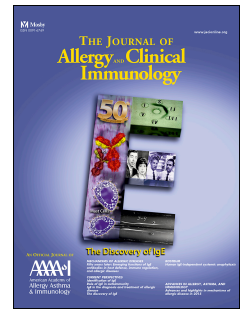


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A randomized trial of egg introduction from 4 months of age in infants at risk for egg allergy.

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ABSTRACT

Background:

Epidemiological evidence suggests delayed introduction of egg may not protect against egg allergy in infants at risk of allergic disease.

Objective:

To assess whether dietary introduction of egg between 4-6 months in infants at risk of allergy would reduce sensitization to egg.

Methods:

We conducted a randomized controlled trial in infants with at least one first-degree relative with allergic disease. Infants with a skin prick test to egg white (EW-SPT) $<2\text{mm}$ were randomized at age 4 months to receive whole-egg powder or placebo (rice powder) until 8-months of age, with all other dietary egg excluded. Diets were liberalized at 8-months in both groups. The primary outcome was EW-SPT $\geq 3\text{mm}$ at age 12-months.

Results:

319 infants were randomized, 165 to egg and 154 to placebo. Fourteen infants reacted to egg within one week of introduction (despite EW-SPT $<2\text{mm}$ at entry) and were unsuitable for intervention. 254 infants (83%) were assessed at 12 months of age. Loss to follow up was similar between groups. Sensitization to EW at 12 months was 20% and 11% in infants randomized to placebo and egg, respectively (OR=0.46, 95%CI 0.22-0.95, $p=0.03$, Chi-Square test). The absolute risk reduction was 9.8% (95%CI: 8.2% to 18.9%) with a number needed to treat of 11 (95%CI: 6 to 122). IgG₄ to egg proteins and IgG₄/IgE ratios were higher in those randomized to egg ($p<0.0001$ for each) at 12 months. There was no effect on the proportion of children with probable egg allergy (placebo 13, egg 8).

Conclusions:

Introduction of whole-egg powder into the diets of high risk infants reduced sensitization to EW and induced egg-specific IgG₄. However 8.5% of infants randomized to egg were not amenable to this primary prevention.

Key words- primary prevention, egg allergy, tolerance

Capsule summary: In a RCT, dietary introduction of egg between age 4-6 months was associated with reduced sensitization and a marked induction of egg specific IgG₄ in infants at risk of allergy.

Clinical Implications:

Epidemiological evidence suggests that delayed introduction of dietary egg may promote rather than protect from egg allergy in infants at risk of allergic disease, as has been recently shown for peanut. We conducted a RCT, which demonstrated dietary introduction of egg between 4-6 months of age was associated with reduced sensitization to egg (SPT) and resulted in marked induction of egg specific IgG₄ at 12 months, in infants at high risk of allergy.

ABBREVIATIONS:

AAP	American Academy of Pediatrics
AE	Adverse Event
AR	Allergic Rhinitis
ASCIA	Australasian Society of Clinical Immunology and Allergy
CI	Confidence Interval
ESPGHAN	The European Society for Paediatric Gastroenterology Hepatology and Nutrition
EW	Egg White
FAS	Full Analysis Set
FPIES	Food protein induced enterocolitis syndrome
NHMRC	National Health and Medical Research Council
OFC	Oral Food Challenge

96	OR	Odds ratio
97	PP	Per protocol
98	PPV	Positive predictive Value
99	RCT	Randomized Controlled Trial
100	SAP	Statistical Analysis Plan
101	SPT	Skin Prick Test
102	WHO	World Health Organization

104 **Introduction:**

105 Egg allergy is common, with an estimated prevalence of 1-9% around the world and the
106 leading cause of IgE-mediated food allergy in Australian children (1) (2) (3). Although
107 spontaneous resolution and tolerance occurs in 60-75% of egg-allergic infants by the time
108 they reach teenage years(4), the burden of the disease is significant in early childhood(5).

109 Early published cohorts examining early introduction of complementary foods within the first
110 4 months of life suggested that early feeding may increase the likelihood of developing
111 allergic disease, but did not specifically examine food allergy(11). Observational cohort data
112 have generally reported no protective effect of delayed introduction of foods such as wheat,
113 egg and milk, and no difference rates of other in atopic disease (asthma, eczema and rhinitis)
114 (reviewed in (7)) or an association between early dietary introduction and reduced food
115 allergy. For egg specifically, two cohorts have described an association between early
116 introduction of egg and reduced egg allergy)(12) (13). Reduction of risk from peanut allergy
117 through early introduction of dietary peanut is supported by epidemiological association(14)
118 and now by robust randomized controlled trial (RCT) findings from the Learning Early
119 About Peanut Allergy (LEAP) study (15). Evidence from the recently published Enquiring
120 About Tolerance (EAT) study(16), with early introduction of multiple allergenic foods within
121 a short time frame, including egg, is less compelling.

122 There are issues with reverse causation in observational cohorts, and several early egg
123 introduction RCTs nearing or just completed, will assist in determining whether early egg
124 introduction plays a role in egg allergy primary prevention. These include STEP
125 (ACTRN12610000388011), HEAP (DRKS00005668) and our own current study.

126 We hypothesised that the introduction of regular egg into the diet of infants who are at high
127 risk of developing allergic disease would result in a decreased sensitization to egg, promoting
128 egg tolerance rather than sensitization in those infants who were not yet egg-sensitized at 4
129 months of age.

METHODS:

Study design:

The BEAT study is a randomized, double-blind, single site, parallel arm, controlled trial conducted in Sydney, Australia (ACTRN-12611000535976). The study was powered to determine whether introduction of dietary egg within the first 8 months of life influenced sensitization to egg at 12 months in infants with a first degree relative with atopic disease. The study was approved by the Sydney Children's Hospital's Network Human Ethics Committee (HREC/10/CHW/4). Written informed consent was obtained from parents.

Enrollment and Study procedures:

Enrollment took place between December 2011 and May 2014. Mothers of newborn infants were approached in postnatal wards after delivery of healthy term infants. Infants with at least one first-degree relative with a history of any atopic disease (food allergy, asthma, atopic eczema or allergic rhinitis (AR) were invited to attend for screening at 4 months of age. Infants with a skin prick test (SPT) to commercial egg white (Stallergenes, France) $\geq 2\text{mm}$ (mean wheal diameter) by lancet method were excluded.

Following screening, infants were randomized (by computer using a permuted block method stratified by sex) to receive daily rice powder (placebo) or 350mg egg protein (given as pasteurized whole egg powder, Farm Pride, Victoria, Australia) from the successful introduction of their first weaning food. With the exception of two dietitians specifically allocated to labelling the study treatments, all other study staff were blind to the treatment allocation. Rice or egg powder were dispensed in identical containers, and not opened by assessing staff. Unblinding (e.g. due to an adverse event) was undertaken for one episode of FPIES (rice) during the study, otherwise allocation remained concealed until the end of study.

Parents were instructed to give their child a strict egg-free diet over the intervention period (4-8 months), and were provided with egg avoidance advice and written instructions. A daily diary was provided to record compliance and any adverse events. Infants were assessed at 8 months and all study participants (except those who had reacted to study powder with IgE-mediated symptoms during the intervention period, or with EW-SPT $\geq 5\text{mm}$) were encouraged to have diets liberalized and introduce egg, as per the Australian National Health and Medical Research Council (NHMRC) Infant Feeding Guidelines. The study intervention was continued until any form of lightly cooked egg had been successfully incorporated into the diet. Blinding allocation remained concealed at 8 months in all subjects.

