

Two-step egg introduction for prevention of egg allergy in high-risk infants with eczema (PETIT): a randomised, double-blind, placebo-controlled trial



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Summary

Background Evidence is accumulating that early consumption is more beneficial than is delayed introduction as a strategy for primary prevention of food allergy. However, allergic reactions caused by early introduction of such solid foods have been a problematic issue. We investigated whether or not early stepwise introduction of eggs to infants with eczema combined with optimal eczema treatment would prevent egg allergy at 1 year of age.

Methods In this randomised, double-blind, placebo-controlled trial, we enrolled infants 4–5 months of age with eczema from two centres in Japan. Exclusion criteria were being born before 37 weeks of gestational age, experience of ingestion of hen's eggs or egg products, history of immediate allergic reaction to hen's eggs, history of non-immediate allergic reaction to a particular type of food, and complications of any severe disease. Infants were randomly assigned (block size of four; stratified by institution and sex) to early introduction of egg or placebo (1:1). Participants in the egg group consumed orally 50 mg of heated egg powder per day from 6 months to 9 months of age and 250 mg per day thereafter until 12 months of age. We aggressively treated participants' eczema at entry and maintained control without exacerbations throughout the intervention period. Participants and physicians were masked to assignment, and allocation was concealed. The primary outcome was the proportion of participants with hen's egg allergy confirmed by open oral food challenges at 12 months of age, assessed blindly by standardised methods, in all randomly allocated participants who received the intervention. This trial is registered with the University Hospital Medical Information Network Clinical Trials Registry, number UMIN000008673.

Findings Between Sept 18, 2012, and Feb 13, 2015, we randomly allocated 147 participants (73 [50%] to the egg group and 74 [50%] to the placebo group). This trial was terminated on the basis of the results of the scheduled interim analysis of 100 participants, which showed a significant difference between the two groups (four [9%] of 47 participants had an egg allergy in the egg group vs 18 [38%] of 47 in the placebo group; risk ratio 0·222 [95% CI 0·081–0·607]; $p=0·0012$). In the primary analysis population, five (8%) of 60 participants had an egg allergy in the egg group compared with 23 (38%) of 61 in the placebo group (risk ratio 0·221 [0·090–0·543]; $p=0·0001$). The only difference in adverse events between groups was admissions to hospital (six [10%] of 60 in the egg group vs none in the placebo group; $p=0·022$). 19 acute events occurred in nine (15%) participants in the egg group versus 14 events in 11 (18%) participants in the placebo group after intake of the trial powder.

Interpretation Introduction of heated egg in a stepwise manner along with aggressive eczema treatment is a safe and efficacious way to prevent hen's egg allergy in high-risk infants. In this study, we developed a practical approach to overcome the second wave of the allergic epidemic caused by food allergy.

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Introduction

A second wave of the allergy epidemic, in the form of a rising prevalence of food allergies, has emerged in high-income countries following the first wave of increasing asthma and allergic rhinitis.¹ Food allergy often occurs in the early stage of the allergic march,² along with atopic dermatitis, and is associated with increased risks of anaphylaxis and asthma.³ Hen's egg allergy is one of the commonest forms of food allergy, with sensitisation to egg at 1 year of age strongly associated with sensitisation to aeroallergens at 3 years of age.⁴ Although food allergy and atopic dermatitis (and subsequent asthma) have been widely believed to be prevented by avoidance of

specific food during the perinatal and infancy periods,^{5,6} such an approach has not been supported by a Cochrane systematic review.⁷

Conversely, findings from observational studies^{8–11} have revealed that early introduction of solid food might decrease the incidence of food allergy compared with late introduction or avoidance. Several randomised controlled trials (RCTs) have been done to test the possible protective role of early introduction of solid foods. Investigators of the Learning Early About Peanut (LEAP) study¹² investigated the effect of introduction of peanuts in high-risk infants and found that early introduction reduced the prevalence of peanut allergy at 5 years of age (relative risk

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Research in context

Evidence before this study

We searched PubMed and Embase between Jan 29, 2016, and March 31, 2016, in the English language, using the following search terms: “infant”, “eczema”, “prevention”, “allergy”, and “randomised controlled trial”. The Food Allergy and Anaphylaxis Guidelines published by the European Academy of Allergy and Clinical Immunology reported that “the present evidence does not justify recommendations about either withholding or encouraging exposure to potentially allergenic foods after the age of 4 months”. The Consensus Communication on Early Peanut Introduction and the Prevention of Peanut Allergy in High-Risk Infants was released in 2015 on the basis of the Learning Early About Peanut Allergy trial data, which provided Level 1 evidence that early introduction of peanuts is safe and effective in selected high-risk infants at reduction of subsequent peanut allergy by up to 81%. The Enquiring About Tolerance study was a randomised controlled trial that recruited infants from the general population, assessing whether or not early exposure might reduce subsequent allergy. The per-protocol analysis suggested a possible protective effect of early introduction of peanuts and hen’s eggs, but adherence to the study protocol was rather low and the results in the intention-to-treat analysis were negative. In addition to the Learning Early About Peanut Allergy and Enquiring About Tolerance studies, we identified only one trial published in 2013 describing the preventive effect of early introduction of eggs for high-risk infants with eczema. Investigators reported that a lower proportion of infants in the early egg introduction group (14 [33%] of 42) developed IgE-mediated egg allergy at

12 months of age than in the placebo group (18 [51%] of 35); however, the difference did not reach statistical significance (relative risk 0.65 [95% CI 0.38–1.11]). Caution is needed when infants with moderate-to-severe eczema are first exposed to eggs because many have already developed sensitisation and clinical reactivity by 4 months of age.

Added value of this study

This trial is the first randomised, double-blind, placebo-controlled trial to produce results that show a safe and effective method of early introduction of hen’s eggs to high-risk infants with eczema to prevent egg allergy. Our findings substantiate that early introduction of a small amount of heated egg in a stepwise manner combined with attentive treatment of eczema can reduce the prevalence of egg allergy in high-risk infants with eczema.

Implications of all the available evidence

Our results provide firm evidence that early introduction of potentially allergenic foods can be done safely by starting from a small dose and that prevention of food allergy can be achieved effectively in a stepwise manner. A small amount of solid food is safe, even for sensitised infants, and this stepwise approach is practical at a population level because infants do not need to be screened by skin prick, serum-specific IgE concentration, or challenge tests before introduction. Additional trials are needed to test this approach for prevention of other types of food allergies. Optimal control of eczema might be an integral part of the preventive programme to minimise the chance of percutaneous sensitisations.

0.19; $p < 0.0001$). Results of another RCT¹³ that investigated the effect of egg introduction at 4 months of age did not show any protection (relative risk of hen’s egg allergy at 12 months 0.65; $p = 0.11$) and highlighted a major problem of such an approach in infants with moderate-to-severe eczema because many might have sensitisation and clinical reactivity to egg by 4 months of age. In both RCTs, a considerable number of participants had allergic reactions to several types of food at first introduction. The Enquiring About Tolerance study¹⁴ assessed the possible preventive effect of early introduction of six types of food, including peanuts and hen’s eggs, in infants from the general population. The complex protocol had rather low adherence and the results in the intention-to-treat analysis were negative. Findings of these RCTs suggest that a safe and practical approach for introduction of allergenic foods to high-risk infants with eczema is still very much needed.

Eczema in infancy is associated with an increased risk of percutaneous sensitisation to food allergens.^{15–17} Attentive and effective treatment of eczema might be needed before and during feeding of allergenic food, such as peanuts and eggs, to infants. Our experience of oral immunotherapy suggests that a very small amount of

solid food can be safely introduced even for patients who have already been sensitised at first introduction and that a stepwise increase in dose might be a safe approach. We did a randomised, double-blind, placebo-controlled trial, the Prevention of Egg Allergy with Tiny Amount Intake study, to investigate if the combination of stepwise introduction of egg with aggressive treatment of eczema reduces the risk of onset of hen’s egg allergy at 12 months of age.

Methods

Study design and participants

In this randomised, double-blind, placebo-controlled trial, we recruited patients from the National Center for Child Health and Development (NCCHD) and Tachikawa Sougo General Hospital (TSGH) in Tokyo, Japan. Participants were eligible for enrolment if they were 4–5 months of age with atopic dermatitis meeting Hanifin-Rajkas’ diagnostic criteria.¹⁸ Exclusion criteria were being born before 37 weeks of gestational age, experience of ingestion of hen’s eggs or egg products, history of immediate allergic reaction to hen’s eggs, history of non-immediate allergic reaction to a particular

type of food, and complications of any severe disease. We did not exclude any individual for introduction of any solid food except for egg. We obtained written informed consent from the parents of the participants at the time of enrolment. The study was approved by the institutional review board of the NCCHD and TSGH. The protocol for this study and the subsequent changes are available at the NCCHD website.

Randomisation and masking

Participants were randomly assigned to the egg or placebo group in a ratio of 1:1. Allocation was carried out with use of the block randomisation method. The block size was four, and participants were stratified into four groups based on institution (TSGH or NCCHD) and sex. The allocation plan was created by the Data Centre located at the NCCHD. The Data Centre was in charge of randomisation of participants and storage of all clinical data. The following procedure was adopted to conceal allocation information: at the time of registration of a participant, the Data Centre received information about institution and sex only, and they allocated an assignment code a or b, without knowing the identity of the participant. Assignment was executed on the basis of a computer-generated random number table. The result of the assignment was orally communicated to a designated member of clinical staff, who was the only person to know which assignment code corresponded to the egg or placebo group. This staff member prepared the trial powder for distribution to participants without being involved in any other aspect of the study. Participants were recruited by those giving the intervention (ON, SK, JN, KY-H, MK, MS, and AK) and PETIT study members (listed in the appendix) and the primary and secondary outcomes were assessed by the other masked outcome assessors (PETIT study members). In this way, participants, their caregivers, those giving the intervention, outcome assessors, and the Data Centre were all masked to assignment information during the trial, and allocation was concealed. Only the Independent Data and Safety Monitoring Committee (IDSMC) could access the unmasked data.

Procedures

Participants consumed orally the allocated trial powder from 6 months of age daily, for 6 months. We assigned the egg group a powder consisting of egg and squash (Japanese pumpkin), whereas we assigned the placebo group a powder consisting of squash, for which we matched colour and volume with the egg powder (appendix). For the egg group, we prepared packages containing two different doses of egg powder. One taken daily for 3 months beginning from 6 months of age consisted of 50 mg of heated egg powder (containing 25 mg of egg protein, which is equivalent to 0.2 g of whole egg boiled for 15 min) and 100 mg of squash. The other egg powder packages consumed for

the last 3 months beginning from 9 months of age consisted of 250 mg heated egg powder (containing 125 mg egg protein, which is equivalent to 1.1 g of boiled whole egg) and squash. Our clinical experience of oral immunotherapy for egg allergy suggested that 50 mg of heated egg powder was a safe dose for first introduction. We adopted the two-step approach to increase the dose of egg powder 3 months after the first egg introduction. Correspondingly, we also provided the placebo group two different doses of trial powder packages containing squash, 150 mg from 6 months of age and 250 mg from 9 months of age (appendix).

