

Randomized controlled trial of early regular egg intake to prevent egg allergy

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Background: The ideal age to introduce egg into the infant diet has been debated for the past 2 decades in the context of rising rates of egg allergy.

Objective: We sought to determine whether regular consumption of egg protein from age 4 to 6 months reduces the risk of IgE-mediated egg allergy in infants with hereditary risk, but without eczema.

Methods: Infants aged 4 to 6 months were randomly allocated to receive daily pasteurized raw whole egg powder (n = 407) or a color-matched rice powder (n = 413) to age 10 months. All infants followed an egg-free diet and cooked egg was introduced to both groups at age 10 months. The primary outcome was IgE-mediated egg allergy defined by a positive pasteurized raw egg challenge and egg sensitization at age 12 months.

Results: There was no difference between groups in the percentage of infants with IgE-mediated egg allergy (egg 7.0% vs control 10.3%; adjusted relative risk, 0.75; 95% CI, 0.48-1.17; *P* = .20). A higher proportion of participants in the egg group stopped taking the study powder because of a confirmed allergic reaction (25 of 407 [6.1%] compared with 6 of 413 [1.5%]). Egg-specific IgG₄ levels were substantially higher in the egg group at 12 months (median, 1.22 mg_A/L vs control 0.07 mg_A/L; *P* < .0001).

Conclusions: We found no evidence that regular egg intake from age 4 to 6 months substantially alters the risk of egg allergy by age 1 year in infants who are at hereditary risk of allergic

disease and had no eczema symptoms at study entry. (*J Allergy Clin Immunol* 2016;■■■:■■■-■■■.)

Key words: Allergy prevention, complementary feeding, hereditary risk, egg, food allergy, oral tolerance, randomized controlled trial

The ideal age to introduce potentially allergenic foods into an infant's diet has been debated for the past 2 decades, particularly in high-income countries where allergic disease has become highly prevalent. Initial approaches to primary prevention of food allergy largely focused on "avoidance" strategies. In 2000, practice guidelines generally recommended that allergenic foods (such as egg, cow's milk, and peanut) be avoided during the first 1 to 3 years of life.¹ As data accumulated from both observational studies and experimental models, it became apparent that avoidance practices may not be beneficial (review in Prescott et al²). In animal studies, it was shown that early, regular exposure to allergens is a requirement for oral tolerance,³ whereas human population studies collectively suggested that earlier introduction of allergenic foods may reduce the risk of allergy.⁴⁻⁶ As a result, avoidance guidelines were disbanded from 2008.^{2,7-9} However, the question of whether allergenic foods should be proactively introduced during infancy has remained unclear, emphasizing the need for randomized controlled trials (RCTs) to examine this question.

In many countries, including Australia, egg allergy is the most common food allergy in the first year of life.¹⁰ Notably, in an observational study, our collaborators in the Australian Health-nuts study¹¹ found that introduction of cooked egg between age 4 and 6 months was associated with a reduced risk of egg allergy at age 12 months by 80% compared with delaying until age 10 to 12 months. Here, we report the largest double-blinded RCT to investigate whether early, regular inclusion of egg into the diets of infants alters the risk of egg allergy: the Starting Time of Egg Protein (STEP) trial. To ensure that the STEP trial was a primary prevention trial and relevant to population-based feeding recommendations, we excluded infants with any allergy symptoms or eczema before trial entry.

METHODS

Study design

This study was a double-blinded multicenter RCT. Singleton infants with atopic mothers (history of a medically diagnosed allergic disease with sensitization to at least 1 common aeroallergen) were recruited before age 6.5 months. This study excluded infants who had a history of allergic disease (eg, eczema) or had any previous known direct ingestion of egg. Infants who had commenced solids before age 4 months or had any congenital or acquired disease or developmental disorder likely to affect infant feeding were also excluded. Initially only those infants who were born at greater or equal to 37 weeks' gestation with a birth weight of 2500 to 4500 g were included. To improve recruitment rates, from August 6, 2012, onward in Adelaide only, the

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Abbreviations used

aRR: Adjusted relative risk
 LEAP: Learning Early About Peanut Allergy
 RCT: Randomized controlled trial
 STAR: Solids Timing for Allergy Reduction
 STEP: Starting Time of Egg Protein

trial inclusion criterion was changed to infants who were born at more than 35 weeks' gestation with a birth weight of more than 2000 g. The incidence of childhood allergies is not known to differ between late preterm infants and term infants, or between lower or higher birth weight infants.

Research assistants and nurses conducted initial participant screening for eligibility at major maternity centers from 2 Australian cities (Adelaide and Perth). Interested families were then recontacted when their infant was age 4 months, and if they remained eligible, a final screening appointment occurred between age 4 and 6.5 months. Written informed consent was obtained before trial participation. Approval was granted by the local institutional review boards (Human Research Ethics Committees) of each center, Women's and Children's Health Network, Adelaide, Flinders Medical Centre, Adelaide, and Princess Margaret Hospital, Perth. The trial was registered with the Australian New Zealand Clinical Trials Registry: ACTRN12610000388011.

