AUSTRALIAN EGG CORPORATION LIMITED

Immunological Changes when Egg Allergic Kids Eat Baked Egg

Final Project Report

A report for the Australian Egg Corporation Limited

by M.J. Netting and I. Penttila

February 2015

AECL Publication No 1WC121A

ISBN 978-1-920835-16-3

Project Title: Immunological Changes when Egg Allergic Kids Eat Baked Egg

AECL Project Number 1WC121

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Published in June 2015

Foreword

Egg allergy affects almost one in ten Australian children. Until recently, children with egg allergy were advised to avoid all forms of dietary egg exposure on the assumption that consumption of egg would prolong their allergy. Recent publications indicate that up to 70% of children with egg allergy can eat egg baked in a cake or muffin without apparent reaction. These publications have prompted some allergy clinics to encourage the introduction of baked egg in the diets of children with raw egg allergy under the premise that this may hasten development of tolerance to raw egg.

The primary aim of this clinical intervention trial was to determine whether raw egg allergy is outgrown earlier in children who regularly consume products containing baked egg, compared with the standard treatment of an egg free diet.

This project was funded from industry revenue that is matched by funds provided by the Australian Government.

This report is an addition to AECL's range of peer reviewed research publications and an output of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Acknowledgments

The CAKE Study was supported by funding from the Ilhan Food Allergy Foundation and the Women's and Children's Hospital Foundation. Merryn Netting received an MS McLeod PhD Scholarship.

We thank the children and families who participated in the study as well as the staff of the Child Nutrition Research Centre, the WCH Medical Day Unit and the WCH Department of Allergy and Clinical Immunology.

The Australian Egg Corporation Limited provided the funds that supported the extensive immune analysis of this project.

About the Authors

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Abbreviations

aOR	Adjusted odds ratio
BE	Baked egg
EW	Egg white
EY	Egg yolk
FPIES	Food Protein Induced Enterocolitis Syndrome
IgE	Immunoglobulin E
IgG_4	Immunoglobulin G4
IL	Interleukin
IQR	Interquartile range
kUA/L	Kilounits of antibody/litre
OFC	Oral food challenge
OR	Odds ratio
OVA	Ovalbumin
OVM	Ovomucoid
mgA/L	Milligrams of antibody/litre
PHA	Phytohemagglutinin-L
RCT	Randomised controlled trial
SOTI	Specific oral tolerance induction
SPT	Skin prick testing
TGFß	Transforming growth factor ß
WCH	Women's and Children's Hospital, Adelaide
WCHN	Women's and Children's Health Network
WCHRI	The Women's & Children's Health Research Institute, Adelaide
WE	Whole egg

Executive Summary

Background

Many egg allergic children tolerate baked egg (BE) before they tolerate less well-cooked forms of egg. Inclusion of BE in their diets is now an accepted clinical practice and is associated with changes in immune markers (skin prick test, egg specific IgE and IgG4 levels) suggestive of developing tolerance. However, the effects of this change in clinical practice have not been tested using a randomised controlled trial.

Objective

This randomised controlled trial (RCT) aimed to compare clinical and immunological outcomes after six months consumption of BE with an egg free diet in 1-5 year old BE tolerant, but raw egg allergic children.

Methods

Children were recruited at the Women's and Children's Hospital, Adelaide, Allergy Clinic and randomised into two groups. The intervention group consumed 10g BE per serve of the provided muffins, biscuits or cake, two to three times per week for six months. The control group consumed identical egg free products. Both groups maintained egg free diets at home during the trial. The final assessment was a medically supervised raw egg oral food challenge (OFC) at 7 months. Immune markers, including skin prick testing (SPT), egg specific IgE and IgG4, Th1/Th2 cytokines and T cell phenotype were assessed at baseline and 7 months.

Results

Forty-three children were randomised into the study (intervention group n=21; control group n= 22). The final analysis included 35 children (intervention group n=17; control group n=18) who had raw egg OFCs. Ten children (4/17 intervention group and 6/18 control group) tolerated raw egg at the end of the intervention. Tolerance was independent of age and the amount of BE consumed. Both groups demonstrated decreased SPT wheal sizes and whole egg, egg white, ovalbumin specific serum IgE titre and increased whole egg IgG4. No difference between the groups was observed in the percentage of naive (CD4+ CD45RA+), central ($CCR7^{-}CD45RA^{-}$) or effector ($CCR7^{+}CD45RA^{-}$) memory T-cells or cytokine excretion after culture of cells with egg allergens.

Overall Conclusions

The results of the RCT suggest that baked egg tolerant 1-5 year old egg allergic children are evolving tolerance to raw egg, which may not be influenced by short-term, regular inclusion of BE. Further trials of larger sample size, including children of different age groups are required.

1 Introduction

1.1 Background

Hen's egg allergy is one of the most common IgE mediated food allergies in children (1), and carries a significant burden in terms of cost to the health system and the quality of life of families and individuals with egg allergy (2). Egg allergy is usually outgrown in early childhood, however, recent evidence suggests an increasing persistence of egg allergies with only 50% resolution of allergy to raw egg by ten years of age (3). Specific oral tolerance induction (SOTI) protocols using raw egg are effective in desensitising egg allergic children, but are associated with high levels of adverse events and may not achieve sustained tolerance (4). SOTI is associated with increases in protective IgG (1 and 4) and IgA, a decrease in IgE and a shift in the Th1/Th2 balance towards Th1, along with a decrease in T cell proliferation and cytokine responses to allergens. Importantly there are increases in regulatory T cells along with an increased production of IL-10 and TGFß, markers linked with the development of oral tolerance (5).

Many egg allergic children tolerate baked egg (BE) before less well-cooked forms of egg (3, 6) as heating causes structural changes in some egg epitopes and digestibility of the egg proteins are reduced when heated with wheat (7, 8). Regular consumption of BE by egg allergic children for as little as 3 months is associated with similar immunological changes observed during SOTI (decreasing skin prick test (SPT) wheal sizes to egg white, and egg white serum specific IgE levels, and increasing ovalbumin and ovomucoid specific IgG4 levels) (6, 9), and individuals consuming BE appear to gain tolerance to lightly cooked egg earlier than those who do not consume BE (10-12).

