a,2]	2.7 ± 0.6[b,2]	2.6 ± 0.5[b,2]	2.7 ± 0.5[b,2]
12 mo	4.8 ± 0.8[a,d,3]	5.1 ± 0.9[a,2]	6.7 ± 1.3[b,3]	3.0 ± 0.5[c,2]	3.3 ± 0.7[c,3]	4.1 ± 1.2[d,3]
AA (% of total phospholipid fatty acids)						
6 mo	15.8 ± 0.9[a,2]	15.6 ± 1.1[a,2]	15.8 ± 0.8[a,2]	14.1 ± 0.8[b,2]	14.2 ± 0.9[b,2]	14.4 ± 0.9[b,2]
12 mo	15.3 ± 0.7[a,2]	15.3 ± 0.8[a,2]	13.9 ± 0.9[c,2]	15.0 ± 0.8[a,2]	15.1 ± 0.7[a,2]	14.5 ± 1.0[b,2]
Cholesterol (mmol/L)						
6 mo	4.4 ± 0.9[2]	4.3 ± 0.8[2]	4.3 ± 0.7[2]	3.8 ± 0.7[2]	3.8 ± 0.7[2]	3.8 ± 0.6[2]
12 mo	4.1 ± 0.9[2]	4.2 ± 0.8[2]	4.4 ± 0.7[2]	3.9 ± 0.8[2]	4.2 ± 0.6[2]	4.2 ± 0.7[2]

[1] $\bar{x}$ ± SD. DHA, docosahexaenoic acid; AA, arachidonic acid. Values with different superscript letters indicate significant differences between dietary groups, $P < 0.05$ (three-factor repeated-measures ANOVA). DHA: dietary group, $P < 0.001$; mode, $P < 0.001$; dietary group × mode, NS; time, $P < 0.001$; time × dietary group, $P < 0.001$; time × mode, $P < 0.001$; time × dietary group × mode, NS. AA: dietary group, $P < 0.01$; mode, $P < 0.001$; dietary group × mode, NS; time, NS; time × dietary group, $P < 0.001$; time × mode, $P < 0.001$; time × dietary group × mode, NS. Cholesterol: dietary group, NS; mode, $P < 0.005$; dietary group × mode, NS; time, NS; time × dietary group, NS; time × mode, $P < 0.005$; time × dietary group × mode, NS.

[2,3] Values with different superscript numbers indicate significant differences by time within each dietary group, $P < 0.05$ (three-factor repeated-measures ANOVA).

DISCUSSION

Our trial was a systematic study of the nutritional value of including egg yolk in the weaning diet of breast-fed and formula-fed infants. With the separate random assignment of the breast-fed and formula-fed infants, the trial was structured to answer 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 LCPUFAs but have been reported to be at risk of depleted

TABLE 6
Iron status of infants[1]

	Breast-fed cohort		Formula-fed cohort	
	Control (n = 23)	Egg yolk intervention (n = 47)	Control (n = 23)	Egg yolk intervention (n = 44)
Hemoglobin (g/L)				
6 mo	124.0 ± 7.6[2]	121.8 ± 12.0[2]	123.8 ± 10.4[2]	123.7 ± 9.8[2]
12 mo	119.9 ± 7.4[3]	120.0 ± 9.1[2]	121.3 ± 6.3[3]	122.8 ± 7.9[2]
Ferritin (μg/L)				
6 mo	43.5 ± 35.6[2]	56.9 ± 44.3[2]	40.0 ± 29.5[2]	56.4 ± 43.1[2]
12 mo	20.6 ± 11.1[3]	26.7 ± 19.0[3]	33.6 ± 17.2[2]	36.6 ± 30.2[2]
Iron (μmol/L)				
6 mo	8.2 ± 3.2[a]	8.9 ± 2.9[a,b]	9.3 ± 3.3[a,b]	10.7 ± 4.1[b]
12 mo	8.3 ± 3.4[a]	10.5 ± 4.5[b]	8.2 ± 2.9[a]	10.5 ± 4.7[b]
Transferrin (g/L)				
6 mo	2.7 ± 0.6[a,2]	2.6 ± 0.3[a,2]	2.7 ± 0.3[a,2]	2.7 ± 0.3[a,2]
12 mo	3.0 ± 0.4[a,3]	3.0 ± 0.4[a,3]	2.9 ± 0.4[a,2]	2.9 ± 0.4[a,2]
Transferrin saturation (%)				
6 mo	12.4 ± 5.6[a,2]	14.0 ± 5.4[a,b,2]	13.8 ± 4.9[a,b,2]	16.1 ± 5.8[b,2]
12 mo	10.8 ± 4.6[a,3]	14.3 ± 6.8[b,2]	11.6 ± 4.5[a,3]	14.6 ± 6.6[b,3]
Iron deficiency (% positive)				
6 mo	8.7 [2/23]	2.1 [1/47]	0 [0/23]	2.3 [1/44]
12 mo	17.4 [4/23]	17.0 [8/47]	8.7 [2/23]	6.8 [3/44]