Clinical assessments, including growth parameters, presence of atopic eczema on examination (by modified Hanifin and Rajika criteria(17)) and eczema severity scores (objective SCORAD(18)) were performed at 4, 8 and 12 months of age. All infants with a history of reaction to the study intervention, egg at dietary introduction after 8 months and/or EW-SPT $\geq 3\text{mm}$ at 12 months of age were invited to undergo an oral food challenge (OFC) to lightly-cooked whole egg (scrambled egg) under medical supervision (cumulative dose 6

grams egg protein, equivalent to one egg, with doses increasing semi-logarithmically at 20 minutely intervals). Staff conducting OFCs were blind to allocation and SPT. Blood samples were collected at 12 months of age.

Immune parameters:

IgG₄ and IgE measurement were performed using ImmunoCAP 250 (Thermo Fisher Scientific) with F245, F1, F233 and F232 (Phadia, ImmunoCAP).

Study Outcomes:

The primary study outcome was the proportion of infants in each group sensitized to egg white on SPT at 12 months (defined as EW-SPT ≥ 3 mm) at 12 months of age.

Secondary outcomes were:

1. Egg allergy at open OFC at 12 months (performed according to international consensus criteria) (19)
2. IgE (whole egg, ovalbumin, ovomucoid), IgG₄ (EW, ovalbumin, ovomucoid), IgG₄/IgE ratio
3. Probable egg allergy at 12 months (defined as)
 - Reacted to egg powder or introduction of egg with typical IgE-mediated symptoms within one hour of ingestion and EW-SPT ≥ 3 mm at 12 months
 - OR reaction to egg OFC at 12 months
 - OR EW-SPT ≥ 5 mm
4. Presence of eczema (as defined above) at 8 and 12 months of age
5. SCORAD at 8 and 12 months of age;
6. SPT ≥ 3 mm at 12 months to commercial extracts for peanut, cow's milk, soy and wheat

Statistical analysis:

The statistical analysis plan (Text E1-online repository) was formulated prior to database lock and study unblinding. The primary outcome was analyzed according to randomized treatment using chi-squared tests on all participants with available data (Full Analysis Set- FAS), and on all infants with outcome data whose parents reported taking the allocation on at least 80% of days (per-protocol- PP). The FAS, is the analysis set which is as complete and as close as possible to the ITT ideal of having primary outcome data on all randomized subjects.

Absolute risk reduction was calculated as risk of not sensitized in egg arm – risk of non-sensitized in placebo arm, and the number needed to treat (NNT) was obtained by calculating reciprocals.

To attempt to account for missing outcome data at 12 months, the primary outcome was also analyzed using logistic regression weighted by the inverse probability of having the observed outcome data. The probabilities were derived from a logistic regression model where selected pre-specified baseline characteristics were tested as predictors of having observed primary outcome data. An additional “pessimistic” sensitivity analysis was performed, in which

missing outcome data were imputed by a random sample from a binomial distribution with probability parameter equal to the sensitization rate in the other treatment arm.

The safety population included all observed adverse events (AEs) in all subjects according to randomized allocation.

Secondary outcomes were analyzed according to randomized treatment in all participants where the relevant outcome was available. There was no adjustment for multiple statistical comparisons. Comparisons between groups used Wilcoxon rank sum non-parametric tests for continuous outcomes and chi-square tests for categorical outcomes. Missing data - baseline characteristics of those infants with missing primary outcome data at 12 months were compared with those in the FAS. Subgroup analysis on the outcomes of EW-SPT ≥ 3 mm and probable egg allergy at 12 months were performed using binary logistic regression to test for any interaction between eczema at 4 months and randomized treatment. Pre-defined exploratory analyzes were performed using binary logistic regression to test for the effects of selected baseline characteristics on the same outcomes and were adjusted for randomized treatment. The planned sample size of 160 patients per group had 80% power at two-sided 5% alpha to detect a difference in sensitization using EW-SPT at 12 months from 15% in the control group to 5% in the egg group with an allowance of 15% for loss to follow-up. All analyzes were performed in SAS, version 9.3.

Study Design Rationale:

Introduction of egg between 4-8 months was chosen as the intervention of interest because of developmental readiness, harmonization (in terms of time points) with other studies investigating early introduction, and following advice from the ethics committee that to prolong avoidance of egg past 8 months of age would not be acceptable. We did not perform OFC at study entry at 4 months for a number of reasons. First, a single large exposure to egg may influence study outcome, and particularly sensitization. Second, it was considered that an oral challenge at 4 months and/or an additional blood collection would be a significant deterrent against participation, and in any event was unlikely to receive ethics approval.

RESULTS:

Study population

The study population is outlined in the consort diagram (Figure 1). Approximately 4000 families were approached, of whom 1434 indicated some interest and 332 attended the initial screening visit. Of the 332 infants screened, 13 were excluded on the basis of EW-SPT ≥ 2 mm, with a median EW-SPT of 6mm (IQR 3.5-9) and 319 infants (166 males) underwent randomization, at a median age of 3.8 months (IQR 3.6-4.0, range 2.7-6.0 months). 26% of infants had eczema evident on examination at randomization. Overall 44% of infants had at least one parent born outside Australasia, with parents born in Greater Asia (South East Asia,

India, China) representing the largest group of those with non-Australasian region of birth. 24% of infants had both parents born outside Australasia. The median age at delivery was 39 weeks, (39.0 egg, 39.1, placebo) and median birth weight 3.4kg (egg-3.3kg, placebo- 3.4kg). Forty-five percent of the cohort was receiving only breast milk at randomization. The baseline demographics of each group are shown in Table 1.

228 (71%) infants had a mother with a history of atopy: AR (45%), asthma (32%), eczema (24%) and food allergy (18%). 197 (62%) infants had a father with a history of atopy: AR (39%), asthma (24%), eczema (19%) and food allergy (11%). In 41% of infants, both parents had a history of asthma, eczema, food allergy and/or AR. 39% of infants had one or more sibling with a history of atopic disease.

The median age of introduction of intervention was 4.0 months (IQR 4.0-4.5) and 4.0 months (IQR 4.0-4.8) for egg and placebo groups respectively, with 75% of infants successfully taking the intervention by 5 months of age. Compliance (>80% of days of intervention from introduction until 8 months) was similar across the two groups: 81% for egg powder and 89% for placebo, in those attending for the assessment at 8 months.

27 infants were randomized but did not receive the intervention at any stage because their parents decided not to participate further (13 egg, 14 placebo). There were 30 infants randomized to receive placebo and 35 infants to egg for whom no data were available at 12 months of age. Baseline characteristics of complete cases (FAS) compared to those lost to follow up/withdrawn/without primary outcome data were not different, except with respect to region of birth of mother and father, where birth outside Australasia of the father ($p<0.001$) or mother ($p<0.02$) was associated with greater loss to follow up at 12 months. All 15 infants who were lost to follow up between the 8 and 12 month visit had EW-SPT of 0mm at the 8 month visit.