The observations related to the changes in immune markers of allergy led to supposition that consumption of BE and other heat denatured proteins by allergic children could be used to promote tolerance to uncooked proteins, in a safe and palatable manner (8, 9). As we reviewed (13), support for this concept also comes from the work of Nowak-Wegrzyn et al. (14) who reported that baked milk tolerant, cow's milk allergic children consuming baked milk products for 3 months had significantly smaller SPT and higher casein – specific IgG4 compared with baseline. On repeat challenges with uncooked cow's milk, heated cow's milk tolerant children outgrew their milk allergy quicker compared with a group who did not tolerate heated cow's milk in their diet. Studies using murine models of allergy demonstrated that heat treatment of ovalbumin affects the intestinal absorption of its intact form, which is capable of triggering basophils and effector T cells (8), and heated egg protein (ovomucoid) is able to desensitise egg allergic mice as effectively as unheated ovomucoid (11). In egg and cow's milk, allergic children's consumption of BE or baked milk is associated with improved quality of life scores (15) and does not affect growth or intestinal permeability (assessed by measurement of urinary clearance of non-metabolised sugars), and although eosinophilic oesophagitis was reported in baked milk studies it was not reported in BE studies (16).

Although both the baked egg and milk studies by Lemon-Mulé et al. and Nowak-Wezgryn et al. (6, 14) are promising, neither trial was an intervention study comparing outcomes in children matched for tolerance to the baked protein, nor did they compare tolerance to the raw protein at the end of the intervention. Results from the BE study group were compared after 6 years with a retrospective comparative group, matched for age, SPT results and clinical history, but not BE tolerance (17). Challenges to raw egg were not performed as it is not generally encountered in children's usual diets (6). However, raw egg OFCs are routine in many countries and give an indication of the overall egg allergy status of the child (1, 18-20). This is in contrast to OFC with cooked egg, as children may be tolerant to cooked egg,

but still react to a raw egg (for example, gelato with raw egg white or uncooked cake mix) or a food that contains egg that is only partially cooked (e.g. an omelette or a soft boiled egg) (3). The baked milk trial compared baked milk tolerant children with those not tolerant to baked milk, and milk allergic children who gained tolerance to baked milk during the study period (14).

The clinical effects of consumption of BE have not been compared using a randomised controlled trial methodology, and the immune changes that occur after ingestion of BE have not been fully investigated. It is unclear if ingestion of heated egg affects the natural history of egg allergy when compared with strict avoidance, or if the effects are related to selection of a group of children moving towards natural resolution of their allergy, changes with time or other unidentified confounders. Despite this, inclusion of BE in the diet of egg allergic children, when tolerated, is now accepted clinical practice (21, 22), which is a change in management paradigm from advising complete dietary avoidance of egg (23, 24).

1.2 Objectives

This study was designed to test the hypothesis that regular consumption of BE by children with raw egg allergy will hasten the resolution of the allergy to raw egg.

The aims of this study were:

1. To determine whether allergy to raw egg is better resolved by regular consumption of BE (intervention group, baked egg exposure) compared with the standard practice of an egg free diet (control group, egg avoidance).
2. To examine the effect of regular BE exposure on immunity, particularly on patterns of evolving allergen-specific responses.

The strength of this study is the focus on a well-defined patient group, ensuring a specific outcome able to be translated into practice.

2 Methods

2.1 Study Design

Children aged between 0.5 to 5 years with IgE mediated egg allergy who were not already consuming BE were recruited from the Allergy Clinic at the Women's and Children's Hospital (WCH), Adelaide, Australia. Ethics approval was obtained from the Women's and Children's Health Network (WCHN) Human Research Ethics Committee (REC2400/9/14) and the trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN 12612000173897). Written informed parent/caregiver consent was obtained prior to trial participation. The study was a randomised, double blind, controlled trial with a 6-month intervention period.

Children were excluded if they: were older than 5 years, 11 months of age; had parents or caregivers unable to provide informed consent; had non IgE mediated egg allergy; had IgE or non IgE mediated wheat allergy; had Food Protein Induced Enterocolitis Syndrome (FPIES) to any foods; had any congenital, acquired or developmental disorder likely to affect their ability to undergo an OFC.

All children had SPT to egg allergens (whole egg, egg white, egg yolk, ovalbumin and ovomucoid). To be eligible for the trial, demonstrated tolerance to BE was required. This was determined via a medically supervised BE OFC (muffin, containing 10 grams egg) according to standard protocol (25). BE tolerant children with egg white skin prick testing <5mm (6 months to 2yo) or <8mm (2 to 5yo), who had not had clinical reactions to raw egg in the previous 12 months had a raw egg OFC according to standard protocol (26) to confirm the existence of an egg allergy. A peripheral blood sample was also collected to measure whole egg, egg white, ovalbumin and ovomucoid serum specific IgE and whole egg specific IgG4 and functional cell response profiles. The specific IgE and IgG4 were analysed at the completion of the trial, and cell culture experiments (maintaining blinding of the study group allocation) were performed in batches as the trial progressed.

2.1.1 Randomisation and Blinding

Each child was allocated a unique identification number and randomly assigned to either the intervention group or the control group using a computer-generated randomisation schedule generated by an independent consultant. The schedule was stratified by age (6 months to 2 years 5 months, and 2 years 6 months to 5 years 11 months). A research assistant (who had no contact with the study participants and who was not involved in any of the outcome assessments) was responsible for the baking and coding of identically packaged egg and egg free dietary products for the trial.

2.2 Dietary Intervention

The study compared the effects of inclusion of egg/egg free baked products in the diet of egg allergic children for 6 months after randomisation. Both randomisation groups maintained an egg free diet at home and were provided with muffins, biscuits (cookies) or cake to be offered to the child by parents or caregivers for consumption two to three times per week for six months. The intervention group consumed the equivalent of 10g BE (approximately 1.3g egg protein) per serve. The control group consumed egg free products identical in terms of appearance, taste, and texture to the intervention group.