[1] $\bar{x}$ ± SD; n in brackets. Values with different superscript letters indicate significant differences between dietary groups, $P < 0.05$ (three-factor repeated-measures ANOVA). Hemoglobin: egg intervention, NS; mode, NS; egg intervention × mode, NS; time, $P < 0.05$; time × egg intervention, NS; time × mode, NS; time × egg intervention × mode, NS. Ferritin: egg intervention, NS; mode, $P = 0.06$; egg intervention × mode, NS; time, $P < 0.001$; time × egg intervention, NS; time × mode, $P < 0.01$; time × egg intervention × mode, NS. Iron: egg intervention, $P < 0.005$; mode, NS; egg intervention × mode, NS; time, NS; time × egg intervention, NS; time × mode, $P = 0.06$; time × egg intervention × mode, NS. Transferrin: egg intervention, NS; mode, NS; egg intervention × mode, NS; time, $P < 0.001$; time × egg intervention, NS; time × mode, $P < 0.05$; time × egg intervention × mode, NS. Transferrin saturation: egg intervention, $P < 0.005$; mode, NS; egg intervention × mode, NS; time, $P < 0.05$; time × egg intervention, NS; time × mode, NS; time × egg intervention × mode, NS.

[2,3] Values with different superscript numbers indicate significant differences by time within each dietary group, $P < 0.05$ (three-factor repeated-measures ANOVA).

TABLE 7
Plasma egg yolk–specific and egg white–specific antibody responses of infants who completed the trial[1]

	Breast-fed cohort		Formula-fed cohort	
	Control	Egg yolk intervention	Control	Egg yolk intervention
	% with RAST ≥ 1		*% with RAST ≥ 1*	
Egg yolk antibody				
6 mo	4.3 [1/23]	8.8 [4/45]	0 [0/23]	2.3 [1/44]
12 mo	4.3 [1/23]	6.4 [3/47]	0 [0/23]	2.3 [1/44]
Egg white antibody				
6 mo	4.3 [1/23]	8.7 [4/46]	0 [0/23]	9.1 [4/44]
12 mo	17.4 [4/23]	10.6 [5/47]	12.5 [3/23]	4.5 [2/44]

[1] *n* in brackets. RAST (radioallergosorbent test; Kabi Pharmacia Diagnostics AB, Uppsala, Sweden). RAST = 1 is defined as 0.35 to <0.7 kU_A/L, where A is allergen-specific antibodies. There were no significant differences between dietary groups.

iron stores during late infancy. Our trial addressed whether egg yolk, which can be a good source of both LCPUFAs and iron, is beneficial for all infants regardless of whether they are breast-fed or fed formula, without adverse consequences on plasma cholesterol concentrations or plasma indexes of allergy.