13 infants were excluded during screening prior to randomization. Of these 13 excluded infants, 70% had eczema evident on examination.

Study outcomes

Primary outcome: 244/319 infants had primary outcome data available for analysis. There was a reduction in the proportion of infants sensitized to EW at 12 months in those receiving egg (10.7%, 13/122), compared with placebo (20.5%, 25/122) with an odds ratio of 0.46 (95%CI: 0.22-0.95) (FAS, $p=0.03$) (Fig 2). The relative risk reduction was 48% (95%CI: 3% to 72%) and the absolute risk reduction (risk of not sensitized in egg arm – risk of non-sensitized in placebo arm) was 9.8% (95%CI: 8.2% to 18.9%) with a number needed to treat (NNT) of 11 (95%CI: 6 to 122). There was also a significant difference between groups by PP analysis in the 208 infants who had consumed study powder on at least 80% of days and had outcome data available (OR=0.24, 95%CI: 0.09-0.61, $p=0.0015$).

The primary outcome analysis was also significant with inverse probability weighted analysis (OR=0.48, 95%CI: 0.26-0.89, $p=0.02$). The only factor that remained after backward selection was father's region of birth (Australia/NZ born versus elsewhere), where 172/207

(84%) of infants with father born in Aus/NZ had primary outcome data, compared to 74/109 (64%) of infants with fathers born elsewhere (father's region of birth was missing for 3 babies). The sensitivity analysis was performed on the 243 cases with outcome data and known father's region of birth and weighted by the reciprocal of the probability of having outcome data. This gives additional weight in the analysis to babies with fathers born overseas as they are under-represented in the dataset. The results of this analysis (OR=0.48, 95%CI 0.26 to 0.89, P=0.02) were comparable to the unweighted analysis (OR=0.46, 95%CI 0.22 to 0.95, P=0.03).

Secondary Outcomes: Outcome for probable egg allergy determination was available on 253 infants at 12 months (egg, 129 and placebo 124) (figure 1). 21 infants were assessed as having probable egg allergy at 12 months (8.5%). There was no evidence of a difference in probable egg allergy between placebo and egg groups at 12 months when analyzed using FAS (13/124 (10.5%) compared with 8/130 (6.2%) infants respectively, p=0.20). By PP analysis there was a reduction in probable egg allergy at 12 months in those infants receiving egg (11 compared with 2, p=0.013) however this is attributable, at least in part, to the exclusion from the PP analysis of those infants randomized to receive egg who reacted to the intervention within the first weeks and did not, therefore, receive ongoing treatment. Of the 8 infants randomized to receive egg with probable egg allergy at 12 months, 6 had reacted to the intervention (and were unable to continue with their allocated treatment), and 2 infants were compliant.

Of the 21 infants classified as having probable egg allergy at 12 months of age, 7 had confirmed allergy at OFC and 13 had a convincing history of allergic reaction to egg, with an EW-SPT > 3mm. One infant had no exposure; the family refused OFC due to an EW-SPT of 6.5mm at 12 months (Table E1-online repository, for detailed characteristics of probable egg allergy). At 12 months, 205/253 infants had successfully incorporated lightly cooked egg into their diet, while 16 were only having products containing baked egg and 32 were avoiding all egg. We invited parents of all 48 children not regularly ingesting lightly cooked egg at 12 months (irrespective of EW-SPT), or with a history of reacting to the study intervention from 4-8 months or egg on introduction at 8 months to have their infants undertake an egg OFC, but only 21 agreed to this. Of these 48 infants who were not classified as probable egg allergic and/or who did not undergo OFC only 3 had EW-SPT >1mm, and none reported a subsequent reaction at home.

We explored whether the age at introduction of the intervention had any impact on compliance. There was no difference between the age at first introduction of the intervention between those infants deemed compliant in the PP group (median age 119 days, IQR 112-124) and those deemed non compliant (median age 119 days, IQR 114-124), who had outcome data at 12 months.

At 12 months, of the 14 infants randomized to receive egg and who initially reacted to the egg powder (and thus unable to continue with the intervention): 4 (29%) were egg tolerant, 7 (50%) were probably egg allergic, 2 (14%) were avoiding and 1 (7%) was lost to follow up.

Thirty two percent and 37% of infants had eczema on examination at 8 months and 12 months, respectively. There was no difference in the prevalence or severity of eczema between groups at 8 ($p=0.57$, $p=0.98$) or 12 months ($p=0.52$, $p=0.77$) respectively. Most eczema was mild, with a median SCORAD at 12 months in both groups of 7.4 (range 1-31) (Table 2).

The rate of overall sensitization to peanut ($SPT \geq 3$) in the combined cohort at 12 months was high at 8.6%, but there was no difference in peanut sensitization between the two intervention groups ($p=0.49$). 4.5% of infants had a peanut $SPT \geq 5$ mm at 12 months. There were no differences between the intervention groups with regards to sensitization to cow's milk, wheat or soy at 12 months, but absolute numbers were very small ($n=6$, 3 and 1 respectively). There was no significant difference in EW- SPT between infants receiving egg or placebo at 8 months of age, directly following the intervention period ($p=0.69$).

Infants in the egg intervention group ($n=91$) had higher IgG_4 and higher IgG_4/IgE ratios to egg, ovalbumin and ovomucoid, when compared to those infants receiving placebo ($n=93$) ($p < 0.0001$ for all analyzes, Figure 3). There was no significant difference in egg specific IgE at 12 months (Figure E1-online repository).

In our pre-specified subgroup analysis we explored whether having eczema at 4 months of age was associated with any treatment modification effect. Eczema at 4 months was highly associated with both EW- $SPT \geq 3$ mm and probable egg allergy at 12 months ($p < 0.001$); however there was no treatment modification effect. Specifically, there was no interaction between treatment and eczema at 4 months on either EW- $SPT \geq 3$ mm or probable egg allergy at 12 months ($p=0.6$ and $p=0.17$ respectively,).

We explored whether there were any predictors of 12-month outcomes, based upon known baseline characteristics, adjusted for randomized treatment. Eczema at 4 months was the only strong predictor of EW- $SPT \geq 3$ mm (Table 3) or probable egg allergy (Table E2-online repository).

Safety

No SAEs occurred during the study. All IgE -mediated reactions occurred within one hour of ingestion and consisted of urticaria, angioedema and erythema, rhinorrhea, and one instance of vomiting, with no cardiorespiratory involvement and no adrenaline required.

15 infants (4.7%) reacted to allocated treatment within one week of commencing the intervention. 14 (4.4%) were mild to moderate IgE -mediated reactions in infants randomized to receive egg (urticaria, angioedema, vomiting). Families of these infants were advised to stop intervention and continue an egg-free diet. They underwent further clinical assessment and repeat EW- SPT following reaction. One infant randomized to placebo (rice powder) had an FPIES-type reaction (delayed, recurrent, profuse vomiting) and was advised stop the allocated treatment.

Discussion

We have demonstrated that the introduction of daily egg into the diets of infants at risk of egg allergy, from 4 to 6 months of age, was associated with a reduction in egg sensitization on skin testing, and induction of a significant IgG₄ response to egg and the major egg protein components, ovalbumin and ovomucoid at 12 months of age. This suggests a tolerogenic effect for those infants who were able to tolerate egg introduction without having an allergic reaction.