To assess compliance with the intervention caregivers maintained an intake and symptom diary. Participants were reviewed in clinic one month after randomisation and telephoned monthly for the study duration.

After 6 months, children ceased consumption of the baked product and continued to follow an egg free diet for an additional month, to differentiate between desensitisation and development of sustained unresponsiveness to egg (27).

2.3 Outcome Assessments

One month after the ceasing the study intervention, the children returned for a final assessment. At this visit children had repeat SPT and blood sampling. To assess tolerance to raw egg, the children had a medically supervised, graded OFC to pasteurised whole raw egg (26). For children with previous history of anaphylaxis to egg a modified protocol was followed, with a slower dosing regimen and an intravenous line in situ.

A positive reaction to an egg challenge was defined by the development of symptoms within 2 hours of the egg challenge, and included at least 3 concurrent non-contact urticarial lesions persisting for at least 5 minutes and/or generalised skin erythema and/or vomiting and/or anaphylaxis (as defined by multi-system involvement, which included circulatory and/or respiratory involvement) (28). Serious adverse events were reviewed and reported to the Human Research Ethics Committee.

After the 7-month assessment all children who failed the raw egg OFC were offered another BE OFC, performed while the group allocation was still blinded, to ensure tolerance to BE was maintained during the intervention.

2.4 Analysis of Immune Subsets and Cytokines

Peripheral Blood Mononuclear Cells (PBMCs) (10^6 cells/ml) isolated from peripheral blood samples collected at baseline and at 7 months were cultured with egg allergens ovalbumin (OVA) and ovomucoid (OVM) (Sigma-Aldrich, Sydney, Australia) to a final concentration of 100µg/ml for five days, isolated, labelled with fluorescent-tagged antibodies and analysed by flow cytometry (BD Biosciences FACS Canto, Becton Dickinson, CA, USA). Phytohemagglutinin-L (PHA) (Roche Diagnostics, Australia or Remel, KS, USA) was used as a positive control in all cell culture experiments. For the immunophenotyping, cells were assessed at baseline for CD4, CD8, CD14, CD19 and HLA DR expression. To assess activation, cells were labelled with CD69 at baseline and after incubation with OVA and OVM. To assess memory cell phenotype, at baseline and after incubation with OVA or OVM, cells were also assessed for CD45RA, CD45RO, CCR7, CD27 and CD28 expression.

The cytokine concentration in the resultant supernatants of cells after incubation with OVA and OVM was assessed by flow cytometry using a BD Cytometric Bead Array Human Inflammatory Cytokine Kit (Interleukin (IL) 8, IL 1, IL 6, TN.F, IL 12 and IL 10), and BD Biosciences Human Enhanced Sensitivity Flex sets for IL 4, IL 5 and IFNγ. Data were analysed using BD FacsDiva™ software version 6.1.3 (BD Biosciences, CA, USA).

2.5 Statistical Analysis

A sample size estimate was calculated based on the known natural history of egg allergy, expecting after six months of treatment with an egg free diet that 90% of children would still be egg allergic (3). We hypothesised regular exposure to BE would result in 30% absolute reduction (i.e. from 90% to 60%) of egg allergy. To detect such a difference with 90% power and p=0.05, we estimated we would need 49 children per group (total n=98) and aimed to recruit 55 children to each group to allow for withdrawals from the study.

Analyses were performed according to the randomised group, as data were available using STATA13 (StataCorp LP) or the InStat program v 6.05 (Graph Pad software, USA). Statistical significance was assessed at the 0.05 level.

The proportion of children with diagnosed IgE mediated egg allergy at the end of the intervention was compared between groups. Secondary comparisons between groups included changes in SPT wheal size, specific IgE and IgG4 results and other immune outcomes.

For skin prick test, specific IgE and IgG4 results, standard linear regression was performed including baseline level as a covariate to ensure that estimated differences between groups were not biased due to differences in baseline wheal size and/or regression to the mean effects, and for 'adjusted' analyses, age stratum was also included. In all cases, sensitivity analyses (removal of outlying/influential observations) were undertaken, and these did not affect the conclusions. Change between groups was assessed using the Wilcoxon Rank-Sum Test.

For the other immune outcomes, a non-parametric approach was used for the analysis because of the highly skewed distributions of all variables and small sample sizes.

3 Results

Enrolment for the trial commenced in May 2012 and ceased at the end of January 2014. The final 7-month follow up appointment was completed in October 2014.

The study flow is shown in Figure 1. Forty-three children were enrolled and randomised into the study:

- 21 into the intervention group: n=13 (0.5-2.5 yrs); n=8 (2.6 to 5 yrs)
- 22 into the control group: n=14 (0.5-2.5 yrs); n=8 (2.6 to 5 yrs).

The clinical characteristics of each group are shown in Table 1.