A major finding of the current trial was that infants fed formulas without DHA but who consumed 4 n−3 egg yolks/wk had erythrocyte DHA percentages that were not significantly different from those of the breast-fed infants. We showed in an earlier study that weaning diets are very low in LCPUFAs, such that formula-fed infants have little chance in the normal course of events to achieve LCPUFA status similar to that seen in breast-fed infants (16). In that earlier study, we calculated that it could take ≥14 regular egg yolks/wk to provide an infant with the same amount of DHA that breast-fed infants receive. The results of the present trial, which showed that 4 regular egg yolks/wk in the weaning diet had no significant effect on erythrocyte DHA status, add credibility to this calculation. However, the addition of 4 n−3 egg yolks/wk, which provided ≈100 mg additional DHA/d in the weaning diets of the formula-fed infants, resulted in erythrocyte DHA amounts not significantly different from those of the breast-fed infants, who also obtained ≈100 mg DHA/d.

It is also interesting to note that erythrocyte DHA concentrations decreased with age in the breast-fed infants in the control group, whereas there was no significant change in DHA status with time in the formula-fed infants in the control group. Although breast milk remains the main dietary source of DHA in 6–12-mo-old infants, the increasing consumption of weaning foods low in DHA and the reduction in breast milk volume consumed during this age bracket probably explain the reduction in DHA status of the breast-fed infants in the control group. On the other hand, DHA status remains low in infants consuming formulas without DHA and DHA-poor weaning foods and does not alter with age. Our data indicate that n−3 egg yolks can be a useful dietary source of DHA for both breast-fed and formula-fed infants, and show for the first time that egg yolks can be incorporated into the weaning diet for a sustained period of time. The implications of these findings remain to be determined because no trials have specifically addressed changes in physiologic functions or clinical outcomes of infants supplemented with DHA during the second 6 mo of life.

Our trial showed no significant effect of egg yolk intervention on hemoglobin and ferritin, the major determinants of iron status, although there was a positive effect of egg treatment on transferrin saturation and plasma iron. This is perhaps not surprising because the infants studied were largely iron sufficient and had adequate hemoglobin and iron stores (as measured by ferritin). The improvements in plasma iron and transferrin saturation with egg treatment reflect increases in iron transportation (17). Given that the additional amount of iron supplied by the egg yolk intervention was ≈0.5 mg/d, it is possible that either a greater dose or a longer treatment period would be necessary before improvements in iron stores could be observed.

Other comparable trials involving largely iron-sufficient infants that investigated the effect of different iron-containing weaning foods on infant iron status had different results. Engelmann et al (18) showed that partially breast-fed infants randomly allocated to a high-meat (27 g/d) diet had improved iron status compared with infants allocated to a low-meat (10 g/d) weaning diet. Note that the iron contribution from meat in the high-meat group is similar to the amount of iron received from the egg yolk treatment in the current trial. Conversely, one of our earlier trials showed that infants fed a high-iron weaning diet (8.2 ± 2.9 mg/d), in which the iron was derived largely from iron-fortified cereal, had iron status that was not different from that of a control group (5.2 ± 3.4 mg/d) at 12 mo of age (19). The infants in the intervention group of this earlier trial received an extra 3 mg Fe/d compared with an extra 0.5 mg Fe from egg yolk in the current trial. Collectively, these data suggest that foods such as egg yolks and meat may make a useful contribution to the weaning diet and that interventions aimed at increasing the intake of such foods in iron-deplete infants deserve to be tested in a large, community-based trial.

Although an egg yolk typically contains ≈200 mg cholesterol and ≈6 g fat (2 g of which is saturated fat), introducing ≈4 eggs/wk to the diets of weaning infants did not significantly alter plasma cholesterol concentrations in either breast-fed or formula-fed infants. This finding parallels observations from a trial involving adults in which the intake of 4 regular or 4 n−3 eggs/wk resulted in no increase in blood lipids (20). One of the largest randomized intervention trials that investigated early diet and lifestyle counseling to reduce atherosclerosis risk factors showed that intervention to reduce fat intake and replace saturated fat with monounsaturated and polyunsaturated fat in the weaning diet of infants resulted in no effect on blood lipids when infants were 13 mo of age (21). On the other hand, breast-fed infants are consistently reported to have higher plasma cholesterol concentrations than formula-fed infants (22–24). Various randomized trials of cholesterol-supplemented formula have been undertaken with the aim of investigating the effect of cholesterol supplementation on blood lipids and lipid metabolism (25, 26). Although both trials reported increases in plasma cholesterol with cholesterol supplementation of infant formula

(25, 26), cholesterol synthesis remained lower in breast-fed infants than in cholesterol-supplemented, formula-fed infants (25). Few data exists regarding the implications of these findings for physiologic functions and clinical outcomes, and these remain controversial. It has been suggested that cholesterol consumption in human milk may promote the delivery of adequate substrate for brain lipids (26), but other work suggests that the rat brain synthesizes its cholesterol de novo (27).