Randomization and allocation concealment

Each eligible infant was randomized to 1 of 2 groups and assigned a unique study number according to an independently generated randomization schedule programmed into a secure Web-based randomization service accessible to all centers. The computer-generated randomization schedule used randomly permuted blocks of sizes 4, 6, and 8 in the ratio 1:2:1, with stratification for city, infant sex, and feeding mode (breast-fed or formula-fed if receiving >200 mL of infant formula per day at study entry).

Dietary intervention

To enable blinding of parents and research staff, the intervention (egg) and control (no egg) powders were mixed with carrot, pineapple, and rice powders to appear identical in color, texture, smell, and taste, and were packaged in individual daily-use sachets of identical appearance. From randomization, infants were given the study powder orally once per day by mixing 1 sachet (2.4 g) of study powder with their solid foods. Sachets from the egg group contained 0.9 g pasteurized raw whole egg powder per day (equivalent to 0.4 g egg protein per day and ½ an egg per week), manufactured by Farm Pride Foods Pty Ltd (Keysborough, Victoria, Australia). Whole egg powder was used to ensure that infants were exposed to all egg proteins (including ovalbumin, ovomucoid, ovotransferrin, ovomucin, and lysozyme) in proportions found in fresh whole eggs. The control group sachets contained egg-free powder. Study powders were easily mixed with regular infant foods resulting in a smooth consistency. For full details of study powder compositions, see this article's Online Repository at www.jacionline.org. Intervention with study powders was from randomization until the infants were aged 10 months. During this time, all infants followed an egg-free diet. From age 10 months, families in both groups were advised to include cooked egg and egg-containing foods in the infants' diets.

Protocol of the intervention phase

Baseline characteristics including basic demographic information, family history of allergic disease, birth order, parental education, smoking in the home environment, total household egg intake, the age of solid food introduction, and detailed infant dietary information on breast-feeding and/or formula feeding were recorded at randomization. A blood sample was collected before the first exposure to the study powder. Baseline whole egg-specific IgE and egg white-specific IgG₄ serum antibody concentrations

were analyzed (for full details, see this article's Online Repository at www.jacionline.org) at the completion of the trial, and did not influence eligibility.

The families were contacted by telephone monthly between randomization and age 11 months. At each contact time point, questions were asked relating to compliance with the dietary intervention, infant feeding, egg intake, and symptoms of allergic disease (including eczema, hives, wheeze, and anaphylaxis). If an allergic reaction to the study powder was suspected, a medical assessment, including an observed ingestion of the allocated study powder, was conducted to confirm any possible allergic reactions to the study powder. Only infants with medically observed confirmed allergic reactions to the study powder were advised to cease the study powder use, and if no reaction was observed the study powder was reintroduced.

Allergic disease outcome assessments

At age 12 months, infants attended an appointment at the Women's and Children's Hospital in Adelaide or Princess Margaret Hospital in Perth. A medical practitioner took a structured history and did a standardized clinical examination to diagnose allergic disease (including eczema, IgE-mediated food allergy, and infant wheeze) for a period covering the first year of life. All were blinded to treatment group allocation and had quality assurance reviews every 6 months with 1 of the investigators (D.J.P.). The infants had skin prick tests (for details, see this article's Online Repository at www.jacionline.org) and a blood sample was collected to measure whole egg-specific IgE and egg white-specific IgG₄ serum antibody concentrations (for details, see this article's Online Repository at www.jacionline.org).

Infants had a medically supervised oral pasteurized raw egg challenge where the infant was given ½ whole egg (for more details, see this article's Online Repository at www.jacionline.org) and observed for at least 2 hours. An *allergic reaction* was defined as at least 3 concurrent noncontact urticaria persisting for at least 5 minutes and/or generalized skin erythema and/or vomiting (forceful/projectile) and/or anaphylaxis (as defined by multisystem involvement that must include circulatory and/or respiratory involvement). Unless the infant experienced an allergic reaction, the families were advised to include all forms of egg-containing foods in the infant's diet. The primary outcome was the diagnosis of IgE-mediated egg allergy at age 12 months defined as an allergic reaction to the egg challenge and sensitization (positive skin prick test result) to egg.

Serious adverse events were defined as any death, admissions to intensive care, or anaphylactic reaction. These events were reviewed by an independent Serious Adverse Event Committee and reported to the Human Research Ethics Committees. Data on other adverse events including febrile illnesses, courses of antibiotics, and hospital admissions longer than 24-hour duration were also recorded.

Sample size and statistical methods

We expected that the prevalence of IgE-mediated egg allergy at age 12 months in a population of infants with atopic mothers would be 9.7% on the basis of previous Australian data. To have 85% power to detect a relative risk reduction of 50% in IgE-mediated egg allergy (such a reduction is meaningful to families and likely to be associated with significant savings to health care costs) from 9.7% in the control group to 4.9% in the intervention group, 567 infants per group were required ($\alpha = 0.05$). Taking a conservative estimate of 5% loss to follow-up, this led to an initial sample size estimate of 597 infants per group (total of 1194 infants). Recruitment ended with 820 infants because of funding constraints. This final sample size provided 68% power to detect a 50% relative risk reduction and 80% power to detect a 56% relative reduction in IgE-mediated egg allergy.