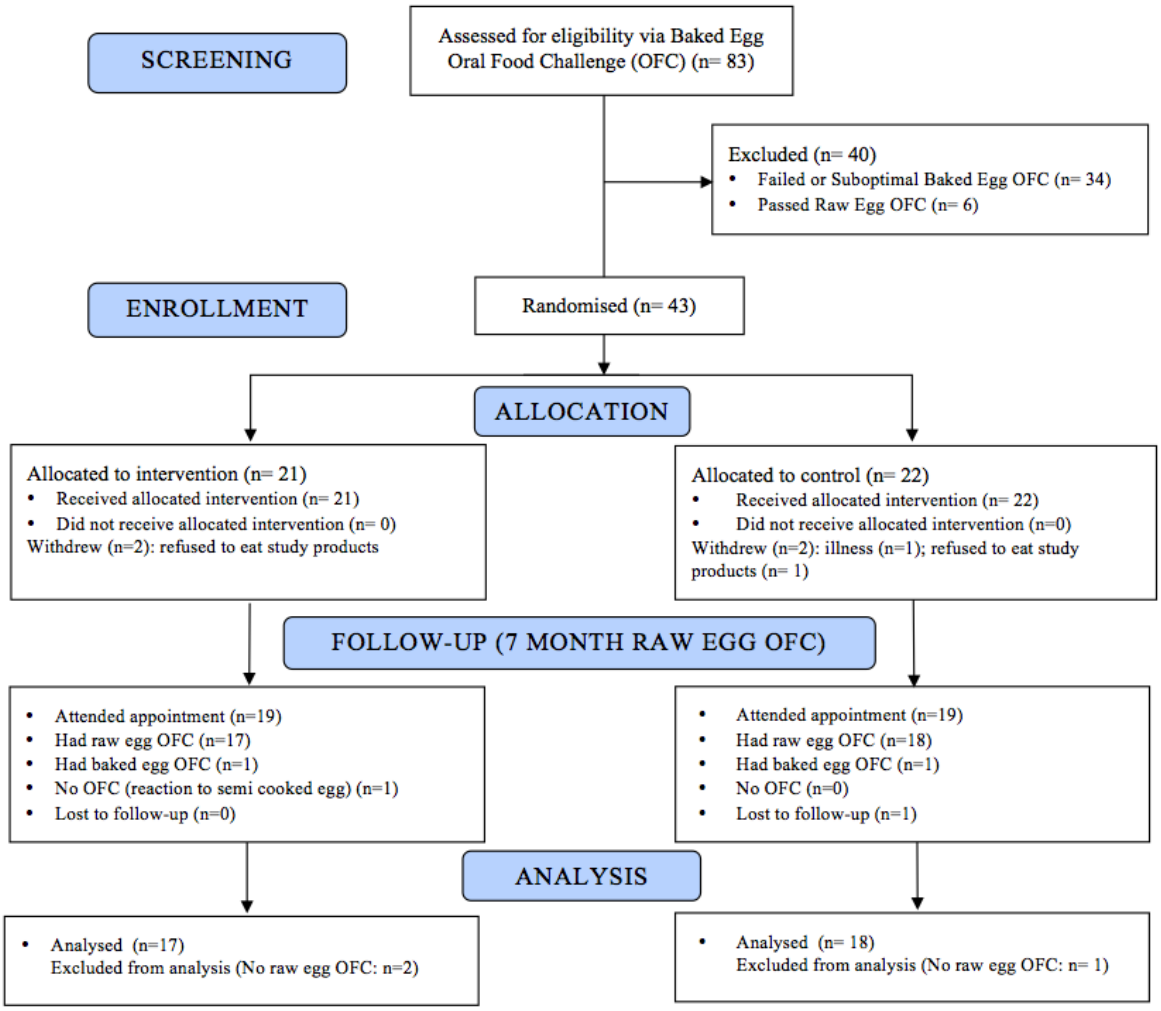

Figure 1 Study flow

Table 1 Demographic and clinical characteristics of children at study entry

Characteristic	Baked Egg Group n=21	Control Group n=22
Maternal age (years)*	35.67 (3.7)	34.14 (3.7)
Maternal ethnicity caucasian †	16 (76%)	19 (86%)
Age at screening (years) ‡	2.00 (1.21-3.25)	2.13 (1.29-3.12)
Male Sex †	14 (67%)	16 (73%)
First degree relative with atopy †	18 (86%)	18 (82%)
Birth weight (grams) *	3509 (538)	3592 (470)
Gestational age at birth (weeks)*	38.9 (1.0)	38.7 (1.0)
Ever breastfed †	21 (100%)	20 (91%)
Breastfed at screening †	2 (10%)	2 (9%)
Age at diagnosis of egg allergy (months)*	9.5 (4.3)	7.7 (3.4)
Clinical reaction to egg †	13 (62%)	8 (36%)
History of anaphylaxis to egg †	3 (14%)	5 (23%)
Egg White SPT ≥ 95%PPV †	7 (33%)	14 (64%)
Other IgE mediated food allergies †	15 (71%)	18 (82%)
Eczema †	15 (71%)	19 (86%)
Eczema severity (Objective SCORAD score) ‡	1.80 (0.00-12.33)	3.90 (0.00-9.0)
Asthma (Doctor diagnosed) †	2 (9.5%)	6 (27%)

Values are presented as follows: *mean (SD), †number (percentages) or ‡median (IQRs).

3.1 Clinical Outcomes

3.1.1 Tolerance to Raw Egg after the Intervention

Thirty-five children had raw egg OFCs (17 from the intervention group and 18 from the control group). Three children attending the 7-month appointment were not given a raw egg OFCs due to clinical decisions not to proceed with the OFC, and were excluded from the analyses. Of these, one child (intervention group) did not have a raw egg OFC due to a recent reaction to semi-cooked egg at home and two children (one from the intervention group and one from the control group) had BE challenges due to refusal to eat the study product at home.

Four of 17 children (23.5%) from the intervention group and 6 of 18 (33.3%) children from the control group passed the raw egg challenge. There was no difference between the groups in the likelihood of passing the raw egg challenge (Odds Ratio (OR) 0.62 CI 0.14-2.73 p=0.523), even when adjusted for age (aOR 0.50 CI 0.11-2.40 p=0.390).

3.1.2 Compliance with the Intervention

Children in the intervention group were offered fewer (1065) serves and consumed a median of 1.6 (Interquartile Range (IQR) 0.7- 2.6) serves per week, compared with the control group who were offered 1259 serves and consumed a median of 2.3 (IQR 1.4 - 2.7) serves per week. The differences between the average number of serves per week (p=0.14) and the total intake of study product (p=0.10) were not significant.

3.1.3 Maintenance of Tolerance to Baked Egg in the Absence of Regular Antigen Exposure

At the end of the study, the children who were not tolerant to raw egg were given another baked egg challenge to check that they maintained BE tolerance during the study. Twenty-three children (11 from the intervention group / 12 from the control group) had these challenges. Twenty-two passed and one child (intervention group) failed the BE challenge. Two children (one from the intervention group and one from the control group) reintroduced and tolerated BE at home.