Despite excluding infants who already had evidence of sensitization to egg (on SPT) at study entry (4-6 months), nearly 10% of infants randomized to receive egg powder were already allergic to egg, albeit some transiently, and therefore not amenable to this form of early intervention. For the remainder, there was a beneficial effect in induction of immunological markers of tolerance, although our study size precluded us demonstrating a definitive effect on clinical egg allergy. Whilst infants randomized to egg were less likely to have probable egg allergy at 12 months by per-protocol analysis, we contend that this is a distortion caused by the removal of infants who had early allergic reactions to intervention in the PP analysis, and therefore cannot be used to conclusively show that egg allergy was reduced through early dietary introduction of egg. Importantly the treatment effect was not influenced by presence of eczema at 4 months, which was the strongest predictor in our study for egg sensitization and probable egg allergy at 12 months. This suggests that where tolerated, early egg introduction is likely to be as beneficial in those infants with existing eczema.

Our findings of a significant increase in IgG₄ and IgG₄/IgE are consistent with those of the smaller STAR study, where infants with moderate-severe eczema underwent early egg introduction, irrespective of baseline SPT(20). We enhance this knowledge by demonstrating a similar phenomenon in infants with predominantly mild or no eczema, and by extending the observations to the major egg protein components, ovalbumin and ovomucoid. Additionally, our findings are consistent with those reported for the introduction of other allergenic foods, such as peanut(15).

Importantly, there was no significant harm associated with the introduction of egg in our study. Early introduction of egg was not associated with higher rates of eczema at 8 or 12 months of age, or higher rates of sensitization to other foods at 12 months. No infant had a serious allergic reaction, either at home, or during OFC to egg, despite the use of pasteurised whole egg powder as the intervention. This is in contrast to the STAR study, where several episodes of anaphylaxis were reported. This may relate to the highly atopic study population in the STAR study, where all participants had moderate to severe eczema. In contrast, although the prevalence of eczema at 4 months in our study was high at 26%, it was mostly mild. Moreover, we used lightly-cooked egg as our challenge substance at 12 months of age, in contrast to the STAR study which used raw egg.

There are several useful additional observations that come from our cohort. We confirm a high rate of probable egg allergy and egg sensitization in Australian infants, first documented by the Melbourne-based HealthNuts cohort(1), but not elsewhere. The HealthNuts cohort was

a population-based cohort, whilst we included only infants at high risk, i.e. all participants having at least one first-degree relative with a history of atopy. One would therefore expect higher rates of sensitization and egg allergy in the placebo arm in our cohort, which is indeed the case. In that context, the intervention could be viewed as reducing the overall rate of egg sensitization and allergy burden of a high-risk cohort down to approximately that of the Australian “general” infant population(1). Peanut sensitization, which has significant relevance given current discussions about the nature and extent of possible screening for peanut sensitization in infants at risk of peanut allergy, was 8.7% at 12 months of age, with 4% of infants above the reported 95% PPV for peanut allergy in infants under 2 years of age ($\geq 5\text{mm}$)(21, 22).

Infant weaning is an emotionally charged area, with many rigid views of “when, what and how” being delivered to parents by family elders, clinic nurses, mother support groups and the like. As such, intervention studies, which seek to interfere with what parents had otherwise planned for early infant weaning – especially directly after the first successful solid food introduction – are difficult. Recruiting to such studies is difficult, and the large number of families we needed to approach during recruitment phase illustrates this. The intervention requires significant encouragement and support for participating families; the commitment required from parents make it unlikely to be easily replicated for each different food allergens, across different regions of the world. Within this context, despite our best efforts, we had 20% loss to follow up over the study period. There are several points worth noting. The largest loss, at nearly 10%, occurred between randomization and commencement of study intervention, where no powder at all was given to the randomized infant. This loss was similar between the two study groups, and it may be considered that these infants were lost at random, unrelated to their allocated treatment, and therefore unlikely to have affected study outcomes. The baseline characteristics of those with missing data and those who completed the 12-month follow up were similar except for region of birth mother and particularly father. This is potentially useful in planning future intervention studies, where additional support and more frequent follow-up may be helpful. Losses during the intervention period and after 8 months were equal between the study groups; specific reasons for this are speculative. Infants who were EW-SPT negative at 8 months and who were successfully eating egg at home thereafter may have been less likely to attend the follow-up visit at 12 months, leading to an over-estimate in the rates of sensitization and eczema in both groups at 12 months. Indeed all infants lost to follow up between 8-12 months were EW-SPT negative.

There are several potential weaknesses of this study. Like other primary prevention studies, the parents most attracted to participate may not be representative of the general population, with a higher proportion of well education, middle-high socio-economic bracket parents in this study. These traits, however, are also associated with higher rates of allergic disease, which confounds any attempt to assess the degree to which this skewing might affect the generalizability of the study findings. We were pleased to recruit a relatively high proportion of non-Caucasian infants, which differentiates this cohort in some respects from other studies.

Although the pre-specified sample size was not met for the 12-month primary outcome, this did not affect our primary outcome because of the high rate of sensitization to egg in this

high-risk cohort. We used a relatively small amount of dietary egg, and asked parents to give it daily, rather than only once or twice a week, as would be the likely natural exposure to dietary egg at 4-8 months. The dose was chosen to approximate $\frac{1}{2}$ a small egg/week, which would be in line with recommended daily intakes. Although 350mg of egg protein is relatively small, it is over 5 times higher than the published dose at which 50% of egg-allergic subjects are estimated to react (ED50) (23), which suggests that the active intervention was not acting as de-facto oral immunotherapy at this dose. Moreover, the reactions we observed within the first week of introduction suggest that this is unlikely. Reassuringly, we did not see any serious allergic reactions due to introduction of pasteurised whole egg, unlike reports from the West Australian STAR study and other (as yet) unpublished studies, such as HEAP, where a much higher dose of egg were used. Whilst egg consumption once-twice weekly may reflect actual real life, we felt that compliance with the intervention would be improved with daily dosing. The majority of parents were able to maintain compliance at >80% of daily doses and were able to introduce the intervention within the first month of solids introduction. 75% of infants in this study had the intervention successfully introduced before 5 months of age, in contrast to 55% in the EAT study. EAT, however, required 5 foods to be incorporated (and not one) and used larger amounts of food rather than blinded powder, so the two studies are not directly comparable.

We were unable to challenge all infants with possible egg allergy at 12 months, as many parents declined a further challenge having recently observed an obvious IgE-mediated allergic reaction to egg in their child. It is possible that some infants labelled as “probable egg allergic” may have been able to tolerate egg at the 12 months OFC; however clinical egg allergy was not the predetermined primary outcome for this study. Not all participants agreed to have blood collected at the 12 month visit, therefore the IgG4 and IgE data may not be entirely representative of the whole cohort, however the differences identified between the two groups were very marked.

There is no perfect study design to assess early dietary introduction of allergenic food, which can circumvent the fact that some infants will already be allergic to the food in question. One possible solution could be for all study infants to undergo randomization only after tolerance has been demonstrated (say) to daily egg for one week. However, this degree of exposure to egg could be sufficient for those who are subsequently randomized to placebo to alter tolerance (and thus such infants could not then be considered as early egg avoidant), or more concerning, might increase sensitization and the risk of subsequent clinical allergy. This issue should be kept in mind in designing future primary prevention intervention studies.