On the first day of trial powder introduction at 6 months of age and the first dose-up day at 9 months of age, participants visited the hospitals to receive an oral food challenge test by using the assigned powder so that they could be carefully observed for 2 h after intake of the powder by a paediatric allergist. Subsequently, participants' consumption of the trial powder was recorded daily by their respective caregivers. We did not place participants under any food restrictions except for eggs and egg products. We did not require their mothers to follow any dietary restrictions, whether they were breastfeeding or not.

We treated participants' eczema attentively, aiming to achieve remission, and gave proactive therapy to some patients with moderate-to-severe eczema to prevent flare-up, as previously described.¹⁹ We treated participants for their eczema when they visited the hospitals as outpatients for the first time before enrolment in this study. Specifically, for infants with moderate-to-severe eczema, we applied topical corticosteroids (0.1% hydrocortisone butyrate ointment for the face and 0.12% betamethasone valerate ointment for everywhere else) daily until the eczema disappeared. Topical steroids would then be used intermittently for a maximum of 2 days a week for maintenance of remission. If remission was maintained for more than a month, we replaced application of steroid ointment with emollients and gradually tapered them off (appendix). Participants had regular visits to outpatient units for assessment in addition to their scheduled visits for this trial to make sure that their eczema was in remission. We obtained blood samples at patients' first visits as outpatients, at enrolment (4–5 months of age), and at 9 months and 12 months of age. We did clinical assessments at the same timepoints. Adverse events were recorded in an event diary by caregivers and physicians checked the diary at every scheduled or unscheduled visit.

The oral food challenge (OFC) consisted of a cumulative dose of 7 g of heated whole-egg powder equivalent to 32 g of boiled whole hen's eggs in both groups. If a participant showed any objective immediate allergic reactions, such as urticaria, continuous cough, wheezing, vomiting, or diarrhoea, the participant would be diagnosed as having hen's egg allergy. Additional information about OFC is provided in the appendix. We measured serum concentrations of IgE specific to egg white and ovomucoid

For the study protocol see http://www.ncchd.go.jp/en/hospital/about/section/medical_subspecialties/pdf/PETITprotocol.pdf

See Online for appendix

with the ImmunoCAP (Thermo Fisher Scientific, Upsala, Sweden) system. We measured serum concentrations of IgG1, IgG4, and IgA specific to egg white and ovomucoid with a microarray system using diamond-like carbon-coated densely carboxylated protein chips.²⁰

We collated baseline data at enrolment (4–5 months old), but measured Scoring Atopic Dermatitis (SCORAD)²¹ and thymus and activation-regulated chemokine (TARC) data at the time of patients' first visits as outpatients, even if this visit was before 4 months of age, to record patients' initial skin conditions before they started eczema treatment. We recorded each participant's skin condition using SCORAD and the patient-oriented eczema measure (POEM).²² We assessed SCORAD at the first visit as baseline data and again at 12 months of age. POEM was assessed by a caregiver weekly during the trial. We used mean POEM score for 4 weeks as the score for each month.

Outcomes

The primary outcome was the proportion of participants with hen's egg allergy confirmed by open OFC at 12 months of age. Secondary outcomes were serum egg

white and ovomucoid-specific IgE, IgG1, IgG4, and IgA concentrations, together with TARC concentration. Although we planned to measure salivary antigen-specific immunoglobulin concentration as a secondary outcome, we were unable to measure it because of technical reasons. We measured egg-specific immunoglobulin concentration at enrolment (4–5 months of age) as baseline data and at 9 months and 12 months of age. We measured TARC concentration at participants' first visit as outpatients before enrolment (as baseline data) and then at 9 months and 12 months of age (appendix). The safety outcome was the proportion of infants with serious adverse events and important medical events during taking of trial powders. Changes of outcomes after the trial was started are described in the protocol.

Statistical analysis

We predicted the prevalence of IgE-mediated egg allergy at 12 months of age to be 7% in the egg consumption group and 20% in the placebo group on the basis of published reports.^{8,23} To detect a relative reduction of 65% (from 20% to 7%), with a power of 80% ($\alpha=0.05$ [two-sided]), we estimated that 92 infants per group were

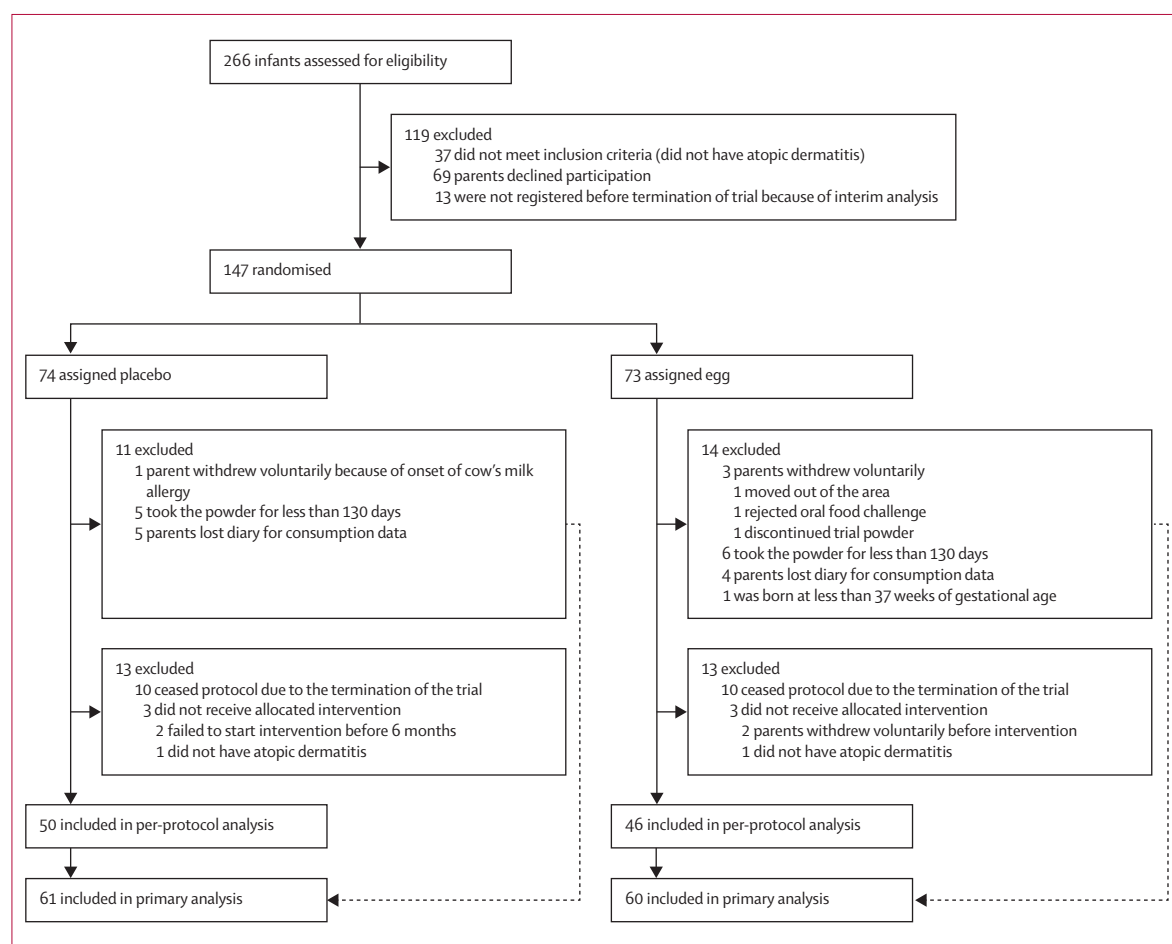


Figure 1: Trial profile

needed. Allowing for about 10% loss to follow-up, we planned to recruit 200 infants.

We analysed the primary outcome in all randomly allocated participants who received the intervention. We excluded participants whose parents voluntarily withdrew them from the analysis before they started the intervention and considered those who were withdrawn after they started the intervention positive for the primary outcome. We included participants who satisfied the recruitment criteria, took a trial powder for more than 130 days, and accidentally ingested hen's egg outside of the trial less than twice in the per-protocol analysis. We excluded participants whose primary outcome was not established when the trial ceased (on the basis of the results of the interim analysis) from the primary analysis population, the per-protocol analysis, and baseline data. We analysed adverse events in the primary analysis population.

Interim analysis by the IDSMC was scheduled for 1 year after the start of the study or when the number of participants reached 100. At interim analysis, we planned a point estimate for the primary outcome and if the estimated intervention effect was different from the value used in the sample size calculation, we would recalculate the required sample size. Decision making about changing of the total number recruited on the basis of the interim analysis would be done by the IDSMC, not by the trial steering committee, which was masked to assignment.

We analysed the primary outcome with the χ^2 test. We set the level of statistical significance for the primary outcome at 0.05 (two-sided). We also did post-hoc logistic regression analysis to take into account stratification factors and imbalances of baseline variables between groups, including SCORAD score, family history of allergic diseases, and start of solid foods. We also did subgroup analyses stratified by concentration of egg white-specific IgE at baseline (we defined lower than 0.35 kUA/L as non-sensitised) in the primary analysis and per-protocol analysis populations. Furthermore, we did analyses stratified by the class of egg white and ovomucoid-specific IgE at baseline in the per-protocol analysis population. We also did logistic regression analysis, adjusting for the following covariates: serum TARC, total IgE, and egg white-specific IgE concentrations at baseline and mean POEM score from 6–12 months of age. We did safety analyses in the primary analysis population using χ^2 and Wilcoxon rank-sum tests. We did all statistical analyses using R version 3.1.0. This trial was overseen by the IDSMC. This trial is registered with the University Hospital Medical Information Network Clinical Trials Registry, number UMIN000008673).

Role of funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of

the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Sept 18, 2012, and Feb 13, 2015, we assessed 266 infants for their eligibility, successfully enrolling 147 (55%) of them on the basis of the inclusion and exclusion criteria (figure 1). We allocated 73 participants (50%) to the egg group and 74 (50%) to the placebo group. We stopped recruitment early at the request of the IDSMC because the interim analysis of the first 100 participants detected a significant difference between the two groups in the primary outcome. Six (6%) participants were excluded from this analysis (and the final analysis) because they never consumed a trial powder (three [6%] in each group): two (2%) were found to not meet the diagnostic criteria for atopic dermatitis (one [2%] in each group) and four (4%) did not start the intervention (two [4%] in each group). Allocation was opened to all investigators and participants after fixing of the data, on May 15, 2015.