Statistical analyses were carried out according to a prespecified statistical analysis plan. Analyses were performed using an intention-to-treat approach; all participants were analyzed as randomized. To handle missing outcome data, multiple imputation implemented under a missing at random assumption was used; for further details on the imputation methodology, see this article's Online Repository at www.jacionline.org. The primary outcome and secondary binary outcomes were analyzed using log binomial regression

models, with treatment effects described using relative risks and 95% CIs. Because of the presence of detection thresholds, whole egg-specific IgE and egg white-specific IgG₄ serum antibody concentrations were analyzed using Tobit regression models, with treatment effects described using ratios of means and 95% CIs. Both unadjusted and adjusted analyses were performed, with adjustment for stratification variables (city, infant sex, and breast-feeding status) as well as paternal history of allergic disease. The adjusted results were used to draw conclusions about the effect of treatment on the outcomes of interest. For the primary outcome only, secondary analyses were performed to test for evidence of effect modification by breast-feeding status at baseline (yes/no), age of the infant at randomization (<6 months, ≥6 months), and egg-specific IgE levels at randomization (<0.1, ≥0.1 kU_A/L; post hoc analysis). A per-protocol analysis for the primary outcome was also undertaken as a sensitivity analysis. Infants were excluded from the per-protocol analysis if they either consumed egg or egg-containing foods on 10 or more occasions during the whole intervention period and/or missed giving study powder for more than 4 d/wk (including ceased giving study powder) for 2 months or more in total over the whole intervention period. Statistical significance was assessed at the 2-sided *P* level of .05 level. All analyses were performed using SAS Software version 9.3 or later (SAS Institute Inc, Cary, NC) and Stata v13 (StataCorp LP, College Station, Tex).

RESULTS

Enrollment began on January 12, 2011, and ended on November 12, 2014. A total of 820 infants were randomized into the trial, 407 infants to the intervention (egg) group and 413 infants to the control (egg-free) group. Fig 1 illustrates the participant flow diagram. The baseline characteristics of the 2 groups are described in Table I. Data collection was completed on May 28, 2015. Ninety-five percent (782 of 820) of infants attended their final appointment, with 748 of 820 (91%) having skin prick tests and undertaking an egg challenge (primary outcome). Twelve (5 in egg group) parents withdrew consent to participate during the study.

Compliance with the dietary intervention

Sixty-five of 407 (16.0%) infants in the egg group and 66 of 413 (16.0%) infants in the control group missed more than 4 d/wk of study powder for 2 months or more. Only 4 of 407 (1.0%) infants in the egg group and 4 of 413 (1.0%) infants in the control group consumed egg-containing foods on 10 or more occasions during the whole intervention period, while attempting to follow the baseline egg-free diet until age 10 months. When asked “which powder do you think your baby was having,” 558 of 798 (69.9%) parents answered “unknown.” More parents of infants in the egg group, 94 of 407 (23.1%), correctly guessed their intervention group assignment compared with the control group, 57 of 413 (13.8%) (adjusted relative risk [aRR], 1.67; 95% CI, 1.23–2.25; *P* = .0009).

Clinical outcomes

For the primary outcome (intention-to-treat), there was no difference between groups in the percentage of infants with IgE-mediated egg allergy, defined by a failed egg challenge at age 12 months (egg 26 of 371 [7.0%] vs control 39 of 377 [10.3%]; aRR, 0.75; 95% CI, 0.48–1.17; *P* = .20; Table II). The smallest dose of pasteurized raw egg after which the infants began to have an allergic reaction was less than 0.09 g egg protein (23 infants, 3 in the egg group). There were only 3 infants (2 in the egg group) who experienced a serious adverse event; all 3

had anaphylaxis after the pasteurized raw egg challenge. Skin symptoms of allergic reaction to the pasteurized raw egg challenges were most common, in particular urticaria/angioedema with at least 3 concurrent hives (all 65 infants), and only 4 infants had emesis (2 in the egg group). Most infants, 60 of 65 (92%), who had a reaction to the pasteurized raw egg challenge were tolerating baked or cooked egg in their diet without an allergic reaction. The effect of treatment was not modified by breast-feeding status, age at randomization, or baseline egg-specific IgE levels (Table III).

The per-protocol analysis found a lower percentage of infants in the egg group, 9 of 305 (3.0%), compared with the control group, 31 of 312 (9.9%) (aRR, 0.32; 95% CI, 0.16–0.65; *P* = .002), had IgE-mediated egg allergy at age 12 months. A higher percentage of participants in the egg group were excluded from the per-protocol analysis because of a perceived adverse reaction (36 of 407 [8.9%] compared with 19 of 413 [4.6%]; *P* = .02) or a confirmed allergic reaction (25 of 407 [6.1%] compared with 6 of 413 [1.5%]; *P* = .0004) to the study powder. There were no anaphylactic reactions to the pasteurized whole egg powder upon initial introduction at age 4 to 6.5 months. Table IV summarizes the clinical outcomes of infants (*n* = 31) who had a confirmed allergic reaction to the study powder.

Secondary outcome analyses found no differences between the 2 groups for the percentage of infants sensitized to egg (positive SPT result) at age 12 months (egg 10.8% vs control 15.1%; aRR, 0.77; 95% CI, 0.54–1.10; *P* = .15; Table II). In total, 11.3% of infants had eczema diagnosed between randomization and age 12 months, and there were no differences between the 2 groups (egg 10.7% vs control 11.9%; aRR, 0.84; 95% CI, 0.57–1.23; *P* = .37; Table II). There were also no differences between the 2 groups for sensitization to peanut, sensitization to any allergen, atopic eczema, or wheeze (Table II). With regard to other post-randomization infant health measures (the number of febrile illnesses, number of courses of antibiotics, and number of hospital admissions longer than 24-hour duration), there were no differences between the 2 groups (data not shown).