Egg allergy and intolerance are among the most common food allergies and intolerances (1). In our group of healthy infants with no known protein allergies or intolerances, only 1 of 82 breast-fed and 1 of 79 formula-fed infants were withdrawn from the trial because the parents perceived that the infants had an adverse reaction to the egg yolk intervention. Both of these infants were ingesting egg protein as part of their usual weaning diet, however, and we did not have the opportunity to confirm egg intolerance by using a double-blind, placebo-controlled challenge. The presence of antigen-specific antibodies to egg yolk and egg white were used as indexes of egg intolerance, although IgE antibody responses to food proteins can appear in healthy infants with no clinical manifestations of allergy or intolerance (28). The results of our trial confirm this because none of the infants had overt signs of egg intolerance, but 4% and 8% of the egg-treated infants had specific antibody responses to egg yolk and egg white, respectively, compared with 2% and 15% of the control infants, respectively. Within the limits of our trial there were no significant differences in specific antibody responses between the treated and control infants. However, given the relatively low rates of positive responses, hundreds of infants per treatment group would have been required to detect differences. Further studies should be conducted to better define the clinical and plasma markers of allergy in response to egg intervention.

In summary, the results of our trial indicate that it is possible and practical for weaning infants to consume ≤4 egg yolks/wk without affects on the intake of other foods such as cereals and meats. The egg yolk intervention resulted in modest improvements in iron status that may be most beneficial to infants who are iron deplete, and this deserves further investigation. Eggs fortified with n−3 fatty acids provide a means of increasing dietary DHA during the second 6 mo of life without altering plasma cholesterol. The emerging work in the field of dietary DHA and infant outcomes will determine whether there are any physiologic or clinical benefits to improved DHA status during the second half of infancy.

We thank Helen Blake, Jenny Osmond, Jane Armstrong, Lyn Pullen, Bronwen Paine, Jacqui Miller, Joanne Hartley, George Blake, Ela Zielinski, and Dani Bryan for their administrative, clinical, and technical support and acknowledge the generous support of the Flinders Medical Centre, the Women's & Children's Hospital, and the City Council Immunization Clinics of Noarlunga, Marion, and Happy Valley, which kindly accommodated the trial.