	Placebo (n=61)	Egg (n=60)
Age at enrolment (days)	163.4 (19.9)	162.2 (21.5)
Male sex	40 (66%)	39 (65%)
Gestational age (weeks)	39.1 (1.28)	38.9 (1.18)
Birthweight (g)	3108 (325.4)	3163 (373.8)
Caesarean section	6/53 (11%)	8/57 (14%)
Age at onset of eczema (months)	1.8 (1.1)	2.0 (1.1)
SCORAD score	42.0 (22.1–52.3)	27.5 (10.3–38.2)
TARC (pg/mL)	3165 (1532–5867)	2297 (1525–3250)
Total IgE concentration (IU/mL)	41.2 (15.9–181.0)	16.5 (5.3–51.2)
Egg white-specific IgE concentration (kUA/L)	4.46 (0.47–24.8)	0.73 (0.17–5.55)
Ovomucoid-specific IgE concentration (kUA/L)	0.35 (0.17–0.56)	0.17 (0.17–0.35)
Paternal history of allergic disease	34/60 (57%)	40/57 (70%)
Maternal history of allergic disease	37/60 (62%)	38/57 (67%)
Have pets	15/59 (25%)	6/53 (11%)
Smoking in the household	12/58 (21%)	14/56 (25%)
Age at start of solid foods (months)	5.6 (0.7)	5.6 (0.7)
Hen's egg elimination by mother during lactation	15/57 (26%)	15/59 (25%)
Breastfeeding		
at 6 months of age	56/60 (93%)	57/60 (95%)
at 12 months of age	43/59 (73%)	37/57 (65%)
POEM at 6 months of age	2.9 (3.2)	2.1 (2.8)

Data are mean (SD), n (%), median (IQR), or n/N (%). SCORAD=Scoring Atopic Dermatitis. TARC=thymus and activation-regulated chemokine. POEM=patient-oriented eczema measure.

Table 1: Baseline characteristics

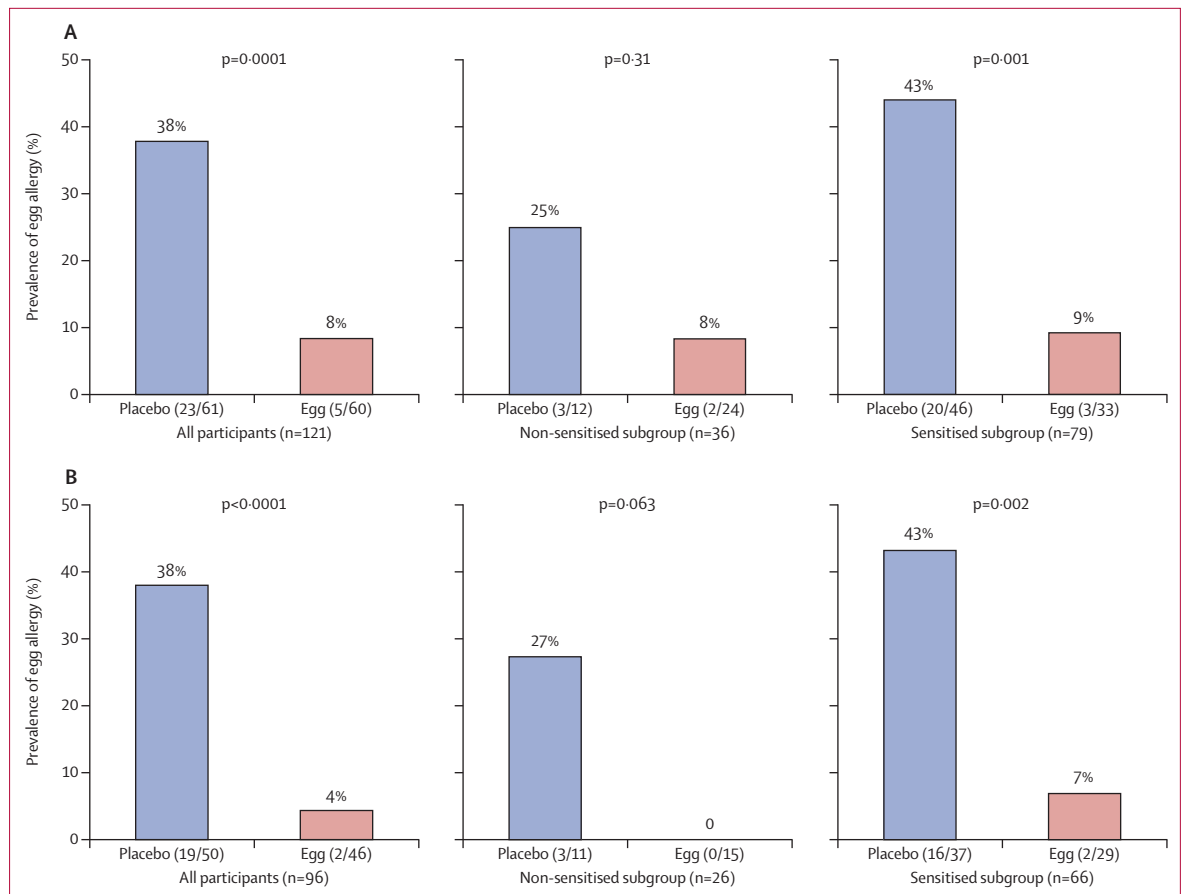


Figure 2: Prevalence of egg allergy

(A) Primary analysis population. (B) Per-protocol analysis. The left panels show all participants. The middle and right panels show the subgroup analysis that stratified by concentration of egg white-specific IgE at baseline into lower (non-sensitised subgroup; middle panels) or higher (sensitised subgroup; right panels) than 0.35 kUA/L. We used χ^2 tests for all participants and Fisher's exact tests for subgroup analyses.

The number of participants included in the primary analysis was 60 (50%) in the egg group and 61 (50%) in the placebo group. We excluded 20 (14%) participants from the final analysis who had not been assessed by the provocation test when the trial ceased and were asked to stop their participation (ten [14%] in each group). 117 (97%) of 121 participants were assessed for the primary outcome by OFC (70 [96%] of 73 in the egg group and 73 [99%] of 74 in the placebo group). One (1%) participant in the placebo group and three (4%) in the egg group withdrew from the trial (appendix); they were included in the primary analysis but excluded from the per-protocol analysis. With regard to adherence, six (8%) participants in the egg group and five (7%) in the placebo group were excluded from per-protocol analysis because they had taken the trial powder for less than 130 days. In the per-protocol analysis population, the mean number of consumption days of the trial powder was 163.5 days (SD 17.9) in the egg group and 167.6 days (16.5) in the placebo group. Baseline characteristics are shown in table 1 and the appendix.

More than 90% of participants received breastfeeding at 6 months of age.

The number of patients with hen's egg allergy confirmed by OFC at 12 months of age was four (9%) of 47 in the egg group and 18 (38%) of 47 in the placebo group at interim analysis. The IDSMC requested the person in charge of statistics in this trial (EI) to calculate the significance of difference, although this calculation was not planned at the interim analysis in the statistical analysis plan. The risk ratio was 0.222 (95% CI 0.081–0.607; $p=0.0012$), which crossed O'Brien-Fleming's boundary; the corresponding significance level was 0.003.

In the primary analysis population, five (8%) of 60 in the egg group and 23 (38%) of 61 in the placebo group had hen's egg allergy confirmed by OFC at 12 months of age. The risk difference was 29.4% (95% CI 15.3–43.4), the number needed to treat was 3.40 (2.30–6.52), and the risk ratio was 0.221 (0.090–0.543; $p=0.0001$; figure 2). The odds ratio of the two groups was 0.113 (95% CI 0.035–0.361) adjusted for SCORAD score, 0.161 (0.056–0.468) adjusted for

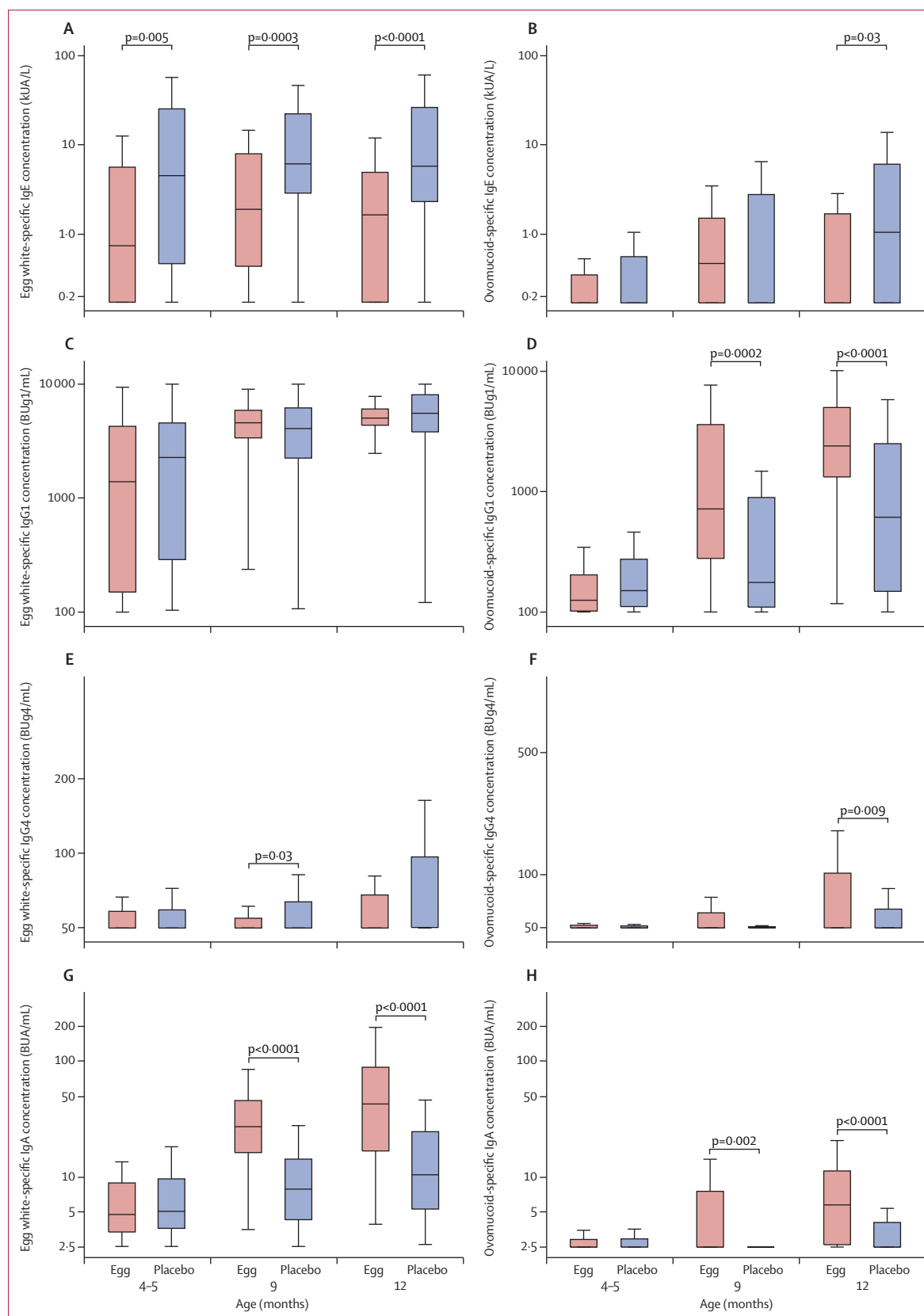


Figure 3: Immunoglobulin concentration
Changes in (A) egg white-specific IgE, (B) ovomucoid-specific IgE, (C) egg white-specific IgG1, (D) ovomucoid-specific IgG1, (E) egg white-specific IgG4, (F) ovomucoid-specific IgG4, (G) egg white-specific IgA, and (H) ovomucoid-specific IgA concentrations. We used Wilcoxon rank-sum tests to compare immunoglobulin concentrations between the placebo and egg groups. * $p < 0.05$.