IgE and IgG₄ antibody measurements

A total of 357 infants had matched blood samples collected at randomization and age 12 months. At randomization (age 4–6.5 months) and before any known ingestion of egg in solid foods, 5.0% (18 of 357) of infants were sensitized to egg with an egg-specific IgE level of more than 0.35 kU_A/L. At age 12 months, egg-specific IgE levels were higher in the egg group than in the control group (adjusted ratio of means, 2.73; 95% CI, 1.09–6.85; *P* = .03) (see Fig 2).

Early ingestion of egg (the egg group) was associated with higher (*P* < .0001) egg-specific IgG₄ levels at age 12 months (Fig 3). This was despite most infants in both groups having egg and egg-containing foods introduced into their diets from age 10 to 11 months (96.2% in the egg group, 97.5% in the control group). Before their 12-month appointment, 394 of 401 (98.3%) infants in the egg group and 402 of 403 (99.8%) infants in the control group had egg introduced into their diet. The reasons for not introducing egg before age 12 months in the other 8 infants were advice from an independent medical doctor due to suspected egg allergy (5 in the egg group and 1 in the control group) or parents who thought their infant had an egg allergy (2 in the egg group). All these 8 infants had the egg challenge at age

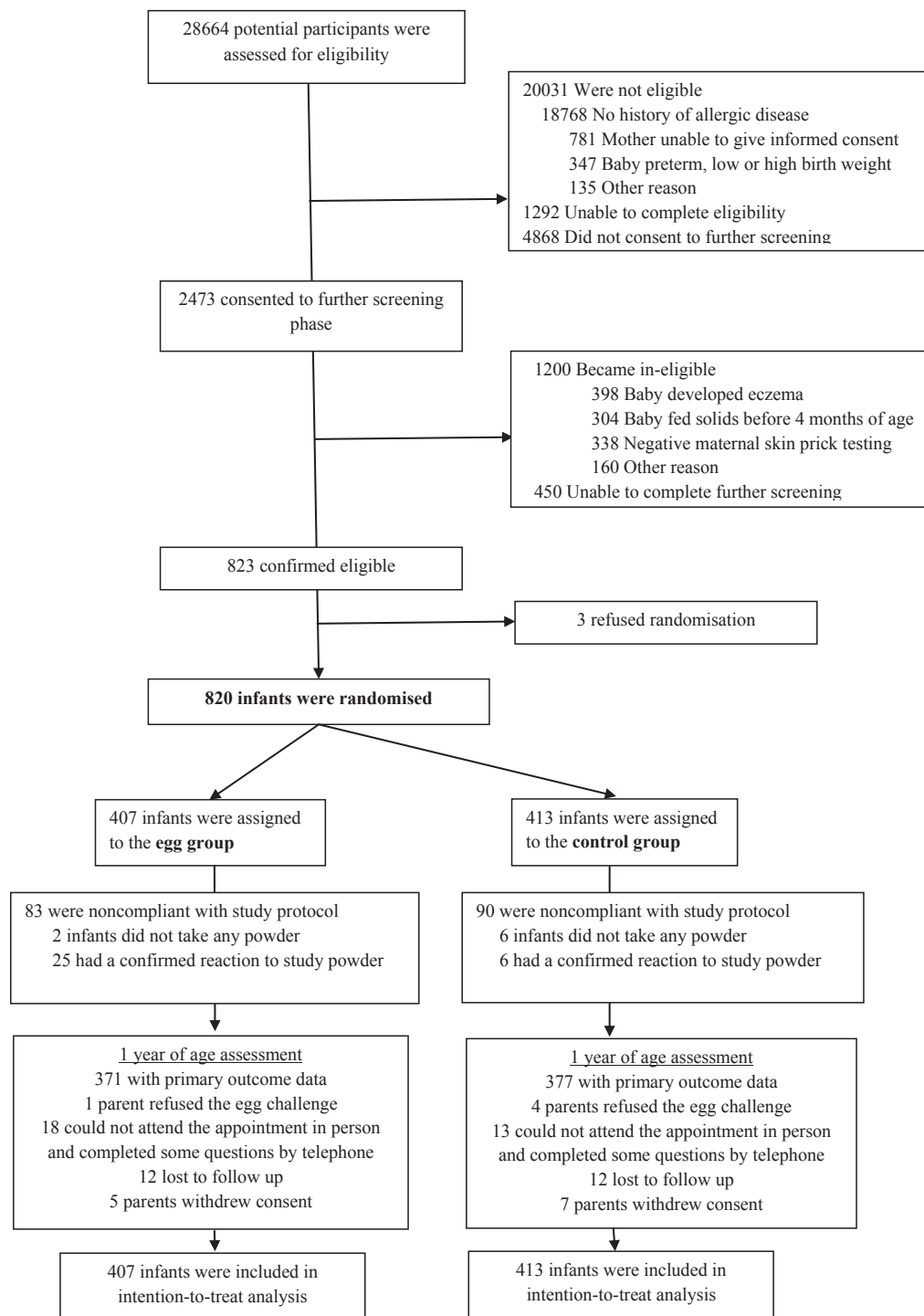


FIG 1. Study flow of participants.