We believe our study differs sufficiently from the STEP and HEAP studies, and provides additional useful information about the relative merits of early introduction of egg into the diet of infants and subsequent egg allergy. We targeted those infants not yet significantly sensitized to egg at 4 months, but most at risk of subsequent egg allergy, by deliberately including infants with eczema and with a family history of allergic disease(24). Unlike the STEP trial, we excluded individuals who were sensitized to egg at 4 months, but importantly included infants with eczema. Given that eczema is strongly associated with the development of egg allergy and potentially more severe reactions to egg (20) we feel this is a very

important population in which to offer an intervention, if tolerated. The HEAP trial did not assess egg SPT prior to intervention, and used a far greater amount of egg protein (2.5g thrice weekly) as the study intervention. In addition, a significant proportion of infants in our study had one or both parents born in Asia, a likely risk factor for food allergy in Australian infants(25, 26).

Overall our data suggest that, for infants at risk of egg allergy and who are able to tolerate it, introduction of regular egg immediately following successful first weaning solids, at a low dose, may be beneficial, with no evidence of harm.

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Figure Legends

Legends

Figure 1

Screening, enrollment and participant flow: Screening visits occurred at 4 months of age. All infants had at least one first degree relative with a history of asthma, eczema, allergic rhinitis or food allergy. Infants with a skin prick test to egg white ≥ 2 mm were excluded. All other infants were randomized 1:1 to receive daily placebo or pasteurized whole egg powder (egg protein 350mg) from the first successful introduction of solids until 8 months of age. Diets were liberalized at 8 months of age. Infants were assessed at 12 months with examination, skin prick test (egg white, peanut, cow's milk, soy, wheat) and open oral food challenge.

Figure 2.

The proportion of participants in the placebo and egg treatment groups who had Egg White SPT ≥ 3 mm at 12 months of age. Graph includes all observed data available at 12 month follow up. A total of 144 participants had EW-SPT data at 12 months (122 placebo and 122 egg groups). P value is based upon chi-squared analysis.

Figure 3

The upper panels shows levels of egg-specific IgG4 (KU/L) and the lower panels show ratio of egg specific IgG4/IgE in serum at 12 months from infants who were randomized to received placebo and egg and include all available observations (93- egg, 91 placebo). Results for Egg white/whole egg, ovalbumin and ovomucoid are shown. Wilcoxon rank sum non-parametric tests were used for analysis.

Figure E1

The panel shows levels of whole egg-specific IgGE (KU/L) in serum at 12 months from infants who were randomized to received placebo and egg and include all available observations (93- egg, 91 placebo). Wilcoxon rank sum non-parametric tests were used for analyzes.

Legends

Figure 1

Screening, enrollment and participant flow: Screening visits occurred at 4 months of age. All infants had at least one first degree relative with a history of asthma, eczema, allergic rhinitis or food allergy. Infants with a skin prick test to egg white ≥ 2 mm were excluded. All other infants were randomized 1:1 to receive daily placebo or pasteurized whole egg powder (egg protein 350mg) from the first successful introduction of solids until 8 months of age. Diets were liberalized at 8 months of age. Infants were assessed at 12 months with examination, skin prick test (egg white, peanut, cow's milk, soy, wheat) and open oral food challenge.

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Figure 3

The upper panels shows levels of egg-specific IgG₄ (KU/L) and the lower panels show ratio of egg specific IgG₄/IgE in serum at 12 months from infants who were randomized to received placebo and egg and include all available observations (93- egg, 91 placebo). Results for Egg white/whole egg, ovalbumin and ovomucoid are shown. Wilcoxon rank sum non-parametric tests were used for analysis.