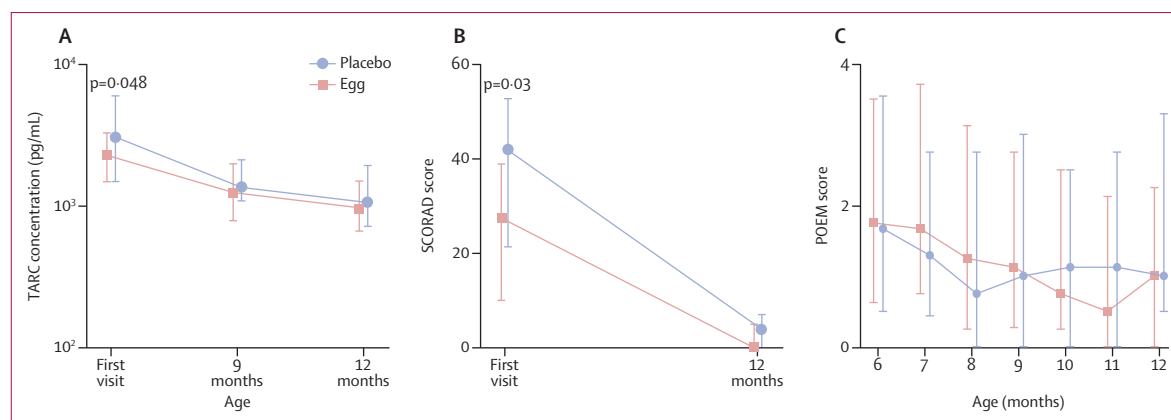


Figure 4: Markers of participants' skin conditions

Change in (A) serum thymus and activation-regulated chemokine concentration, (B) Scoring Atopic Dermatitis score, and (C) patient-oriented eczema measure (POEM) score. We measured serum thymus and activation-regulated chemokine concentration and Scoring Atopic Dermatitis score at the first visit as outpatients. We recorded POEM score during treatment (6–12 months of age). We treated the mean POEM score during 4 weeks as the score for each month. We used Wilcoxon rank-sum tests. Error bars are IQRs. POEM=patient-oriented eczema measure. Error bars are IQRs. SCORAD=Scoring Atopic Dermatitis. TARC=thymus and activation-regulated chemokine. * $p < 0.05$

allergic history of father and mother, and 0.083 (0.023–0.297) adjusted for start of solid foods (appendix). For worst scenario analysis in which withdrawers from the egg group are considered positive for the primary outcome and those from the placebo group as negative, five (8%) of 60 participants in the egg group had confirmed egg allergy compared with 22 (36%) of 61 in the placebo group ($p = 0.0002$). In the per-protocol analysis, two (4%) of 46 participants in the egg group were diagnosed as having hen's egg allergy compared with 19 (38%) of 50 participants in the placebo group (figure 2). The risk difference was 33.7% (95% CI 19.0–48.3) and the risk ratio was 0.114 (0.028–0.464; $p < 0.0001$). The number needed to treat was 2.97 (2.07–5.27) and the relative reduction in the prevalence of hen's egg allergy in the egg group compared with the placebo group was 89%.

Ovomucoid-specific IgE concentration at 12 months of age in the egg group was lower than that in the placebo group, whereas ovomucoid-specific IgG1, IgG4, and IgA concentrations at 12 months of age were higher in the egg group than in the placebo group (figure 3, appendix). Sequential changes of egg white-specific and ovomucoid-specific IgE concentrations of individual participants are shown in the appendix. Egg white-specific IgE and ovomucoid-specific IgE and IgG4 concentrations of participants with egg allergies in the placebo group were increased compared with those without egg allergies (appendix). TARC concentration at 9 months and 12 months of age and SCORAD score at 12 months of age were decreased from baseline in both groups (figure 4). POEM scores during the intervention were low in both groups, suggesting that the eczema was well maintained during the trial in both groups. The two (3%) participants who developed egg allergy in the egg group in the per-protocol analysis had a higher POEM score than did the other participants (appendix).

The only difference between groups in adverse events was the total number of admissions to hospital (table 2). Five (8%) participants in the egg group were admitted to hospital (a total of six times) during the intervention. The number of participants diagnosed as being affected with asthma according to the Japanese guideline's criteria⁴ was not significantly different between groups. Four (3%) participants (three [5%] in the egg group and one [2%] in the placebo group; details shown in the appendix) withdrew from the trial after commencement of the intervention. No participants withdrew because of adverse reactions caused by the trial powder (appendix). No acute sign occurred when participants ingested the trial powder for the first time at the outpatient unit of the hospital at 6 months and 9 months of age. Although a few participants had urticaria 4 h or longer after ingestion of trial powder, skin rash around the mouth, angioedema of the lip, vomiting, transient worsening of eczema, bloody stool, or diarrhoea lasting 10 days or longer after intake of the trial powder at home, we noted no difference in reported numbers between the two groups (19 events in nine [15%] infants in the egg group versus 14 events in 11 [18%] infants in the placebo group). Furthermore, infants who showed acute signs of angioedema of the lip or vomiting were able to continue taking the trial egg powder without developing the same symptom again. Other adverse events were also not different between groups.

We carried out post-hoc analyses to explore the effects of stratification factors and baseline imbalances between the two groups. For the subgroup analysis by stratification with use of the concentration (0.35 kUA/L) of egg white-specific IgE at baseline, in the non-sensitised subgroup, two (8%) of 24 participants in the egg group and three (25%) of 12 in the placebo group were diagnosed as being affected with hen's egg allergy (risk difference 16.7% [95% CI –10.2 to 43.5]; $p = 0.31$; figure 2). In the

sensitised subgroup, three (9%) of 33 participants in the egg group and 20 (43%) of 46 participants in the placebo group were diagnosed as being affected with hen's egg allergy (risk difference 34.4% [17.0–51.7]; $p=0.001$). The subgroup analysis in the per-protocol analysis population showed similar results to those of the subgroup analysis in the primary analysis population (figure 2). We also analysed the primary outcome in the per-protocol analysis population with stratification by the class of egg white and ovomucoid-specific IgE concentration at baseline (appendix). Comparing both groups in the participants of the same IgE class, the positive ratio of OFC in the placebo group tended to be higher than that in the egg group at 12 months of age.

The odds ratio of positive OFC by logistic regression analysis was 0.149 (95% CI 0.052–0.428) adjusted for sex, 0.156 (0.054–0.451) adjusted for TARC concentration, 0.206 (0.068–0.622) adjusted for total IgE concentration, 0.189 (0.063–0.565) adjusted for egg white-specific IgE concentration at baseline, and 0.063 (0.014–0.287) adjusted for mean POEM score from 6 months to 12 months of age in the primary analysis population, and findings were similar in the per-protocol analysis population (appendix).

Discussion

In this trial, we found that hen's egg could be introduced to high-risk infants with atopic dermatitis with use of a two-step approach without immediate allergic reaction, even for participants who had IgE sensitisation to hen's egg before starting the intervention. In a previous RCT¹³ a high proportion (31%) of infants had allergic reactions to pasteurised raw egg powder. In our trial, no participants had an allergic reaction to the heated egg powder. Such a difference might be due to the higher allergenicity of raw than heated egg powder. Use of a lower starting dose than in that trial might also contribute to the safety of our approach. In the LEAP study,¹² six (13%) of 47 infants who were skin prick test positive reacted to baseline peanut challenge. Infants who were already sensitised to egg before introduction might have developed an allergic reaction if they were given a high dose of heated or raw egg powder at the start. These findings suggest that our two-step approach starting from low dose introduction might be a safer method than that used in the LEAP study, which more naturally mirrors real life rather than screening high-risk infants with skin prick test or serum food-specific IgE concentration or a challenge test before introduction.

In our analyses for safety, we noted a difference in the total number of admissions to hospital between groups, but the incidence of urinary tract infection and Kawasaki disease was within the general prevalence found in Japanese children.^{25,26} Admissions to hospital for moderate wheezing attack is common in Japan and the incidence was within the normal range found in Japan.²⁴ The incidence of asthma diagnosed with Japanese guideline's

	Placebo (n=61)	Egg (n=60)	p value
All-cause admission to hospital			
Total number of admissions to hospital	0	5 (8%); 6	0.02
Moderate wheezing attack	0	2 (3%); 3	0.16
Urinary tract infection	0	2 (3%); 2	0.16
Kawasaki disease	0	1 (2%); 1	0.32
Immune system disorders at 12 months of age			
Asthma*	2 (3%); 3	3 (5%); 12	0.68
Food allergy other than hen's egg allergy†	13 (21%); 13	9 (15%); 9	0.48
Cow's milk allergy†	7 (11%); 7	6 (10%); 6	1
Wheat allergy†	3 (5%); 3	1 (2%); 1	0.62
Other food allergy†	6 (10%); 6	3 (5%); 3	0.49
Acute signs after intake of the trial powder at home during the trial			
Urticaria within 4 h after ingestion of trial powder	0	0	1
Urticaria 4 h or longer after ingestion of trial powder	5 (3%); 7	2 (3%); 7	0.27
Rash around the mouth	2 (3%); 3	0	0.16
Respiratory symptom	0	0	1
Angioedema of the lip	0	1 (2%); 2	0.32
Vomiting	1 (2%); 1	1 (2%); 4	0.98
Transient worsening of eczema	0	3 (5%); 3	0.08
Bloody stool	1 (2%); 1	0	0.32
Diarrhoea lasting 10 days longer	2 (3%); 2	3 (5%); 3	0.64
Infections until 12 months of age			
Acute gastroenteritis	20 (33%); 28	15 (25%); 23	0.41
Fever	14 (23%); 27	17 (28%); 35	0.51
Upper respiratory infection	30 (49%); 44	31 (52%); 51	0.60
RS virus infection	0	2 (3%); 2	0.16
Influenza virus infection	1 (2%); 1	1 (2%); 1	0.99
Adenovirus infection	1 (2%); 1	0	0.33
Hand-foot-and-mouth disease	6 (10%); 6	4 (7%); 4	0.53
Herpangina	2 (3%); 2	1 (2%); 1	0.58
Acute bronchitis	1 (2%); 1	1 (2%); 1	0.99
Exanthema subitum	4 (7%); 4	1 (2%); 1	0.32
Acute otitis media	0	3 (5%); 5	0.08
Otitis media with effusion	1 (2%); 1	0	0.33
Impetigo	2 (3%); 2	1 (2%); 1	0.58
Suppurative lymphadenitis	0	1 (2%); 1	0.31
Data are number of patients (%); number of events. We compared groups with Fisher's exact tests and Wilcoxon rank-sum tests. RS=respiratory syncytial. *Defined as more than three events of clinical wheezing. ²⁴ †Defined as a clear clinical history of immediate allergic reaction.			
Table 2: Adverse events			

criteria²⁴ at 12 months of age was not different between groups and wheezing attacks did not occur within 4 h of ingestion of egg powders. For the other safety measures, we noted no significant difference between groups. No

participant withdrew from this trial because of adverse events, substantiating the safety of our approach.