12 months, and only 3 (all in the egg group) failed the egg challenge.

DISCUSSION

In the largest RCT of its type, investigating the early, regular inclusion of egg in solid foods from age 4 to 6.5 months, we found no evidence for a difference in the risk of IgE-mediated egg allergy in the first year of life. Our STEP RCT was designed as a

primary prevention trial including infants at hereditary risk (atopic mothers) but without any allergy symptoms or eczema at the time of recruitment. Hence, we believe that the results are relevant to a major proportion of the population who are seeking guidance on complementary feeding strategies for the primary prevention of food allergy. It is important to distinguish the generalizability of our STEP trial from the previously published Solids Timing for Allergy Reduction (STAR)¹² and Learning Early About Peanut Allergy (LEAP) trials.¹³ Although STAR

TABLE I. Baseline characteristics

Characteristic	Egg group (n = 407)	Control group (n = 413)
Maternal age (y), mean $\pm$ SD	32.0 $\pm$ 5.4	32.1 $\pm$ 5.5
Maternal white race	362 (88.9%)	373 (90.5%)
Mother completed secondary school	337 (82.6%)	334 (81.1%)
Cesarian-section birth	146 (35.9%)	151 (36.6%)
Paternal history of allergic disease	188 (47.1%)	206 (50.7%)
First-degree relative history of food allergy	71 (17.9%)	66 (16.2%)
Infant male sex	197 (48.4%)	201 (48.7%)
Infant gestational age at birth (wk), mean $\pm$ SD	39.6 $\pm$ 1.2	39.5 $\pm$ 1.2
Parity = 1	211 (52.1%)	205 (49.8%)
Other children in the home, median (interquartile range)	1.0 (0.0-1.0)	1.0 (0.0-1.0)
Ever breast-fed	397 (97.8%)	398 (96.8%)
Breast-fed at randomization	274 (67.5%)	267 (65.0%)
Infant age at randomization (mo), median (interquartile range)	5.8 (5.3-6.3)	5.9 (5.4-6.3)
Age at introduction to infant formula (mo), median (interquartile range)	1.0 (0.3-3.0)	0.8 (0.3-3.0)
Age at introduction to solid foods (mo), median (interquartile range)	4.5 (4.3-5.3)	4.8 (4.3-5.3)
Dog as a pet	185 (45.6%)	183 (44.4%)
Cat as a pet	84 (20.7%)	93 (22.6%)
Total household egg intake per week (eggs), median (interquartile range)	12.0 (7.0-19.0)	12.0 (7.5-19.0)
Maternal egg intake per week (eggs), median (interquartile range)	4.5 (3.0-7.0)	4.5 (3.0-7.0)
Maternal smoking	42 (10.3%)	36 (8.7%)
Other smoking in the household	84 (20.7%)	68 (16.5%)
No antibiotics use before randomization	369 (90.9%)	384 (93.4%)

Values are numbers (percentages) unless otherwise indicated.

TABLE II. Clinical allergy assessment outcomes at age 12 mo

Outcomes	Egg group (n = 407)	Control group (n = 413)	Unadjusted RR (95% CI)*	Unadjusted P value	Adjusted† RR (95% CI)	Adjusted† P value
IgE-mediated egg allergy	26 of 371 (7.0%)	39 of 377 (10.3%)	0.76 (0.48-1.20)	.24	0.75 (0.48-1.17)	.20
Anaphylaxis to egg challenge‡	2 of 365 (0.6%)	1 of 377 (0.3%)		.62		
Nonanaphylactic allergic reaction to egg challenge	28 of 365 (7.7%)	41 of 377 (10.9%)	0.80 (0.51-1.24)	.31	0.79 (0.51-1.21)	.28
Positive skin prick test to egg	40 of 371 (10.8%)	57 of 377 (15.1%)	0.77 (0.53-1.11)	.16	0.77 (0.54-1.10)	.15
Sensitization to any allergen	55 of 360 (15.3%)	68 of 368 (18.5%)	0.87 (0.63-1.19)	.38	0.87 (0.64-1.19)	.38
Sensitization to peanut	16 of 370 (4.3%)	17 of 376 (4.5%)	0.96 (0.50-1.87)	.91	0.95 (0.49-1.85)	.89
Eczema diagnosis	40 of 374 (10.7%)	45 of 378 (11.9%)	0.84 (0.57-1.24)	.38	0.84 (0.57-1.23)	.37
Atopic eczema	10 of 372 (2.7%)	20 of 379 (5.3%)	0.53 (0.26-1.10)	.09	0.53 (0.26-1.09)	.09
Wheeze	92 of 378 (24.3%)	84 of 382 (22.0%)	1.12 (0.86-1.44)	.41	1.12 (0.87-1.45)	.39

RR, Relative risk.

*RRs, CIs, and P values are based on an analysis of 100 imputed data sets unless otherwise indicated.

†Adjusted for city, infant sex, breast-feeding status, and paternal history of allergic disease.