The participant who failed the BE challenge had refused to consume the study product and, although the criteria for the screening BE challenge were satisfied, passed a loose bowel action that evening at home, so may have been incorrectly classified as BE tolerant.

3.2 Immunological Outcomes

The immunological characteristics of children consuming BE and those in the control group were compared at baseline and at the end of the intervention. To assess sensitisation, SPT wheal size and egg specific serum IgE levels were measured. Whole egg specific serum IgG_4 was measured as a marker of tolerance development. Immune memory development was assessed by CD45RA/CD45RO, and staining with CCR7 allowed assessment of changes in effector and central memory to be detected (29). To further assess immune activation, peripheral blood mononuclear cells (PBMCs) sampled at baseline or at 7 months were incubated with OVA or OVM and assessed for T cell CD69 expression (30), and cytokine excretion from the PBMCs was also measured to assess changes in the Th1/Th2 balance.

3.2.1 Skin Prick Testing to Egg Allergens

Baseline and post-intervention SPT results are available for all children who attended the 7-month appointment (intervention group n=19 and control group n=19; Figure 2). The mean SPT wheal size decreased for all egg allergens except for OVM in the BE group. Significant differences for egg white (p=0.03) and OVA (p=0.01) were observed. The control group also demonstrated a decrease in the mean wheal sizes for EW (p=0.01) and OVA (p=0.03) but no change in the mean wheal sizes for whole egg, egg yolk and OVM. To compare the effect of group allocation on SPT wheal size, a linear regression model adjusting for baseline SPT wheal size and age stratification was used and no difference between groups was observed.

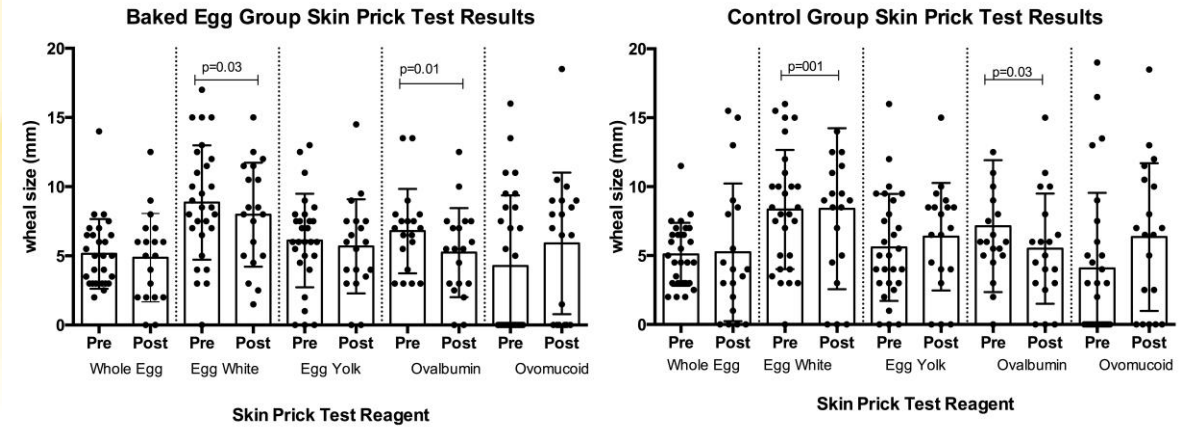

Figure 2 Changes in skin prick test wheal sizes from baseline to end of intervention

Skin prick test results from baseline to end of intervention.
Bars denote mean and standard deviation.

3.2.2 Serum Specific IgE results to Egg Allergens

Baseline and post-intervention serum specific IgE results are available for all children who attended the 7-month appointment (intervention group n=17 and control group n=18; Figure 3). Significant decreases in serum specific IgE levels for whole egg (p=0.04), egg white (p=0.04) and OVA (p<0.01) were observed for both the BE group and the control group (p=0.01, p<0.01 and p<0.01) over the period of the intervention. The serum specific IgE for OVM for the intervention group decreased but not significantly. On the other hand, a slight but non-significant increase in OVM IgE was noted for the control group. When the differences between groups were compared using a logistic regression model, no significant difference between groups was observed.

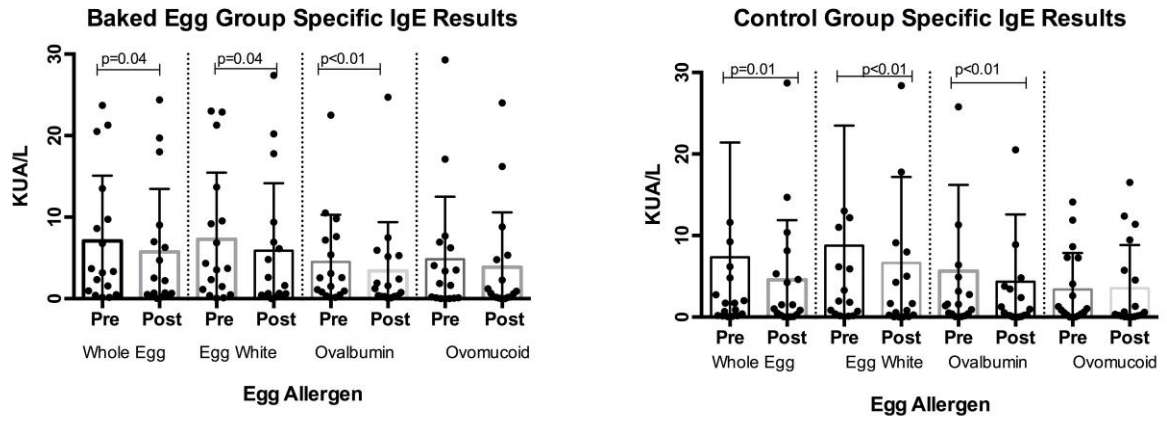

Figure 3 Changes in egg specific IgE levels from baseline to end of intervention

Specific IgE results from baseline to end of intervention.
Bars denote mean and standard deviation.