Another important feature of this study was the assessment and treatment of atopic dermatitis prospectively. All participants received optimal eczema treatment throughout the trial, resulting in very low POEM scores.²² The prevalence of egg allergy in this trial was lower than that in a previous study¹³ (33% in the egg group and 51% in the placebo group). In our study, the two participants who developed egg allergy in the egg group in the per-protocol analysis had a higher POEM score than did the other participants. The risk of percutaneous sensitisation to egg might be higher in infants with uncontrolled eczema than in those with controlled eczema.²⁷ However, further RCTs will be needed to test the role of optimal eczema treatment in prevention of subsequent development of food allergy.

Our study was terminated early because of an unexpectedly large group difference at the planned interim analysis, and caution is needed when the results are interpreted. First, the estimated group difference is possibly biased, resulting in a bigger difference between the two groups than if the trial was not terminated early. Second, the two groups had differences in some baseline characteristics, despite our strict masking and randomisation procedures. These differences might be attributed to the incidental difference in severity of eczema, which have affected the concentration of serum IgE and TARC.^{27,28} We examined whether or not the differences at baseline might have affected our results. Reassuringly, the results of the adjusted and subgroup analyses were consistent with those of the unadjusted analysis, except that the subgroup analysis in the non-sensitised subgroup was not significantly different between groups. The imbalance of baseline variables between groups has the potential to affect the results, but our post-hoc analyses revealed that the effect of such imbalance was small. The strength of our study was the excellent adherence, implying the practicability of our approach. The adherence rate in the Enquiring About Tolerance study¹⁴ was only 43%, suggesting that development of high-adherence approaches like ours is needed to improve the efficacy of early introduction of high-risk foods such as eggs and peanuts.

Our trial is the first to show that stepwise introduction of eggs combined with attentive eczema treatment reduces the prevalence of egg allergy in high-risk infants. Our approach also shows the practicality and effectiveness of this approach. Further studies are needed to establish if this stepwise approach might be effective in prevention of other food allergies and in the general population.

Contributors

SK, JN, and YO conceived and designed the trial. YO coordinated the trial. ON, SK, and JN designed the contents of the trial powders. MN handled the trial powder to conceal assignment. TT managed the Data Centre at the National Center for Child Health and Development, including randomisation and concealment of allocation. ON, SK, JN, KY-H, MK, MS, and AK recruited patients and collected data.

HK designed and supervised measurement of antigen-specific IgG and IgA concentration in the serum of participants. EI analysed the data as a statistician. All authors interpreted results. ON, SK, JT, KM, HS, GWKW, and YO drafted the manuscript. KM and YO obtained funding. All authors revised the article for important intellectual content, are accountable for the accuracy and integrity of the report, and provided final approval to submit for publication. YO is the principal investigator of the study.

Declaration of interests

ON and KY-H have Japan Society for the Promotion of Science KAKENHI grants. EI receives payment for lectures from Merck Serono and Tokyo Women's Medical University. GWKW receives payment for lectures from Merck Sharp and Dohme (MSD), Takeda, Mundipharma, and Teva Pharmaceuticals. KM receives payment for lectures from MSD, Ono Pharmaceutical, GlaxoSmithKline, Kyorin Pharmaceutical, Ohtsuka Pharmaceutical, Mitsubishi Tanabe Pharma, AstraZeneca, Siemens Healthcare, Abbott Japan, Sumitomo Dainippon Pharma, Maruho, and Teijin Pharma and receives payment for development of educational presentations from Gifu Pharmaceutical University. HS receives payment for lectures from MSD, Shiseido, GlaxoSmithKline, Kyowa Hakko Kirin, AstraZeneca, Daiichi Sankyo, and Ono Pharmaceutical. YO receives payment for lectures from Maruho, MSD, Kyorin Pharmaceutical, Kyowa Hakko Kirin, Sysmex, Shiseido, and GlaxoSmithKline, and has grants from the Ministry of Health, Labour and Welfare and National Centre for Child Health and Development. MN reports personal fees from Merck Sharp and Dohme, Kyorin Pharmaceutical, AstraZeneca, Miyarisan Pharmaceutical, Mitsubishi Tanabe Pharma, GlaxoSmithKline, and Maruho outside the submitted work. All other authors declare no competing interests.

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References

- Prescott S, Allen KJ. Food allergy: riding the second wave of the allergy epidemic. *Pediatr Allergy Immunol* 2011; **22**: 155–60.
- Wong GW. Epidemiology: international point of view, from childhood to adults, food allergens. *Chem Immunol Allergy* 2015; **101**: 30–37.
- Schroeder A, Kumar R, Pongracic JA, et al. Food allergy is associated with an increased risk of asthma. *Clin Exp Allergy* 2009; **39**: 261–70.
- Dean T, Venter C, Pereira B, et al. Patterns of sensitization to food and aeroallergens in the first 3 years of life. *J Allergy Clin Immunol* 2007; **120**: 1166–71.
- American Academy of Pediatrics. Committee on Nutrition. Hypoallergenic infant formulas. *Pediatrics* 2000; **106**: 346–49.

- 6 Fiocchi A, Assa'ad A, Bahna S. Food allergy and the introduction of solid foods to infants: a consensus document. Adverse Reactions to Foods Committee, American College of Allergy, Asthma and Immunology. *Ann Allergy Asthma Immunol* 2006; **97**: 10–20.
- 7 Kramer MS, Kakuma R. Maternal dietary antigen avoidance during pregnancy or lactation, or both, for preventing or treating atopic disease in the child. *Cochrane Database Syst Rev* 2012; **9**: CD000133.
- 8 Koplin JJ, Osborne NJ, Wake M, et al. Can early introduction of egg prevent egg allergy in infants? A population-based study. *J Allergy Clin Immunol* 2010; **126**: 807–13.
- 9 Katz Y, Rajuan N, Goldberg MR, et al. Early exposure to cow's milk protein is protective against IgE-mediated cow's milk protein allergy. *J Allergy Clin Immunol* 2010; **126**: 77–82.e1.
- 10 Roduit C, Frei R, Depner M, et al. Increased food diversity in the first year of life is inversely associated with allergic diseases. *J Allergy Clin Immunol* 2014; **133**: 1056–64.
- 11 Kull I, Bergstrom A, Lilja G, Pershagen G, Wickman M. Fish consumption during the first year of life and development of allergic diseases during childhood. *Allergy* 2006; **61**: 1009–15.
- 12 Du Toit G, Roberts G, Sayre PH, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J Med* 2015; **372**: 803–13.
- 13 Palmer DJ, Metcalfe J, Makrides M, et al. Early regular egg exposure in infants with eczema: a randomized controlled trial. *J Allergy Clin Immunol* 2013; **132**: 387–92.e1.
- 14 Perkin MR, Logan K, Tseng A, et al. Randomized trial of introduction of allergenic foods in breast-fed infants. *N Engl J Med* 2016; **374**: 1733–43.
- 15 Lack G. Epidemiologic risks for food allergy. *J Allergy Clin Immunol* 2008; **121**: 1331–36.
- 16 Brough HA, Liu AH, Sicherer S, et al. Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitization and likely peanut allergy. *J Allergy Clin Immunol* 2015; **135**: 164–70.
- 17 Lack G, Fox D, Northstone K, Golding J. Factors associated with the development of peanut allergy in childhood. *N Engl J Med* 2003; **348**: 977–85.
- 18 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; **92** (suppl): 44–47.
- 19 Fukuie T, Nomura I, Horimukai K, et al. Proactive treatment appears to decrease serum immunoglobulin-E levels in patients with severe atopic dermatitis. *Br J Dermatol* 2010; **163**: 1127–29.
- 20 Suzuki K, Hiyoshi M, Tada H, et al. Allergen diagnosis microarray with high-density immobilization capacity using diamond-like carbon-coated chips for profiling allergen-specific IgE and other immunoglobulins. *Anal Chim Acta* 2011; **706**: 321–27.
- 21 Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 1993; **186**: 23–31.
- 22 Charman CR, Venn AJ, Williams HC. The patient-oriented eczema measure: development and initial validation of a new tool for measuring atopic eczema severity from the patients' perspective. *Arch Dermatol* 2004; **140**: 1513–19.
- 23 Ito K, Morishita M, Ito A, Sakamoto T, Torii S. [Immediate type food hypersensitivity associated with atopic dermatitis in children]. *Arerugi* 2004; **53**: 24–33 (in Japanese).
- 24 Hamasaki Y, Kohno Y, Ebisawa M, et al. Japanese Guideline for Childhood Asthma 2014. *Allergol Int* 2014; **63**: 335–56.
- 25 Nakamura Y, Yashiro M, Uehara R, et al. Epidemiologic features of Kawasaki disease in Japan: results of the 2009–2010 nationwide survey. *J Epidemiol* 2012; **22**: 216–21.
- 26 e-Stat Portal Site of Official Statistics in Japan. Patients statistics in Japan 2016. http://www.e-stat.go.jp/SG1/estat/GL08020103.do?_toG_L08020103_&listID=000001141596&requestSender=dsearch (accessed Feb 2, 2016).
- 27 Flohr C, Perkin M, Logan K, et al. Atopic dermatitis and disease severity are the main risk factors for food sensitization in exclusively breastfed infants. *J Invest Dermatol* 2014; **134**: 345–50.
- 28 Hill DJ, Hosking CS, de Benedictis FM, Oranje AP, Diepgen TL, Bauchau V. Confirmation of the association between high levels of immunoglobulin E food sensitization and eczema in infancy: an international study. *Clin Exp Allergy* 2008; **38**: 161–68.