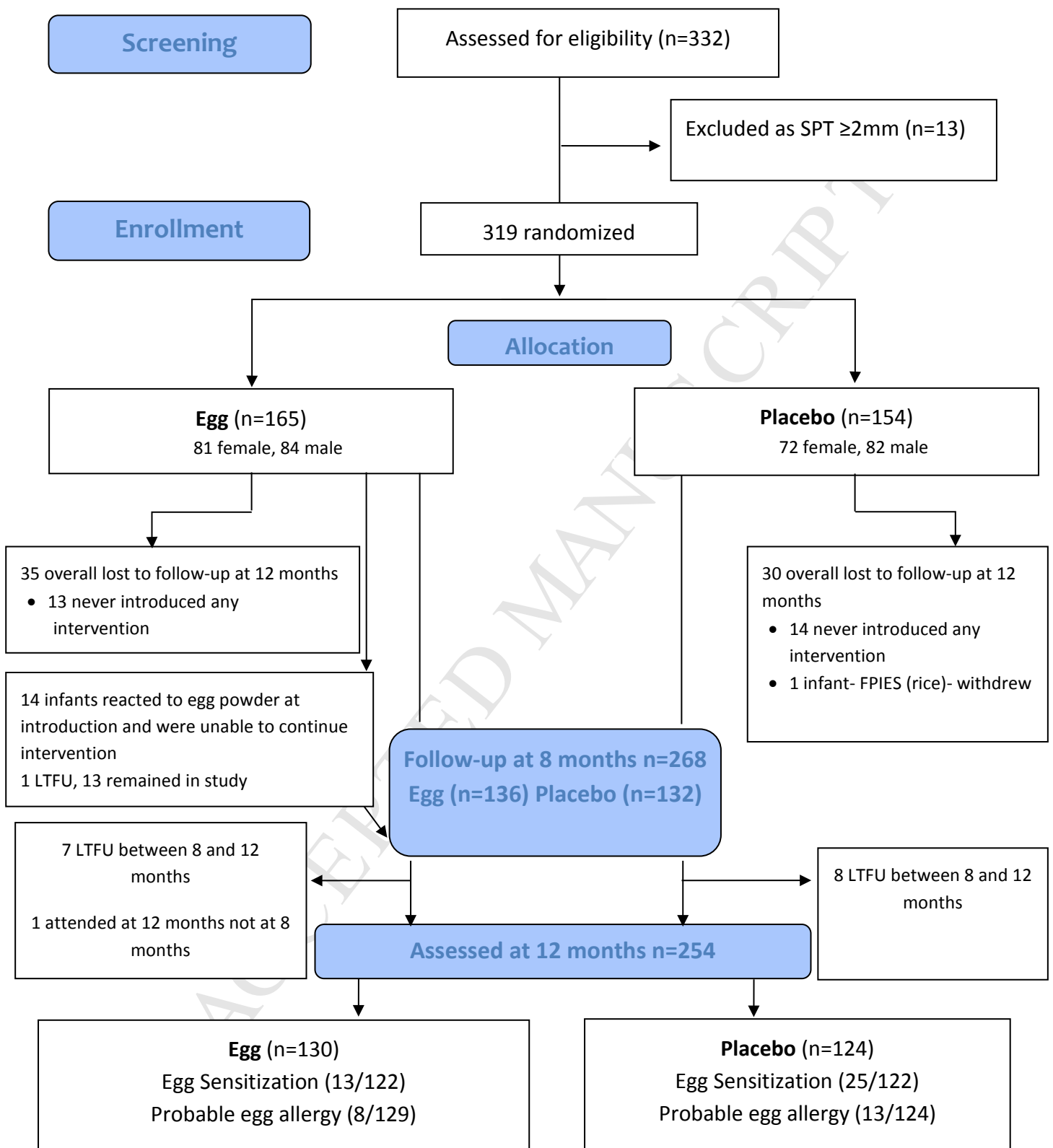


Figure 2

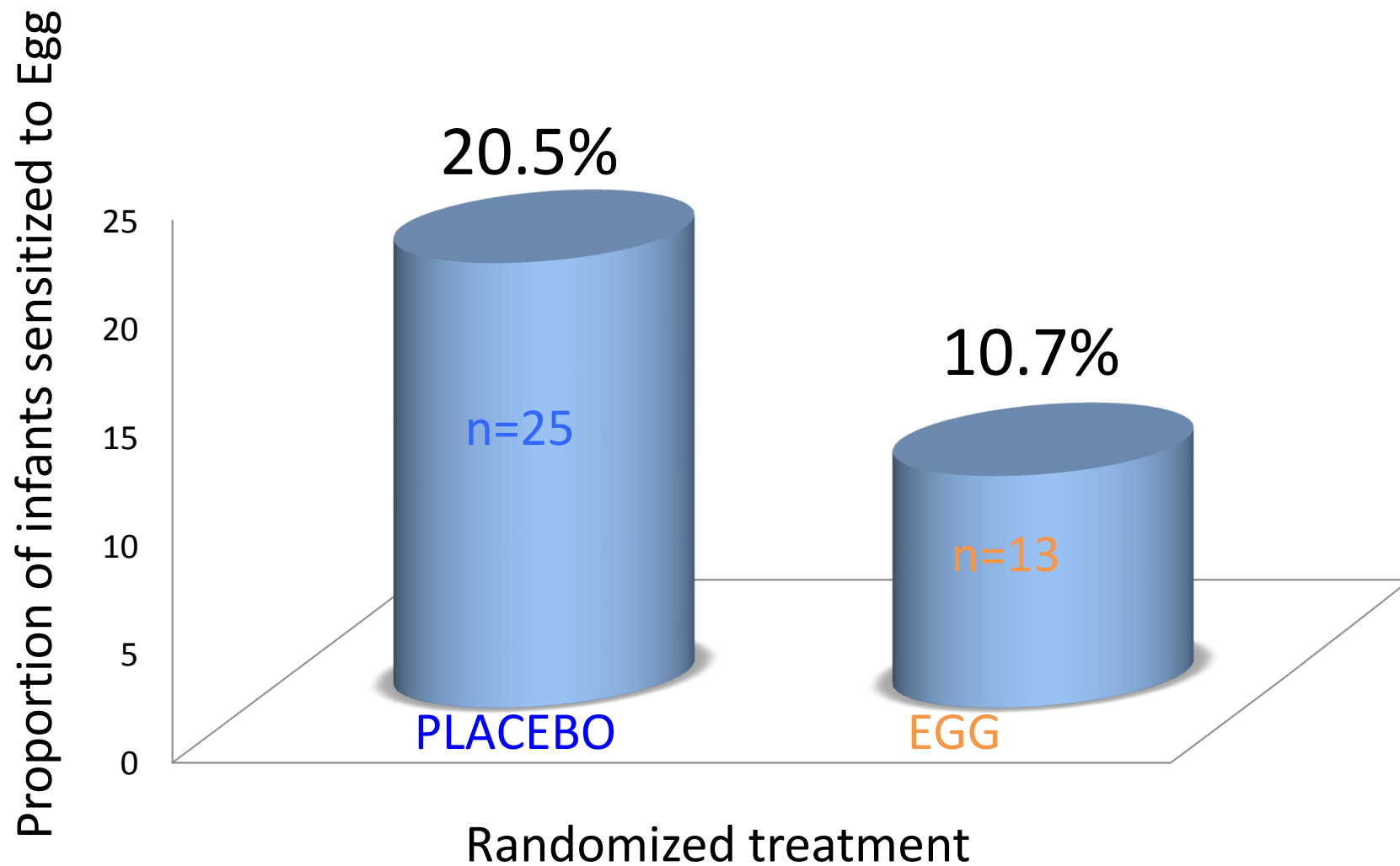
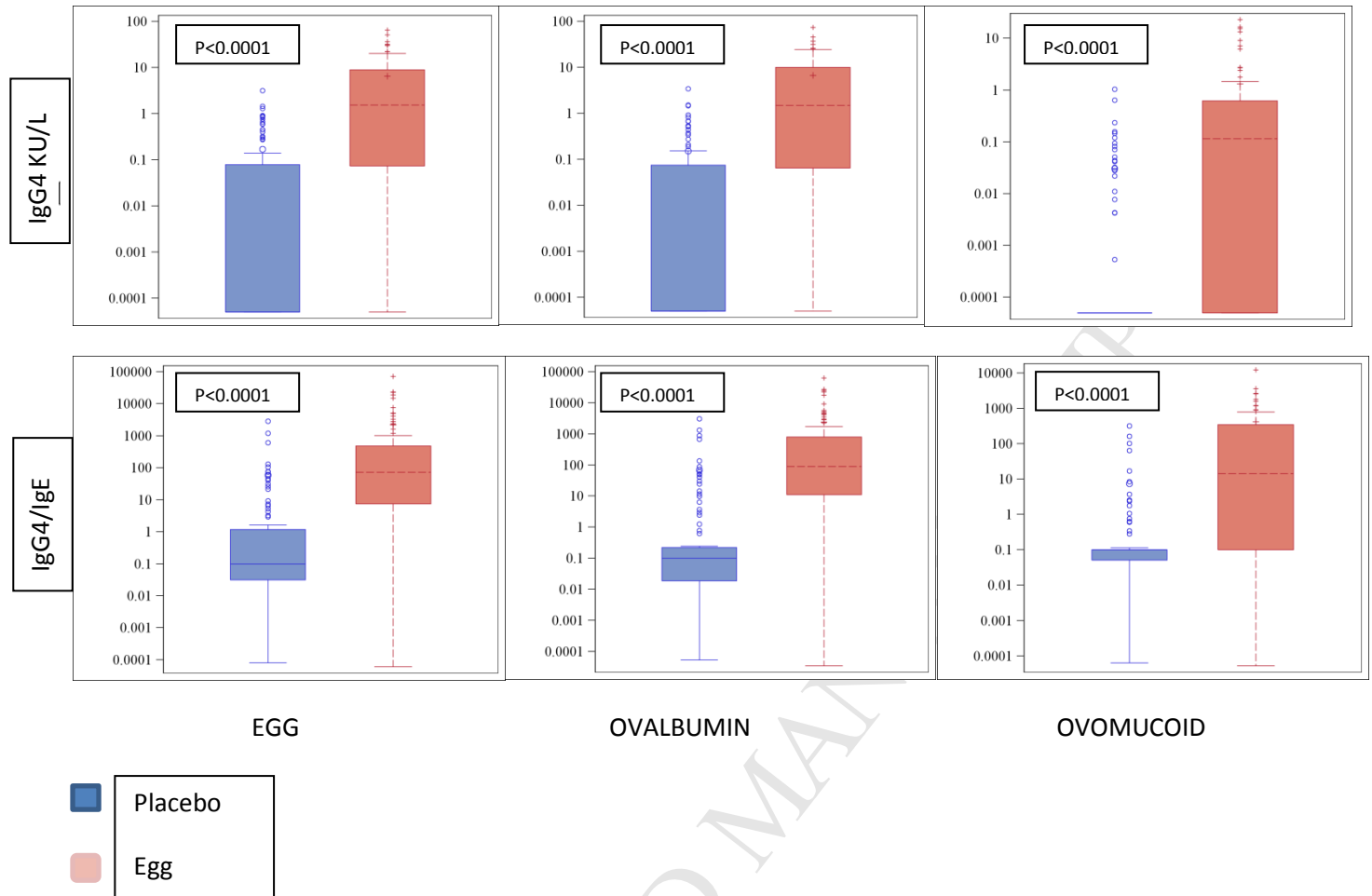