REFERENCES

1. Report of the Working Group on the Weaning Diet of the Committee on Medical Aspects of Food Policy, Department of Health. Weaning and the weaning diet. London: Her Majesty's Stationery Office, 1994. (Report on Health and Social Subjects 45.)
2. Simopoulos AP, Salem N Jr. Egg yolk as a source of long-chain polyunsaturated fatty acids in infant feeding. Am J Clin Nutr 1992; 55:411–4.
3. Michaelsen KF, Weaver L, Branca F, Robertson A. Feeding and nutrition of infants and young children. Guidelines for the WHO European Region, with emphasis on the former Soviet countries. Copenhagen, Denmark: World Health Organisation Regional Office for Europe Copenhagen, 2000.
4. The British Nutrition Foundation's Task Force. Iron: nutritional and physiological significance. London: Chapman and Hall, 1995.
5. Hallberg L, Hulthen L. Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. Am J Clin Nutr 2000;71:1147–60.
6. Hurrell RF, Lynch SR, Trinidad TP, Dassenko SA, Cook JD. Iron absorption in humans: bovine serum albumin compared with beef muscle and egg white. Am J Clin Nutr 1988;47:102–7.
7. Gibson RA, Makrides M. Polyunsaturated fatty acids and infant visual development: a critical appraisal of randomized clinical trials. Lipids 1999;34:179–84.
8. Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. Am J Clin Nutr 1994;60:189–94.
9. Gibson RA, Neumann MA, Makrides M. Effect of dietary docosahexaenoic acid on brain composition and neural function in term infants. Lipids 1996;31:S177–81.
10. Cashel K, English R, Lewis J. Composition of foods: Australia. Vol 1. Canberra, Australia: Australian Government Publishing Service, 1989.
11. van Elswyk ME. Comparison of n−3 fatty acid sources in laying hen rations for improvement of whole egg nutritional quality: a review. Br J Nutr 1997;78:S61–9.
12. Daniel A. Power, privilege and prestige: occupations in Australia. Melbourne: Longman-Cheshire, 1983.
13. Broekhuyse RM. Improved lipid extraction of erythrocytes. Clin Chim Acta 1974;51:341–3.
14. Makrides M, Neumann MA, Jeffrey B, Lien EL, Gibson RA. A randomized trial of different ratios of linoleic to α-linolenic acid in the diet of term infants: effects on visual function and growth. Am J Clin Nutr 2000;71:120–9.
15. Centers for Disease Control. CDC criteria for anemia in children and childbearing-aged women. MMWR Morb Mortal Wkly Rep 1989; 38:400–4.
16. Jackson KA, Gibson RA. Weaning foods cannot replace breast milk as sources of long-chain polyunsaturated fatty acids. Am J Clin Nutr 1989;50:980–2.
17. Yip R, Dallman PR. Iron. In: Ziegler EE, Filer LJ Jr, eds. Present knowledge in nutrition. Washington, DC: ILSI Press, 1996:277–92.
18. Engelmann MD, Sandström B, Michaelsen KF. Meat intake and iron status in late infancy: an intervention study. J Pediatr Gastroenterol Nutr 1998;26:26–33.
19. Makrides M, Leeson R, Gibson RA, Simmer K. A randomised controlled clinical trial of increased dietary iron in breast-fed infants. J Pediatr 1998;133:559–62.
20. Ferrier LK, Caston LJ, Leeson S, Squires J, Weaver BJ, Holub BJ. α-Linolenic acid– and docosahexaenoic acid–enriched eggs from hens fed flaxseed: influence on blood lipids and platelet phospholipid fatty acids in humans. Am J Clin Nutr 1995;62:81–6.
21. Lapinleimu H, Vukari J, Nunikoski H, et al. Impact of gender, apolipoprotein E phenotypes, and diet on serum lipids and lipoproteins in infancy. J Pediatr 1997;131:825–32.
22. Akeson PM, Axelsson IE, Ralha NC. Plasma lipids and apolipoproteins in breastfed and formula-fed Swedish infants. Acta Paediatr 1999;88:1–6.
23. Kallo MJ, Salmenpera L, Siimes MA, Perheentupa J, Miettinen TA. Exclusive breast-feeding and weaning: effect on serum cholesterol and lipoprotein concentrations in infants during the first year of life. Pediatrics 1992;89:663–6.
24. Wong WW, Hachey DL, Insull W, Opekun AR, Klein PD. Effect of dietary cholesterol on cholesterol synthesis in breast-fed and formula-fed infants. J Lipid Res 1993;34:1403–11.
25. Bayley TM, Alasmi M, Thorkelson T, et al. Influence of formula versus breast milk on cholesterol synthesis rates in four-month-old infants. Pediatr Res 1998;44:60–7.

26. Van Biervliet JP, Vinaimont N, Vercaemst R, Rosseneu M. Serum cholesterol, cholesteryl ester, and high-density lipoprotein development in newborn infants: response to formulas supplemented with cholesterol and gamma-linolenic acid. J Pediatr 1992;120:S101–8.
27. Lee WN, Bassilian S, Ajie HO, et al. In vivo measurement of fatty acids and cholesterol synthesis using D20 and mass isotopomer analysis. Am J Physiol 1994;266:E699–708.
28. Hattevig G, Kjellman B, Johansson SG, Bjorksten B. Clinical symptoms and IgE responses to common food proteins in atopic and healthy children. Clin Allergy 1984;14:551–9.

The American Journal of Clinical Nutrition