3.2.3 Serum Specific IgG4 results to Whole Egg

Baseline and post intervention whole egg serum specific IgG4 results are available for all children attending the 7-month appointment (intervention group n=17 and control group n=18; Figure 4). There was a slight, non significant increase in whole egg IgG4 levels for both intervention (p=0.31) and control (p=0.31) groups from baseline to the end of the intervention. No difference was observed in WE IgG4 levels between children who were tolerant to raw egg at the end of the intervention compared with those who were not tolerant to raw egg.

IgE/IgG4 ratios have been used to investigate development of tolerance (10, 26). The ratios of all of the egg allergens measured to whole egg IgG4 were calculated. A decrease in mean egg allergen specific IgE/IgG4 ratios was observed for both the BE group and the control group over the duration of the intervention, however, no difference was observed between groups when compared using a logistic regression model.

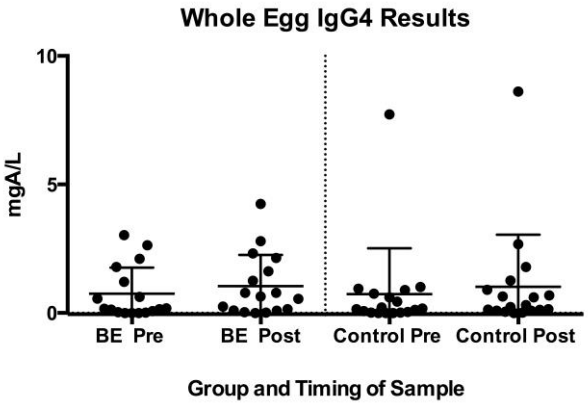

Figure 4 Change in whole egg specific IGg4 from baseline to end of intervention

Whole egg specific IgG4 results from baseline to end of intervention.
Bars denote mean and standard deviation.

3.2.4 Cellular Immune Outcomes

3.2.4.1 T cell phenotypes in the baked egg and control groups at baseline and 7 months

There was no significant difference in the ratio of CD4/CD8 T cells between the control and baked egg group. To further differentiate T cells into naive (CD45RA) or memory (CD45RO) T cells, the CD4+ cells were stained and CD45RA and CD45RO cells assessed by flow cytometry. There was no significant difference in the percentage of CD4+ CD45RA+ or $CD4^{+}CD45RO^{+}$ cells in both groups from baseline to 7 months (see Figure 5 A. and B.).

After culture with OVA and OVM, $CD4^{+}$ cells were assessed for expression of CCR7 to discriminate between central memory ($CCR7^{-}CD45RA^{-}$) and effector memory ($CCR7^{+}CD45RA^{-}$) T cells (29). As tolerance develops there is a decrease in the number of effector memory T cells in the periphery (31). We observed no difference from baseline to 7 months in the mean percentage of central or effector memory cells, in either the baked egg or control group, indicating that there was no overall effect on immunological memory (OVA stimulated cells; Figure 5 C. and D.).

After culture with ovalbumin OVA and ovomucoid OVM, CD4+ cells were assessed for activation status by assessing CD69 expression (see Figure 5 E. and F.). No significant difference in CD69 expression and T cell activation was observed between groups at baseline and at 7 months.