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Supplementary appendix

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Supplementary Appendix

Two-step Egg Introduction for preventing egg allergy in High-risk Infants with eczema (PETIT study): a double-blind, placebo-controlled, parallel-group randomised clinical trial.

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1. Authorship

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3. Supplement to the Methods

Procedures

Intervention

During the two-week period before and after six months of age, participants assigned to the egg group started daily ingestion of an egg trial powder consisting of 50 mg heated whole egg powder (Kewpie Corporation, JAPAN), which is equivalent to 0.2 g of whole egg boiled for 15 minutes, mixed with 100 mg of squash powder (Kabotcha powder®, Mikasa Sangyo Co, JAPAN) and 150 mg of glucose. Starting at the same age, participants in the placebo group started daily ingestion of a trial powder consisting of 150 mg of squash powder mixed with 150 mg of glucose.

During the two-week period before and after nine months of age, the quantity of the egg-containing trial powder was increased to 250 mg of heated whole egg powder (equivalent to 1.1 g of whole boiled egg) mixed with 250 mg of glucose, while the placebo powder was increased to 250 mg of squash powder mixed with 250 mg glucose. Participants in both groups were instructed to keep taking the trial powders once a day until they underwent the OFC.

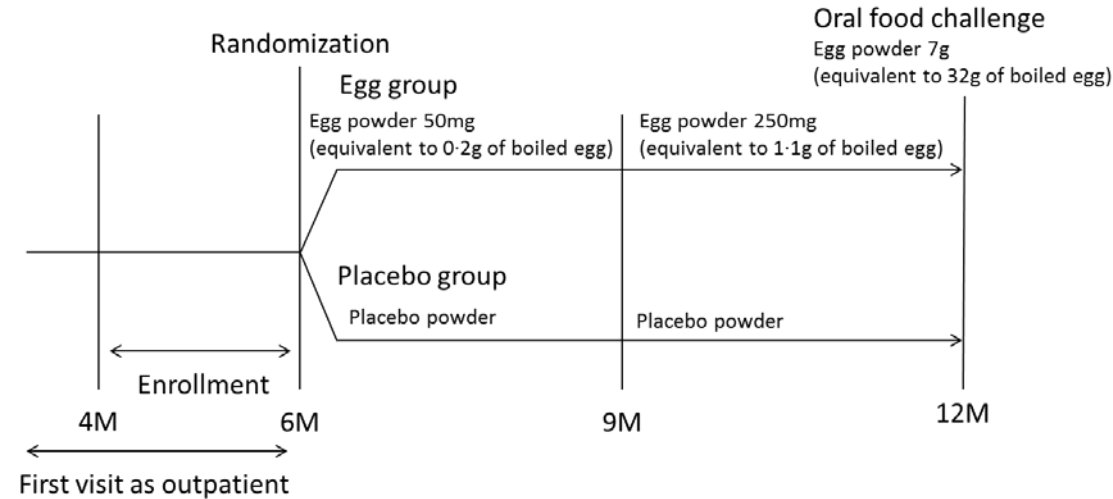
Outcome

Oral food challenge

The primary outcome was the incidence of hen's egg allergy proven by provocation test at 12 months of age. In the open oral food challenge, participants in both groups ingested heated whole egg powder divided with 3 doses every 30 minutes. The amount of the egg powder ingested was 1 g for the first time, 2 g for the second time and 4 g for the third time. The cumulative dose of powder was equivalent to 32 g of whole hen's egg boiled for 15 minutes. If a participant showed any objective immediate allergic reactions, such as urticaria, continuous cough, wheezing, vomiting and/or diarrhea, he/she was diagnosed as having hen's egg allergy.

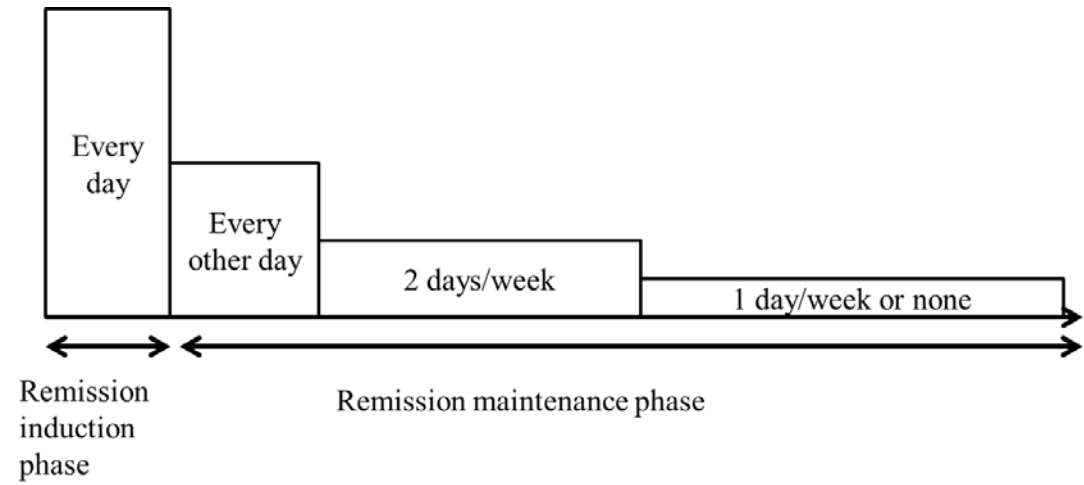
4. Supplementary Figures

Figure E1. Trial design.



Participants were enrolled between four and five months of age. Participants in both groups took the trial powder daily from six to 12 months of age, with an increase in the amount of the trial powder onwards from nine months of age. At 12 months of age, participants in both groups underwent oral food challenge with a total of 7 g of heated whole egg powder.

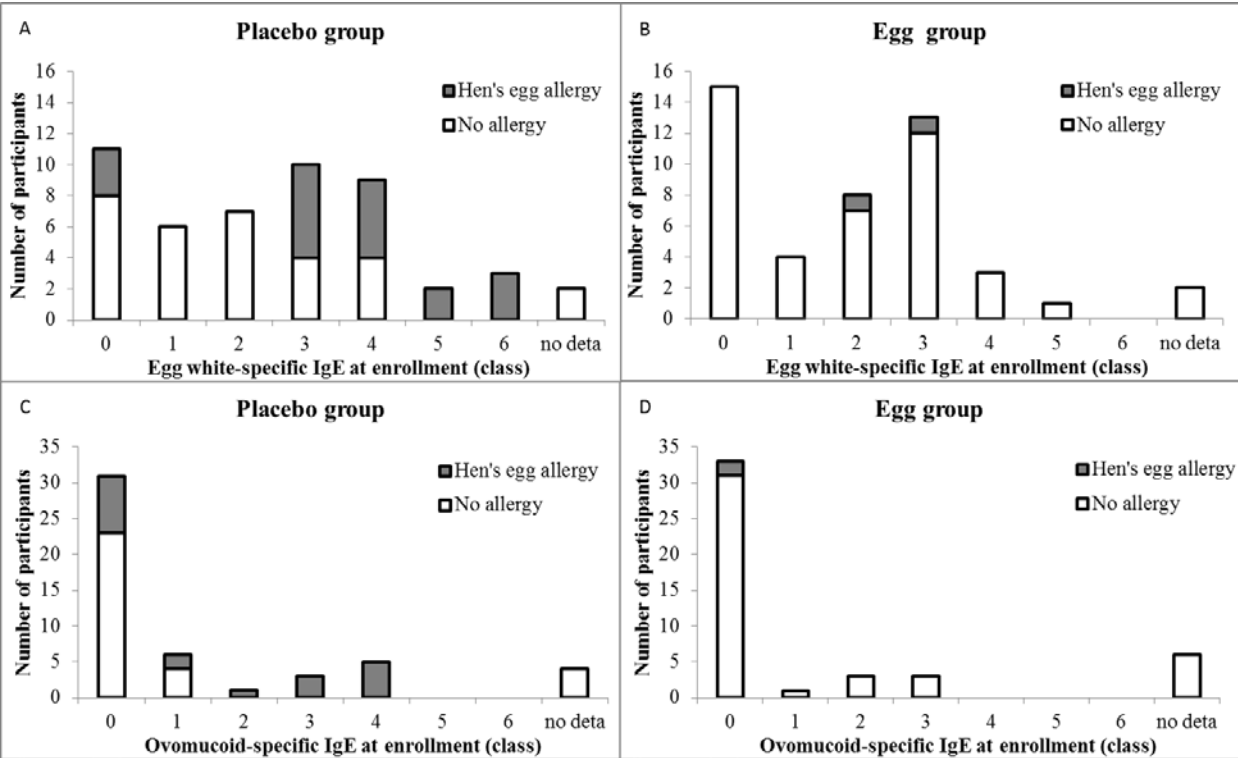
Figure E2. Application of corticosteroid ointment for eczema



Topical Corticosteroid used: 0.1% Hydrocortisone butyrate ointment on face and neck, 0.12% Betamethasone valerate ointment on trunks and extremities