Figure 3- Egg specific IgG4 and IgE at 12 months



E1-Online repository

BEAT (Beating Egg Allergy Trial) study statistical analysis plan

Study design

Randomised double-blind parallel arm study

- **Population:** babies 4 months old, negative to egg white on skin prick test, who have a first degree relative with atopic disease
- **Intervention:** freeze-dried whole raw pasteurized egg powder (0.35g egg protein)per day mixed with water, breast milk or first weaning food daily from 4-6 months of age
- **Control intervention:** rice powder per day mixed with water, breast milk or first weaning food daily from 4-6 months of age
- **Outcome:** IgE sensitisation to egg white measured by skin prick test ≥ 3 mm at 12 months of age

Outcomes

Primary outcome

Reaction to egg white defined by skin prick test greater than or equal to 3 mm diameter at 12 months of age

Secondary clinical outcomes

Clinical egg allergy at 12 months: at least one of the following

- Reacted to study egg powder or egg (parent report, IgE mediated as decided by CHW staff)
- Reacted to oral food challenge in the clinic

Probable egg allergy at 12 months: at least one of the following

- Reacted to study egg powder or egg (parent report, IgE mediated as decided by CHW staff)
- Reacted to oral food challenge in the clinic
- Skin prick test greater than or equal to 5mm at 12 months of age

Egg white skin prick test ≥ 2 mm at 8 months of age

Presence of eczema at 8 months of age

Eczema severity (SCORAD) at 8 months of age

Egg white diameter of skin prick test at 12 months of age

Milk, wheat or peanut skin prick test ≥ 3 mm diameter at 12 months of age

Presence of eczema at 12 months of age

Eczema severity (SCORAD) at 12 months of age

Tertiary and other outcomes

Immunological markers

Safety outcomes/ adverse events

Allergic reaction to powder (egg or rice)

Reaction to oral food challenge

Diary reported outcomes: diarrhoea, eczema flare

Statistical considerations

Analysis principles

All analyses will be performed in SAS Version 9.3.

There will be no adjustment for multiple statistical comparisons.

There will be no direct imputation of missing data but the primary analysis will be performed on the complete cases alone, and on the complete cases with an inverse probability weighting (see details below).

Analysis populations

Full Analysis Set (FAS) – observed data – population

All patients for whom there is primary outcome data (SPT egg white at 12 months), analysed according to their randomised treatment groups.

Per-protocol population

All patients who adhered to study treatment (consumed study treatment on at least 80% of days during the intervention period (4month to 8 month)) and for whom outcome data are available, analysed according to their randomised treatment groups.

Safety population

All randomised patients – all observed adverse events will be described according to randomised treatment

Analysis plan

Baseline characteristics

Baseline characteristics will be described separately in the safety population (all randomised patients) within in each randomised treatment group. Categorical variables will be described by frequency and percentage, continuous variables will be described by median and interquartile range.

No comparison of baseline characteristics between randomised treatment arms is planned.

Comparison of key* baseline characteristics and randomised treatment between randomised patients who have primary outcome data at 12 months versus those without will be performed using chi-square tests or Wilcoxon rank sum (Mann-Whitney) non-parametric tests.

*Baby Eczema, family history food allergy, birth order, region birth father, region birth mother, duration exclusive breastfeeding up to 4 months, SPT egg white diameter at 4 months, gender

Categorical variables:

- Child's gender
- Region of birth of father and mother
 - Australasia, Greater Asia, Europe/US, Middle East, Other
- Highest level of education of father and mother
- Annual income range
- Presence of smokers in the household
- Mode of delivery
- Maternal smoking during pregnancy
- Current feeding
- If formula-fed
 - type of formula,
- Ever-breastfed
- Ever formula-fed
- If ever formula-fed but not currently formula-fed
 - Formula type
- Any food avoidance during breastfeeding
- Introduction of solids
- Diagnosis of reflux
- On thickened formula for reflux
- On anti-reflux medication
- Other children, and if so
 - Number of other children
- Birth order of study participant
- Maternal allergy/atopy
- Paternal allergy/atopy
- Sibling allergy/atopy
- Cats at home
- Dogs at home

- Reaction to food introduced
- IgE-mediated allergy reaction to any food, and if so
 - o List of foods
- Diagnosis of FPIES, and if so
 - o List of foods
- Had scheduled immunisations
 - o Birth
 - o 2 months
 - o 4 months
- Other medical illness, and if so
 - o specify
- On regular medications, and if so
 - o List names
- Eczema evident on examination, and if yes
 - o SCORAD>0
- SPT Egg white $\geq 2\text{mm}$ (note should be excluded)

Continuous variables

- Gestational age (weeks)
- Birthweight (kg)
- If formula-fed
 - o age (months) when started
- If ever breastfed but not currently breastfed
 - o Duration (months)
 - o Duration (months) exclusive breastfeeding
 - o Age (months) when stopped breastfeeding
- If ever formula-fed but not currently formula-fed
 - o Age (months) when started
 - o Duration (months)
- If solids introduced
 - o Age at introduction
- If on anti-reflux medication
 - o Age (months) when medication started
- Weight (kg) – overall and separately by clothed/bare
- Length (cm)
- Head circumference (cm)
- If eczema evident on examination
 - o SCORAD
- SPT Egg white (mm)

Categorical baseline variables that will be tabulated in their raw form but may require recoding or collapsing of categories for publication or analysis

- Postcode
- Country of birth of father, mother and child
- Type of formula (current and ever given)
- Foods avoided during breastfeeding
- First weaning solid
- Other solids introduced
- Name of anti-reflux medication
- Reflux diagnosed – entered as text – needs tidying up
- On thickened formula – entered as text – needs tidying up
- Medication details
- Medical illness specify

Analysis of outcome data

Primary analyses

The primary analysis of sensitisation to egg white at 12 months (SPT greater than or equal to 3mm) will be performed on the FAS by two methods:

- All patients in the FAS will have their primary outcome analysed according to randomised treatment using a chi-square test
- An inverse probability weighted logistic regression analysis will be performed on the FAS population according to randomised treatment.
 - The weights will be the inverse of the probability of having a 12 month egg white SPT value.
 - The probabilities will be obtained from a logistic regression analysis of the binary outcome of having a 12 month egg white SPT value with the “key” baseline characteristics listed above (Baby Eczema, family history food allergy, birth order, region birth father, region birth mother, duration exclusive breastfeeding up to 4 months, SPT egg white diameter at 4 months, gender) and randomised treatment as candidate predictors.
 - Candidate predictors will be tested in univariable logistic regression models and all candidates having p-values less than 0.1 will be included in a multivariable logistic regression with backward selection.
 - The final model after backward selection will be used to generate the predicted probabilities and the area under the ROC (receiver operating characteristic) curve will be calculated.

and on the per-protocol population according to randomised treatment using a chi-square test. The difference in prevalence between treatments will be described by the odds ratio for sensitisation in the active treatment group relative to controls and a 95% confidence interval. A p-value of <0.05 will

be considered significant. Absolute risk reductions and confidence intervals will also be calculated from the chi-square tests to enable comparisons with published studies.