‡Fisher exact test using original data; adjusted/imputed analyses not done because of rarity of outcomes.

and LEAP trials also compared regular consumption of allergenic solid foods (egg/peanut) with avoidance in early life, in requiring an existing allergic disease at study entry (eczema and/or egg allergy) both were secondary prevention trials, making the results applicable to a smaller proportion of the overall population.

We acknowledge that a major limitation of the STEP trial was the inability to reach the original sample size estimate of 1194 infants, designed to detect a clinically meaningful reduction of 50% in the risk of IgE-mediated egg allergy. Although the sample size was sufficient to rule out large increases in egg allergy risk (upper 95% confidence limit for the relative risk of 1.17), we cannot rule out potentially important benefits, because with a lower 95% confidence limit of 0.48, this is consistent with a risk that is more than twice as great in the control group as compared to that in the egg group. Indeed, the wide CI may suggest that there are greater effects in particular subgroups of infants and further investigation is warranted.

The dose of egg (0.4 g egg protein per day) may not have been enough to induce egg tolerance in all infants, especially as the infants increased in body size over the course of the intervention period. Future trials in this area may need to consider an increasing dose approach in their study design. Another possible limitation may have been that the 4- to 6-month difference in egg introduction age between the groups, age 4 to 6.5 months in the egg group compared with age 10 months in the control group, may not have been long enough. This can be considered in context of the LEAP trial results,¹³ where there was a more than 4-year difference in age of introduction of peanut between the intervention group at age 4 to 11 months compared with the peanut avoidance group at age 5 years. However, we specifically designed this STEP trial to be able to maximize future translation of the results in our community. It was important for the control group to reflect the contemporary practice of introducing egg around age 10 months. Furthermore, on the basis of findings of

TABLE III. IgE-mediated egg allergy effect modification by feeding mode, age at randomization, and egg-specific IgE levels at randomization

Baseline characteristic	Egg group	Control group	Unadjusted RR (95% CI)*	Unadjusted P value	Interaction unadjusted P value	Adjusted† RR (95% CI)	Adjusted† P value	Interaction adjusted† P value
Feeding mode: breast-fed	19 of 215 (8.8%)	28 of 224 (12.5%)	0.81 (0.48-1.38)	.44	.65	0.75 (0.48-1.17)	.37	.68
Feeding mode: formula-fed	7 of 156 (4.5%)	11 of 153 (7.2%)	0.63 (0.26-1.57)	.33		0.63 (0.26-1.55)	.32	
Age at randomization <6 mo	18 of 220 (8.2%)	21 of 208 (10.1%)	0.88 (0.49-1.59)	.67	.44	0.84 (0.47-1.50)	.56	.49
Age at randomization ≥6 mo	8 of 151 (5.3%)	18 of 169 (10.7%)	0.60 (0.28-1.28)	.19		0.60 (0.29-1.26)	.18	
Randomization IgE level <0.1 kU _A /L	5 of 151 (3.3%)	8 of 161 (5.0%)	0.67 (0.22-1.99)	.47	.78	0.62 (0.21-1.84)	.39	.53
Randomization IgE level ≥0.1 kU _A /L	10 of 23 (43.5%)	12 of 22 (54.6%)	0.80 (0.44-1.46)	.46		0.91 (0.54-1.55)	.74	

RR, Relative risk.

*RRs, CIs, and P values are based on analysis of 100 imputed data sets unless otherwise indicated.

†Adjusted for city, infant sex, breast-feeding status, and paternal history of allergic disease.

TABLE IV. Clinical outcomes of infants (n = 31) who had a confirmed allergic reaction to the study powder

Allocated study powder	Age at randomization (mo)	Symptoms after study powder ingestion	IgE-mediated egg allergy at age 12 mo
Control	4.9	Urticaria	Yes
Control	5.3	Urticaria, vomiting	Yes
Control	5.7	Angioedema	No
Control	5.7	Vomiting	No
Control	6.0	Urticaria, angioedema	Yes
Control	6.4	Urticaria	No
Egg	4.4	Urticaria	Unknown, lost to follow-up
Egg	4.8	Urticaria, angioedema	Yes
Egg	4.9	Urticaria	Yes
Egg	4.9	Urticaria	No
Egg	5.1	Urticaria	No
Egg	5.3	Urticaria, vomiting	No
Egg	5.3	Urticaria	Yes
Egg	5.4	Urticaria	Yes
Egg	5.6	Urticaria	Yes
Egg	5.7	Vomiting	Unknown, lost to follow-up
Egg	5.7	Urticaria	Unknown, parent refused
Egg	5.8	Vomiting	No
Egg	5.8	Urticaria, vomiting	Yes
Egg	5.9	Urticaria, angioedema	Yes
Egg	6.0	Urticaria	Yes
Egg	6.0	Urticaria	Yes
Egg	6.2	Urticaria, vomiting	Yes
Egg	6.2	Urticaria, vomiting	Yes
Egg	6.2	Vomiting	Yes
Egg	6.2	Urticaria, angioedema	Yes
Egg	6.2	Urticaria, vomiting	No
Egg	6.4	Urticaria	Unknown, lost to follow-up
Egg	6.4	Urticaria	No
Egg	6.5	Urticaria	No
Egg	6.5	Vomiting	No

another previous Australian cohort,¹¹ which showed that delaying the introduction of egg after age 10 months was associated with a higher risk of egg allergy (adjusted odds ratio, 1.6; 95% CI, 1.0-2.6) compared with the earlier introduction at age 4 to 6 months, we were confident that an intervention period ranging from between age 4 and 6.5 months to age 10 months was relevant and suitable.