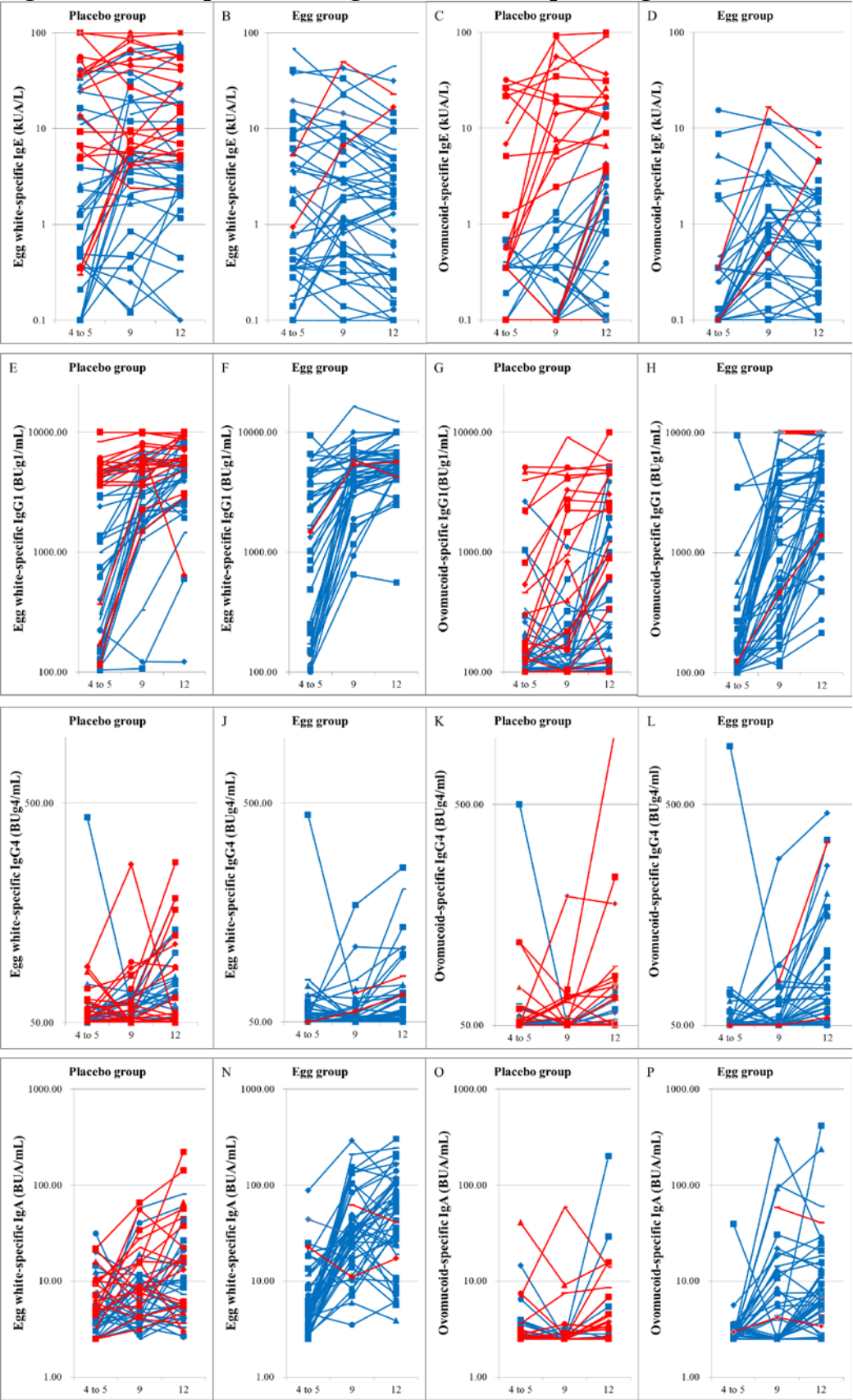
Topical corticosteroids were applied until eczema disappeared and were used intermittently to maintain remission as shown in the figure. Remission was maintained with maximum of steroid applications 2 days a week for most of the participants.

Figure E3. Primary outcome (hen's egg allergy at 12 months of age) stratified by serum egg-specific IgE at baseline (four to five months of age) in per-protocol population.



(A) and (B) were stratified by the class of egg white-specific IgE at baseline (four to five months of age). (C) and (D) were stratified by the class of ovomucoid-specific IgE at baseline. Gray histograms indicate hen's egg allergy at 12 months of age.

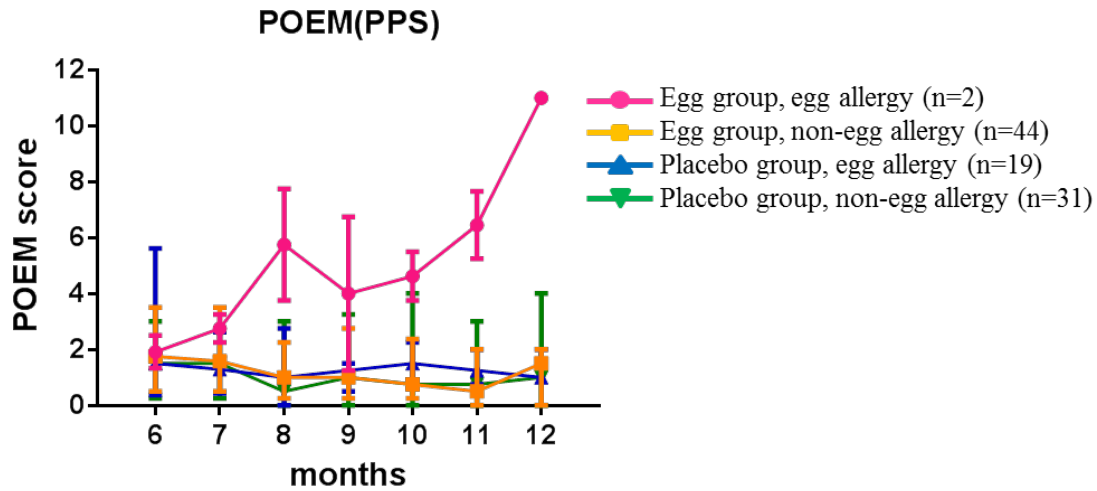
135 **Figure E4. The sequential changes of individual specific IgE level.**



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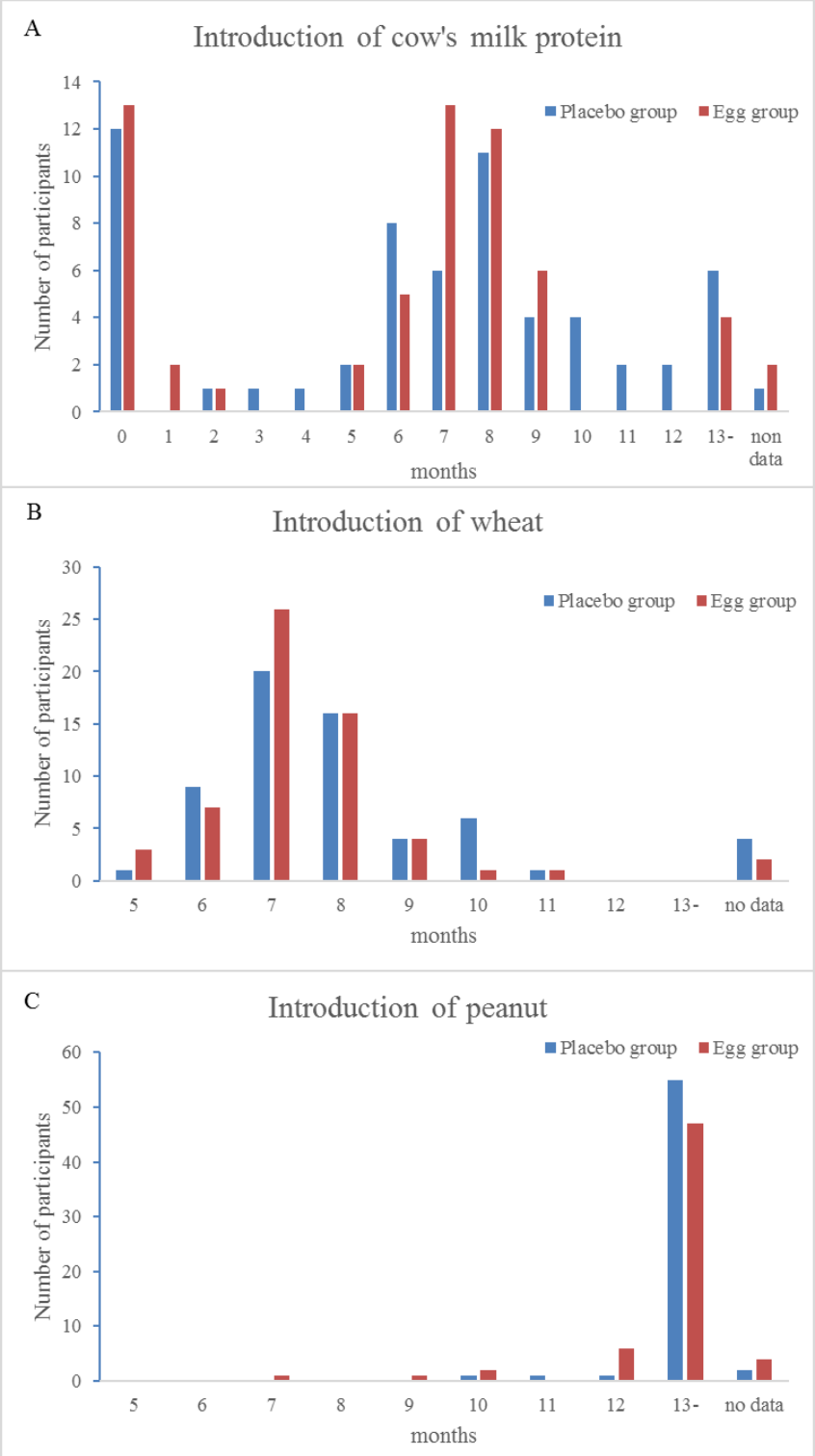
(A) and (B) shows the results of sequential changes of individual egg white-specific IgE level in the per protocol set population. (C) and (D) shows the changes of ovomucoid-specific IgE level in the same population. The results of IgG1 (E, F, G, H), IgG4 (I, J, K, L), and IgA (M, N, O, P) were shown similarly. Red lines represent the results of the participants with egg allergy at 12 months of age. Blue lines represent the result of the participants without egg allergy. Specific IgE was measured with immunoCAP (Thermo Fisher Scientific). Specific IgG1, IgG4, and IgA were measured with a recently established microarray system using DLC (diamond-like carbon)-coated densely carboxylated protein chips.

Figure E5. POEM scores of participants with or without egg allergy



Among the egg group, two Participants with egg allergy showed higher POEM score than the others between 6 and 12 months of age. Some participants in the placebo group developed egg allergy, although their POEM scores were low. Data are shown as the median and the 75th percentile. We used the mean POEM score during four weeks as the score of a particular month.

Figure E6. The timing of the other introduction.



The timing of introduction of the solid foods other than hen's egg was evaluated by questionnaire at enrollment, 6, 9, 12 and 18 months of age. (A) Introduction of cow's milk protein was defined as continuous consumption of non-hydrolysed formula milk or dairy products. If a case was fed with non-hydrolysed formula milk until two months of age and started to eat dairy products from six months of age, the timing of introduction was defined as six month of age. (B) and (C) shows the timing of introduction of wheat and peanut, respectively.

160 **5. Supplementary Tables**

Table E1. Participants who withdrew from the trial.