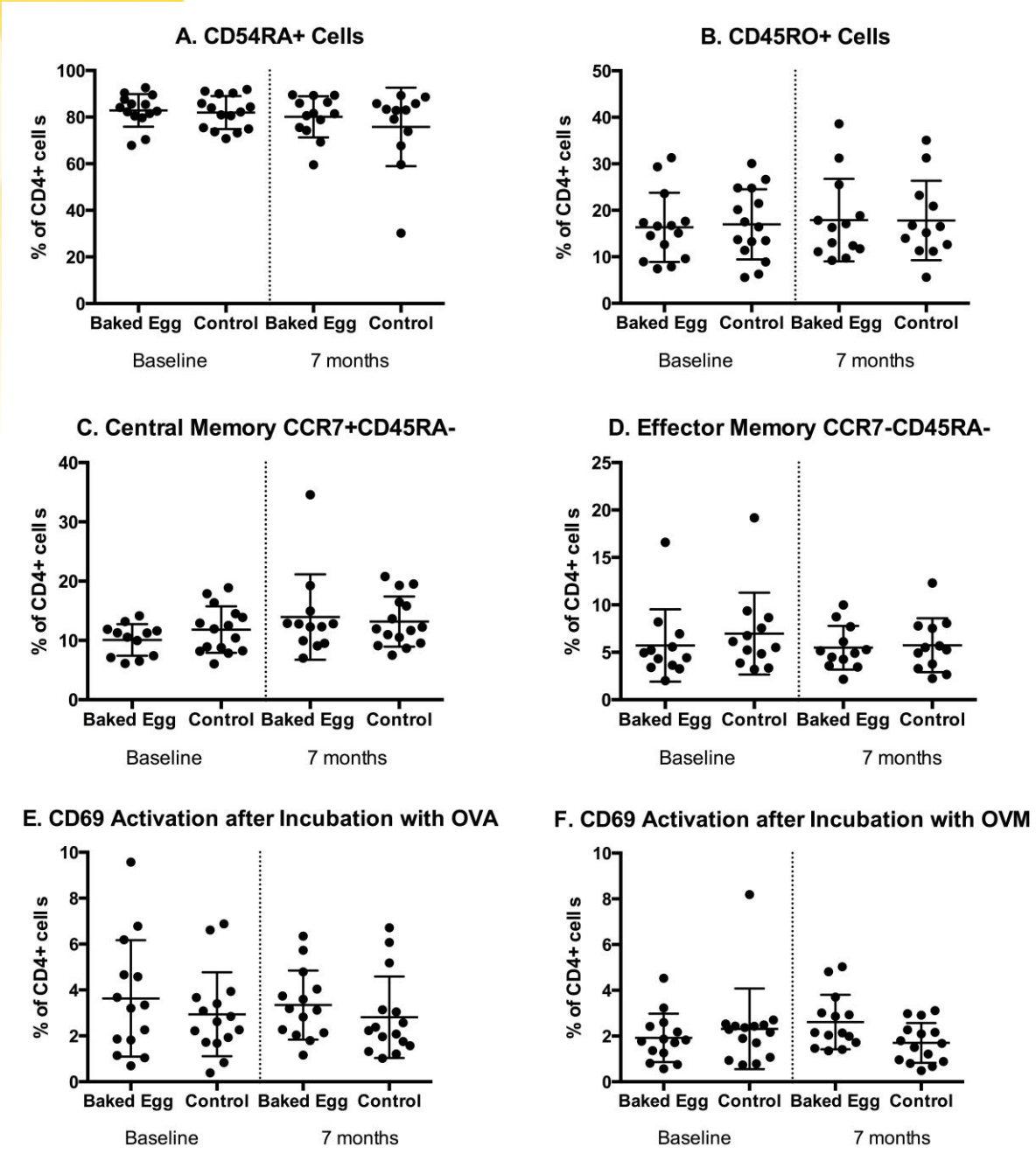

Figure 5 T Cell phenotypes in the baked egg and control groups at baseline and 7 months

A-B: Percentage of CD4+ CD45RA+ and B. CD4+ CD45RO+ T cells at baseline and at 7 months.
C-D: Central Memory (CD4+ CCR7+CD45RA-) and Effector Memory (CD4+ CCR7-CD45RA-) T cells in the baked egg and control groups after incubation with OVA at baseline and 7 months.
E-F: Percentage of CD4+CD69+ Activated T cells after incubation with OVA and OVM in the baked egg and control groups at baseline and 7 months.

Bars denote mean and standard deviation.

3.2.4.2 Cytokine Excretion by PBMCs after incubation with Egg Allergens OVA and OVM

There were no significant differences in cytokine excretion between the baked egg and control groups at baseline and at the 7 month level. The concentration of IL 5, IFN□, IL 8, IL 1, IL 6, IL 10 and TNFα detected after incubation with OVA less the amount secreted by untreated cells incubated with media alone are shown in Figure 6. No IL 4 or IL 12 was detected. There were no significant differences in cytokine excretion by PBMCs after incubation with OVM (data not shown).

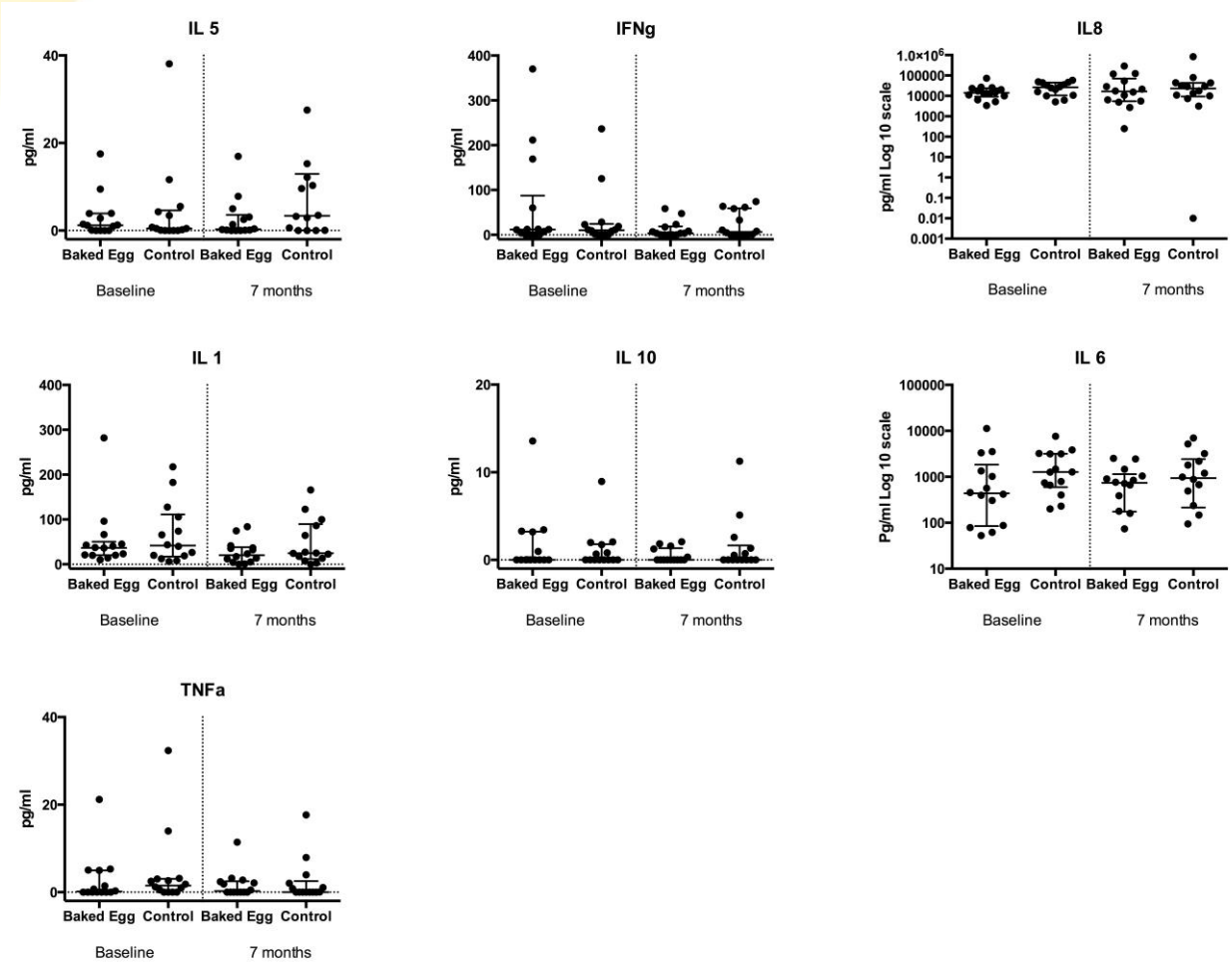

Figure 6 Cytokine excretion after incubation of PBMCs with OVA

Cytokine excretion by PBMCs from the baked egg and control group at baseline and 7 months after incubation with OVA.
Bars denote median and interquartile range.
Note that scales for the y-axis vary between cytokines and that results are presented in log10 scale for IL 8 and IL 6.