Our per-protocol analysis found reduced prevalence of IgE-mediated egg allergy by age 12 months for infants who

tolerated and regularly consumed egg from age 4 to 6.5 months until 10 months. It is important to exercise caution when interpreting this result. We acknowledge possible bias by excluding those infants from the per-protocol analysis who had a reaction to and discontinued use of the egg-containing study powder because of egg allergy. To maximize future translation of the results, we designed the trial as to not exclude infants who were already sensitized to egg before randomization, because testing infants in the community before the introduction of solid

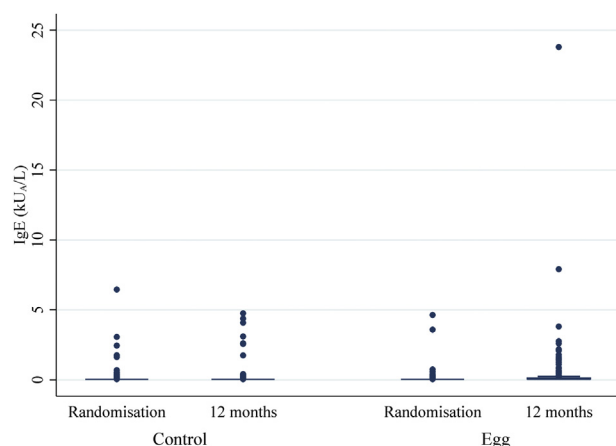


FIG 2. Egg-specific IgE (kU_A/L) concentrations at randomization (4-6.5 months) and primary outcome (age 1 year) for the intervention (egg) group compared with control group.

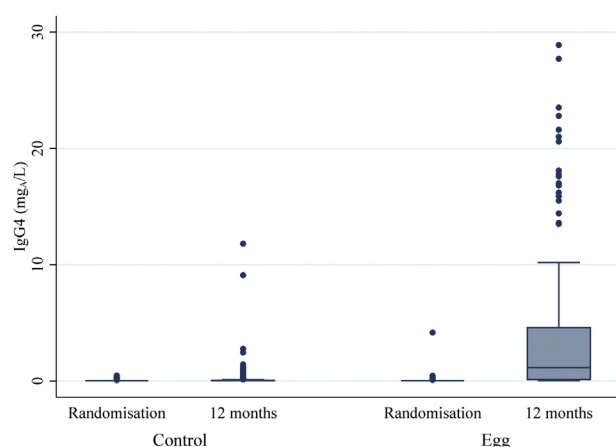


FIG 3. Egg-specific IgG₄ (mg_A/L) concentrations at randomization (4-6.5 months) and primary outcome (age 1 year) for the intervention (egg) group compared with control group.

foods in their diet (especially those without any symptoms of eczema) is not feasible (practically or financially) in most countries. Overall, 5.0% infants were sensitized to egg, with an egg-specific IgE level of more than 0.35 kU_A/L, before any egg in solid foods ingestion and we acknowledge that excluding these infants before randomization may have led to different trial results. Interestingly, our per-protocol result finding was consistent with those from both the LEAP¹³ and Enquiring about Tolerance trials,¹⁴ also investigating the effects of regular food allergen ingestion. This reinforces the notions arising from animal studies that the development of oral tolerance is driven by regular exposure to allergens.³ However, it could also be postulated that introducing egg at age 4 to 6 months does not reduce the risk of egg allergy but actually leads to the earlier presentation and diagnosis of egg allergy in infants already predisposed. We have recently reported from the STAR trial that elevated egg-specific T_H2 cytokine responses, particularly IL-5 and IL-13, were established before egg introduction in infant solid foods, were not altered by the introduction of egg, and these T_H2 cytokine responses at age 4 months also predicted egg allergy at age 1 year.¹⁵ Hence, for many infants, the development of egg

allergy may be “preset” before the introduction of egg and egg-containing foods in the solid foods diet.

Our STEP RCT has much strength in study design, including the double-blinded approach where considerable efforts were made to ensure that the 2 study powders were identical in color, texture, smell, and taste. We also conducted standardized egg challenges to confirm egg allergy at age 12 months, as the “criterion standard” for egg allergy diagnosis. Importantly, our STEP RCT also had high completion rates, with 95% of participants having attended their final appointment at age 12 months and 91% of infants with a primary outcome result. Furthermore, given the long intervention period of 4- to 6-month duration, the overall compliance rates of 84% for regular study powder use and 99% for following an egg-free infant diet in both groups were excellent.

It is important to note that in this STEP trial, involving infants without any symptoms of eczema before the introduction of egg in solid foods, there were no anaphylactic reactions to the pasteurized whole egg powder on initial introduction at age 4 to 6.5 months. This highlights a key public health message: that egg introduction at age 4 to 6.5 months for infants without eczema is safe to do so at home without the need for prior egg sensitization testing. In comparison to our previous STAR RCT,¹² which only randomly allocated infants with moderate to severe eczema, 31% of the infants had a confirmed reaction to the egg-containing study powder, whereas in this STEP trial only 6% of infants in the egg group had a confirmed reaction. Finally, it is also worth noting that more than 90% of the infants who had a reaction to the pasteurized raw egg challenge were tolerating baked or cooked egg in their diet. This raises the question as to how many of these infants would have had an egg allergy diagnosed if they had not had a raw egg challenge. This result is consistent with the previous finding from Koplin et al,¹¹ and highlights the lower frequency of allergic reaction to baked or cooked compared with raw egg. Hence, specifying that the introduction and continued inclusion of *cooked* egg in infant diets would appear advisable.