Other clinical outcomes

All other clinical outcomes will be analysed where observed outcome data are available according to randomised treatment. Binary clinical outcomes (all except SPT diameter and SCORAD eczema severity) will be analysed using chi-square tests. SPT diameter and SCORAD will be analysed by the Wilcoxon rank sum (Mann-Whitney) non-parametric test.

Sensitivity analysis population

All randomised patients analysed according to their randomised treatment groups. For patients who are not also in the ITT population (those with missing or unavailable outcome data) the outcome will be imputed according to the with the rate from the other randomised treatment group:

- those missing from the control (rice) group will have their outcome imputed by a random sample from a binomial distribution with probability parameter equal to the observed rate in the active (egg) group
- those missing from the active (egg) group will have their outcome imputed by a random sample from a binomial distribution with probability parameter equal to the observed rate in the control (rice) group

Other outcomes

Blood parameters at 12 months (whole egg IgE, ovalbumin IgE and IgG4, ovomucoid IgE and IgG4, egg white IgG4) where available will be analysed on the FAS (observed) population and the per-protocol populations according to randomised treatment.

Additional derived parameters will be the ratio of IgG4 divided by IgE for egg (egg white IgG4/whole egg IgE), ovalbumin and ovomucoid.

Descriptive statistics will be median, interquartile range, minimum and maximum values for each parameter in each treatment and plotted using histograms and scatterplots.

Comparisons between groups will use Wilcoxon rank sum (Mann-Whitney) non-parametric tests.

Subgroup analyses

SPT 12m ≥ 3 mm and Probable egg allergy outcomes will be analysed in the FAS (observed) population with "Eczema present at 4 months" as a subgroup using binary logistic regression to test for randomised treatment, baseline eczema and their interaction. If an interaction found then treatment effects will be calculated separately according to baseline eczema status using chi-square tests.

Exploratory analyses

Predictors of allergy outcomes: binary logistic regression analyses will be used in the FAS (observed) population to explore the effect of maternal education, presence of smokers in the household, presence of pets in the household and ethnicity on the outcomes of SPT reaction and allergy. These analyses will be adjusted for randomised treatment.

Table- E1- Characteristics of infants classified as probable egg allergic at 12 months of age.

Gender	Tx	Reaction to intervention	possible cofactor	Sibling with FA	Other egg exposure	Eczema at 4 mth	EW SPT 12 mth (mm)	OFC	Other food sensitization at 12 mth	Other clinical food allergy
F	rice	no		no	avoiding	no	4	Yes-reacted	no	
M	rice	no		yes	avoiding	yes	7	Yes-reacted	no	yes
M	rice	no	Anti-reflux med	yes	reaction on exposure cooked egg-generalised	yes	6	Yes-reacted	cows milk, peanut	yes
M	rice	no		yes	Tolerated 1 teaspoon of cooked egg prior to challenge	no	7	Yes-reacted	peanut	
M	rice	no		no	Generalized reaction to egg on introduction at 8 months	yes	4.5	Yes-reacted	peanut	yes
M	rice	no		no	avoiding	yes	1	Yes-reacted	no	
M	rice	no		no		yes	9	Yes-reacted	Cows milk, wheat, peanut	yes
F	rice	no		no	Lip and eye angioedema, facial urticarial and erythema after 1tsp scrambled egg at 13 months. tolerated baked egg in cake	no	5	Not performed	no	
F	egg	Slight peri-oral erythema on initial doses	Anti-reflux med	yes	facial angioedema and widespread urticaria at 8 months	yes	6	Not performed	peanut	
F	egg	Widespread erythema and facial		no	no	yes	7	Not performed	no	

Table- E1- Characteristics of infants classified as probable egg allergic at 12 months of age.

		angioedema on first powder exposure								
M	egg	Immediate facial and trunk urticarial after first dose egg powder	no	no	yes- generalised allergic reaction to subsequent cooked egg exposure	yes	8.5	Not performed	cows milk	yes
M	rice	no	Anti-reflux med	no	11 months- egg containing pancake- immediate erythema, urticarial and angioedema.	no	8.5	Not performed	Wheat, peanut	
M	rice	Mild perioral erythema		no	10 months of age- ingested small amount meringue- urticarial and angioedema.	yes	7	Not performed		
M	rice	constipation		no	Reaction to small amount of cooked egg white at 10 months with facial urticaria.	no	8.5	Not performed	peanut	no
M	rice	no		yes	Accidental exposure to cooked egg at 7 months- lip and eye angioedema, facial erythema, itch and urticaria. Reaction to egg baked in cake 12 months- similar symptoms	no	7.5	Not performed	no	
M	egg	no		yes	Urticaria and angioedema and cough on introduction of small amount scrambled egg at 8 months. Tolerated baked egg in cake. Subsequent	yes	5.5	Not performed	peanut	

Table- E1- Characteristics of infants classified as probable egg allergic at 12 months of age.

					generalised allergic reaction to undercooked egg in pudding.					
M	rice	no		no		yes	6.5	Not performed	cows milk, wheat	yes
M	egg	Facial urticaria with first exposure to intervention, more extensive widespread urticaria following day 2 exposure		no	Tolerates baked egg	no	7	Not performed	cows milk	yes
M	egg	Generalized urticaria and erythema after first exposure, repeated with same reaction after second and third exposure		no	Tolerates baked egg in cake	yes	7	Not performed	no	
M	egg	Facial urticaria with first exposure to intervention, generalized urticaria following one week of ingestion	Anti-reflux med	no		yes	5.5	Not performed	no	
M	egg	Peri-oral reaction on first exposure. Continued but after 2 weeks-generalized urticaria erythema and itch.		no	Reacted to one whole cooked egg at home with facial urticaria and angioedema at 12 months	yes	2	Not performed	peanut	yes

Table E2- Online repository- Effect of baseline characteristics on probable egg allergy at 12 months from multivariable binary logistic regression.

Baseline Characteristic	Value (reference level)	OR(95% CI) relative to reference level	P-value
Highest Education Mother	High School (reference level)	1.00	0.52
	Certificate/diploma	0.61 (0.09 - 4.34)	
	University	1.34 (0.26 - 6.79)	
Smokers in Household	Any smokers in household (versus none)	1.27 (0.35 - 4.52)	0.72
Pets	Dogs or cats in household (versus neither)	2.41 (0.89 - 6.53)	0.084
Parents' region of birth	Both parents born outside Australasia (versus either or both parents born within Australasia)	1.27 (0.39 - 4.14)	0.69
Eczema at 4 months	Eczema observed at 4 months (versus not observed)	6.62 (2.52 - 17.4)	0.0001
Randomized treatment	Egg (versus Placebo)	0.58 (0.22 - 1.58)	0.29