4 Summary of Main Findings

We studied the effect of BE in the diets of 1-5 year olds as these children have a high incidence of egg allergy. There are no reports of controlled studies considering the effects of BE in the diets of older cohorts of egg allergic children alone, even though older children and young adults were enrolled in other BE studies (6, 9). Further studies investigating inclusion of BE in older children with egg allergy are warranted as BE may modulate the immune system in children with more resistant phenotypes of egg allergy.

This double blind RCT compares the effect of consumption of BE (with avoidance of all egg) in 1-5 year old BE tolerant, egg allergic children on the development of tolerance to raw egg. These children have a high incidence of egg allergy. Our results imply that development of tolerance to egg may be independent of consumption of BE. Previous studies have demonstrated clinical tolerance to egg in children consuming BE (6, 9, 12, 17). Whilst our study was underpowered, we did not see trends suggesting any effects on either clinical or immunological outcomes. Our results are consistent with a population based study of 2 year old BE tolerant, egg allergic children that reported frequent ingestion of BE was associated with earlier resolution of egg allergy (12). This may reflect a phenotype outgrowing their egg allergy more quickly than those self-limiting their BE intake. Once BE intolerant children developed BE tolerance they were as likely to gain tolerance to regular egg as children initially tolerant to BE (12).

Decreased SPT wheal sizes and egg sIgE, and increased sIgG4 have been reported after three to six months exposure to BE (6, 9), leading to conjecture that inclusion of BE, when tolerated, in the diets of egg allergic children may modulate the immune system (13, 15, 24, 32). In our group of BE tolerant children, there was no difference between groups in reduction in egg allergen SPT wheal size and egg sIgE levels, or increase in WE sIgG4 levels. Our results are consistent with Tey et al. (33), who reported no difference in the rate of decline in EW SPT wheal size in 3-6 year old egg allergic children consuming BE compared with an egg free diet, indicating that this change may be independent of consumption of BE.

The strengths of our study include design of the blinded intervention and the consistent dosing protocol. Randomised groups were of similar age, allergy background and egg allergy phenotype, and the timing of assessments for clinical and immunological outcomes add additional strength to our results. To comply with Australian healthy eating guidelines related to consumption of ‘discretionary foods’ (34), we asked the children to consume the study foods two or three times per week. This dose rate is consistent with the maintenance phase of several egg SOTI studies (35-37), but less frequent than Lemon-Mulé et al. (6) who dosed one to three times daily. The amount of baked egg protein and the dose rate may have been too low or not frequent enough, however, adjusting for total consumption of BE in our final analysis made no difference to the outcome.

Our study has some limitations due to its small sample size, and the nul finding could be due to chance. Fifty percent of the children screened for inclusion in this study tolerated BE, which was less than expected (6, 12). In our study, three children (6%) (n=2 intervention group; n=1 control group) refused to consume intervention products. This may have been due to finicky eating, or related to the texture of the study products. This is similar to refusals in other BE trials (38). It is possible some children in our study may have lost tolerance to BE, reflected by their refusal to consume the study product (39). Development of symptoms when consuming BE and subsequent refusal to consume BE have been reported in children passing BE OFCs (25). Equivalent poor compliance has also been reported in other immunotherapy trials (40, 41).

There are no reports of controlled studies considering the effects of BE in the diets of older cohorts of egg allergic children, and such studies are warranted as BE may modulate the immune system in children with more resistant phenotypes of egg allergy.

5 Conclusions and Recommendations

The results of this RCT suggest that BE tolerant 1-5 year old children with IgE mediated egg allergy are evolving tolerance to raw egg and this does not appear to be hastened by short-term, regular inclusion of BE in the diet. Further trials of larger sample size, including children of different age groups are required to further test this hypothesis.

5.1 Dissemination Strategy

A manuscript detailing the results of this study will be submitted to a peer reviewed allergy journal. Findings will also be disseminated to the media. Media stories generated by our results are likely to be highly topical, resulting in high levels of media interest and exposure.

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7 Plain English Summary

Project Title:	Immunological Changes when Egg Allergic Kids Eat Baked Egg
AECL Project No	1WC121
Researchers Involved	M. J. Netting, I. Penttila, M. Gold, P. Quinn and M. Makrides
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Objective	• To compare oral tolerance to raw egg in children who have been consuming baked egg, with those who have been on a completely egg free diet. • To compare immune markers of tolerance to egg in children who have been consuming baked egg, with those who have been on a completely egg free diet.
Background	Egg allergy affects almost one in ten Australian children. Until recently, children with egg allergy were advised to avoid all forms of dietary egg exposure based on the assumption that consumption of egg would prolong their allergy. Recent publications indicate that up to 70% of children with egg allergy can eat egg baked in a cake or muffin without apparent reaction. These publications have prompted some allergy clinics to encourage the introduction of baked egg in the diets of children with raw egg allergy under the premise that this may hasten development of tolerance to raw egg.
Research	We studied 43 children with allergy to raw egg and tolerance to baked egg. The effects of regular consumption of baked egg (intervention group) were compared to the standard treatment of an egg free diet (control group) on clinical tolerance to raw egg and immune markers of tolerance.
Outcomes	The results of this RCT suggest that baked egg tolerant 1-5 year old children with IgE mediated egg allergy are evolving tolerance to raw egg and this does not appear to be hastened by short-term, regular inclusion of baked egg in the diet.
Implications	Further trials of larger sample size, including children of different age groups are required to further test this hypothesis.
Key Words	egg allergy; egg allergic children; heated allergens; tolerance; tolerance to raw egg; oral; immunotherapy

Publications