Conclusions

We found no evidence that regular egg intake from age 4 to 6.5 months substantially alters the risk of egg allergy by age 1 year in infants who are at hereditary risk of allergic disease and had no eczema symptoms at study entry. There is no need for routine testing of infants without eczema in the community to determine egg sensitization status before the introduction of egg and egg-containing foods when solids are introduced. Future research aimed at food allergy prevention should focus on strategies to reduce the development of eczema and elevated food allergen-specific T_H2 cytokine responses in early life before the introduction of solid foods.

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Clinical implications: Regular intake of egg protein from age 4 to 6 months does not substantially alter the risk of egg allergy in infants without symptoms of eczema.

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METHODS

Study powder composition

The suppliers of the study powder ingredients were Farm Pride Foods Pty Ltd (Keysborough, Victoria, Australia) for the spray-dried pasteurized whole egg powder, Four Leaf Milling Pty Ltd (Tarlee, South Australia, Australia) for the white rice flour and brown rice flour, and Morlife Pty Ltd (Arundel, Queensland, Australia) for the supply of the carrot juice powder and pineapple juice powder. The study powders were measured out, mixed together, packaged, and labeled in accordance with Good Manufacturing Procedures by Pharmaceutical Packaging Professionals (BioSA Incubator, Thebarton, South Australia, Australia). Both the egg group and the control group study powder sachets each contained 2.4 g of powder. The formulation of the powder in grams (g) per sachet is presented in [Table E1](#).

Antibody measurements

Whole egg-specific IgE and egg white-specific IgG₄ serum antibody concentrations were measured with the ImmunoCAP 250 system (Phadia AB, Uppsala, Sweden). For specific IgE, the lower limit of detection was 0.1 kU_A/L and for specific IgG₄ the lower limit of detection was 0.07 mg_A/L. All researchers remained blinded to the blood sample results throughout the trial to avoid any study bias.

Skin prick tests

At age 12 months, on the same day but before the egg challenge, the infants had skin prick tests performed by experienced nurses. The allergens tested were rice (Stallergenes, France), whole egg (Stallergenes, France), egg white (Stallergenes, France), pasteurized raw whole egg (actual food not an extract), cow's milk (ALK-Abello, Denmark), wheat (Stallergenes, France), tuna and cod fish (Stallergenes, France), peanut (Stallergenes, France), cashew nut (Stallergenes, France), grass pollen perennial ryegrass (Stallergenes, France), cat hair (ALK-Abello, Denmark), and house dust mite, *Dermatophagoides pteronyssinus* (Stallergenes, France). Glycerin and histamine (Stallergenes, France) were used as negative and positive controls, respectively. A response was considered positive if the mean of the horizontal and perpendicular wheal diameters was 3 mm or greater in size than the mean wheal of the negative control site at 15 minutes. *Sensitization* was defined as a positive skin prick test result to at least 1 of the allergens assessed.

Pasteurized raw egg challenge

At age 12 months, the pasteurized raw egg challenge was given according to a low-risk or high-risk protocol. Infants who were already eating egg as part of their regular diet followed the low-risk protocol, which entailed a single dose of 30 mL of pasteurized raw egg (equivalent to ½ an egg). The high-risk protocol was reserved for infants who had previously had a suspected allergic reaction to egg, never eaten any egg, or who had a positive skin prick test result to 1 or more of the egg allergens tested. This high-risk protocol entailed 6 doses of increasing amount of pasteurized raw egg (drop inside lip, 1, 2, 5, 10, and 20 mL) with the doses at 15-minute intervals. The challenge was ceased if the infant had an allergic reaction.

Multiple imputation methods

Multiple imputation was performed separately by treatment group using chained equations,^{E1} with auxiliary variables added to the imputation model to help satisfy the missing at random assumption and to improve the prediction of missing values. A total of 100 imputed data sets were generated, with treatment effect estimates combined across data sets using Rubin's rules.^{E2} For the primary outcome of IgE-mediated egg allergy, key auxiliary variables in the imputation model included noncompliance with the protocol and a perceived or adverse reaction to egg powder. Conditional on these and other auxiliary variables in the imputation model, no additional predictors of missing data on the primary outcome could be identified, and hence a missing at random assumption was deemed plausible. The sensitivity of results to the missing at random assumption was explored using pattern mixture models applied under a missing not at random assumption. In these models, the risk of IgE-mediated egg allergy was assumed to be between half and twice as high in infants with missing data compared with infants with observed data, with differences applied to one or both treatment groups. All analyses produced similar treatment effect estimates, and so only the results of the primary imputed analysis are presented.

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TABLE E1. Formulation of the study powders

Study group	Egg powder (g)	White rice flour (g)	Brown rice flour (g)	Carrot juice powder (g)	Pineapple powder (g)
Egg	0.87	0.63	0.63	0.24	0.02
Control	—	—	2.02	0.36	0.02