Allocation	Age at withdrawal (months)	Reason for withdrawal	Allergic reaction caused by the trial powder
Egg group	11	Moved	None
Egg group	11	Refused to undergo oral food challenge for assessment of primary outcome	None
Egg group	10	Stopped taking the powder and started to eat whole hen's egg	None
Placebo group	10	Onset of cow's milk allergy caused voluntarily withdrawing	No

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Table E2. Details of Baseline Characteristics

Characteristic	Placebo group (N=61)		Egg group (N=60)		P value
	Missing value	n (%)	Missing value	n (%)	
Male sex	0	40 (65.6%)	0	39 (65.0%)	1.00
Caesarean section	8	6 (11.3%)	3	8 (14.0%)	0.78
Paternal history of allergic disease	1	34 (56.7%)	3	40 (70.2%)	0.13
Maternal history of allergic disease	1	37 (61.7%)	3	38 (66.7%)	0.57
Having pets from birth to enrollment	2	15 (25.4%)	7	6 (11.3%)	0.088
Smoking in the household	3	12 (20.7%)	4	14 (25.0%)	0.66
Hen's egg elimination by mother during lactation at baseline	4	15 (26.3%)	1	15 (25.4%)	1.00
Continuation of lactation					
at 6 month of age	1	56 (93.3%)	0	57 (95.0%)	0.70
at 12 month of age	2	43 (72.9%)	3	37 (64.9%)	0.35

Comparing both groups by Fisher's exact test

Table E3. Details of Baseline Characteristics

Characteristic	Placebo group (N=61)						Egg group (N=60)						P value
	Missing value	Mean	SD	Median	25%	75%	Missing value	Mean	SD	Median	25%	75%	
Age at enrollment (day)	0	163·4	19·9	168	153	179	0	162·2	21·5	167·5	152	177·25	0·87
Gestational age (weeks)	9	39·1	1·3	39	38	40	4	38·9	1·2	39	38	40	0·44
Birth weight (g)	7	3108·3	325·4	3125	2841	3313	3	3162·8	373·8	3154	2934	3480	0·40
Age at onset of eczema (mo)	5	1·8	1·1	2	1	3	1	2·0	1·1	2	1	3	0·40
SCORAD score	1	36·1	21·0	42	22·1	52·3	2	28·3	20·3	27·5	10·3	38·2	0·030
TARC (pg/mL)	0	5133	6733	3165	1532	5867	0	3053	3340	2297	1525	3250	0·048
Total IgE (IU/mL)	4	146·8	232·2	41·2	15·9	181	3	46·5	110·3	16·5	5·3	51·2	0·00024
Egg white-specific IgE (kUA/L)	3	16·21	25·02	4·46	0·47	24·83	3	5·89	11·90	0·73	0·17	5·55	0·0051
Ovomucoid-specific IgE (kUA/L)	5	3·04	7·63	0·35	0·17	0·56	8	0·98	2·55	0·17	0·17	0·35	0·051
Age at start of solid foods (mo)	3	5·6	0·7	6	5	6	2	5·6	0·7	6	5	6	0·92
POEM at 6 months of age	7	2·9	3·2	2	0·3	4	8	2·1	2·8	1	0	2·3	0·15

Comparing both groups by Wilcoxon rank-sum tests· SCORAD=Scoring atopic dermatitis· TARC=Thymus and activation-regulated chemokine·
POEM=Patient-Oriented Eczema Measure·

Table E4. The details of participants who were positive in oral food challenge to heated whole egg at 12 months of age.

No.	Allocation	Cumulative egg powder dose† at first symptom (g)	Symptoms	Medication	Total IgE at 12 months (IU/mL)	Egg white-specific IgE at 12 months (kUA/L)	Ovomucoid-specific IgE at 12 months (kUA/L)
1	EG	7	V	None	125	22.9	6.32
2	EG	7	PU	None	108	16.7	4.69
3	PG	1	SU, cough	Antihistamine	731	100	18.1
4	PG	1	SU	Antihistamine	250	55.1	31.3
5	PG	3	SU, V	Antihistamine	423	57.1	90.5
6	PG	3	PU	None	86.2	9.98	0.11
7	PG	3	SU	Antihistamine	114	56.2	36.8
8	PG	3	SU, wheeze	Antihistamine, salbutamol	19.5	5.34	8.94
9	PG	4	SU	Antihistamine×2	261	44.9	13
10	PG	6	SU, rash around mouth	Antihistamine	118	40.9	21.1
11	PG	7	PU	Antihistamine	53.5	5.16	0.17
12	PG	7	PU	None	20.6	2.31	1.91
13	PG	7	Partial rash, continuous cough	Salbutamol	452	29.6	26.2
14	PG	7	V, diarrhea	None	4797	100	100
15	PG	7	PU	None	57.8	10.2	0.17
16	PG	7	SU	Antihistamine	131	16.8	13.8
17	PG	7	SU	Antihistamine	116	29.8	20.7
18	PG	7	SU	Antihistamine	29.7	6.05	8.99
19	PG	7	PU	None	44.9	4.72	6.55
20	PG	7	SU	Antihistamine×2	96.8	6.98	4.01
21	PG	7	SU	Antihistamine	107	14.7	3.54
22*	PG	1	SU, V, hypotension	Antihistamine, adrenaline	144	25.6	10.9
23*	PG	7	SU	Antihistamine	13.6	2.06	1.04
24*	PG	7	SU	None	104	19.4	5.6

EG, egg group; PG, placebo group; PU, partial urticaria; SU, systemic urticaria; V, vomiting

*Excluded from per-protocol analysis because they took the trial powder for less than 130 days; †Seven g of heated whole egg powder is equivalent to 32 g of boiled egg.

164 **Table E5. Adjusted analysis of primary outcome by the logistic regression model**

	Factor of adjustment for primary outcome	Odds ratio (95% CI)	P value
ITT	None (crude)	0.150 (0.052-0.430)	0.0002
	SCORAD*	0.113 (0.035-0.361)	0.0002
	History of father and mother	0.161 (0.056-0.468)	0.0008
	Start of solid foods (mo)	0.083 (0.023-0.297)	0.0001
	SCORAD*, allergic history of father and mother, start of solid foods (mo)	0.087 (0.024-0.322)	0.0003
	Gender	0.149 (0.052-0.428)	0.0004
	TARC*	0.156 (0.054-0.451)	0.0006
	Log total IgE†	0.206 (0.068-0.622)	0.0051
	Log Egg White-sIgE†	0.189 (0.063-0.565)	0.0029
	POEM *	0.063 (0.014-0.287)	0.0004
	SCORAD* and log Egg white-sIgE†	0.131 (0.039-0.446)	0.0012
	SCORAD*, log Egg white-sIgE†, allergic history of father and mother, start of solid foods (mo) and POEM *	0.076 (0.014-0.408)	0.0026
PPA	None (crude)	0.074 (0.016-0.342)	<0.0001
	SCORAD*	0.080 (0.017-0.376)	0.0014
	History of father and mother	0.084 (0.018-0.393)	0.0017
	Start of solid foods (mo)	0.069 (0.015-0.320)	0.0006
	SCORAD*, allergic history of father and mother, start of solid foods (mo)	0.087 (0.018-0.411)	0.0021
	Gender	0.074 (0.016-0.342)	0.0009
	TARC*	0.078 (0.016-0.369)	0.0013
	Log total IgE†	0.107 (0.022-0.518)	0.0055
	Log egg white-specific IgE†	0.083 (0.017-0.404)	0.0021
	POEM *	0.080 (0.017-0.372)	0.0013
	SCORAD* and log egg white-specific IgE†	0.082 (0.016-0.419)	0.0026
	SCORAD*, log egg white-specific IgE†, allergy history of father and mother, start of solid foods (mo) and POEM**	0.098 (0.018-0.533)	0.0072

ITT, intention-to-treat analysis; PPA, per-protocol analysis; SCORAD, Scoring atopic dermatitis; POEM, Patient-Oriented Eczema Measure. *evaluated at first visit; †evaluated at enrollment; **average during intervention (6 to 12 months of age)

Table E6. Secondary outcome

	Egg group	Placebo group	P value		Egg group	Placebo group	P value
Egg white-specific IgE (kUA/L)				Ovomucoid-specific IgE			
4 to 5 months of age	0.73 (0.17-5.55)	4.46 (0.47-24.83)	0.0051	4 to 5 months of age	0.17 (0.17-0.35)	0.35 (0.17-0.56)	0.051
9 months of age	1.87 (0.46-7.61)	6.04 (2.84-22.00)	0.0003	9 months of age	0.47 (0.17-1.51)	0.17 (0.17-2.78)	0.86
12 months of age	1.62 (0.17-4.67)	5.70 (2.30-25.78)	<0.0001	12 months of age	0.17 (0.17-1.68)	1.05 (0.17-5.84)	0.025
Egg white-specific IgG1 (BUg1/mL)				Ovomucoid-specific IgG1 (BUg1/mL)			
4 to 5 months of age	1392.94 (150.43-4196.76)	2074.97 (274.87-4424.75)	0.28	4 to 5 months of age	125.05 (101.94-198.97)	150.20 (110.89-273.57)	0.18
9 months of age	4477.97 (3374.49-5852.59)	4001.61 (2196.50-5480.49)	0.36	9 months of age	564.66 (258.83-3233.34)	175.57 (109.76-888.39)	0.0002
12 months of age	4896.63 (4227.33-5781.54)	5033.92 (3337.28-6251.88)	0.77	12 months of age	2132.01 (1294.82-4611.93)	603.97 (148.25-2292.46)	<0.0001
Egg white-specific IgG4 (BUg4/mL)				Ovomucoid-specific IgG4 (BUg4/mL)			
4 to 5 months of age	50.00 (50.00-58.17)	52.55 (50.00-59.16)	0.22	4 to 5 months of age	50.00 (50.00-51.69)	50.00 (50.00-51.28)	0.82
9 months of age	51.21 (50.00-54.75)	52.80 (50.00-63.73)	0.033	9 months of age	50.00 (50.00-60.88)	50.00 (50.00-50.73)	0.18
12 months of age	54.71 (50.00-67.93)	62.22 (50.38-93.10)	0.30	12 months of age	57.05 (50.00-102.39)	50.00 (50.00-63.27)	0.0087
Egg white-specific IgA (BUA/mL)				Ovomucoid-specific IgA (BUA/mL)			
4 to 5 months of age	4.73 (3.37-8.66)	5.03 (3.59-9.60)	0.75	4 to 5 months of age	2.50 (2.50-2.88)	2.51 (2.50-2.94)	0.47
9 months of age	27.15 (16.21-45.65)	7.83 (4.26-14.23)	<0.0001	9 months of age	2.50 (2.50-7.56)	2.50 (2.50-2.50)	0.0021
12 months of age	42.66 (16.75-88.64)	10.42 (5.36-23.67)	<0.0001	12 months of age	5.77 (2.62-11.33)	2.50 (2.50-3.97)	0.0001

Median (IQR). Comparing both groups by Wilcoxon rank-sum tests.

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Table E7. Specific IgE and IgG4 at 12 months of age in placebo group

	Placebo group		P value
	Without egg allergy	With egg allergy	
Egg white-sIgE	3.75 (2.02 -15.15)	18.1 (5.87-47.45)	0.0009
Egg white-sIgG4	65.18 (50.57-86.46)	54.27 (50.00-113.6)	0.71
Ovomucoid-sIgE	0.59 (0.10-2.43)	9.95 (3.13-22.38)	<0.0001
Ovomucoid IgG4	50.00 (50.00-52.94)	60.66 (50.00-83.47)	0.0039

Median (IQR). Comparing by Wilcoxon rank-sum tests.