NUTRITIONAL STRATEGIES FOR ALLERGY PREVENTION, DIAGNOSIS AND TREATMENT, WITH A SPECIFIC FOCUS ON EGG ALLERGY

A thesis submitted for the degree of Doctor of Philosophy

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ABSTRACT

Food allergy affects up to 10% of Australian children. This thesis addresses questions related to the prevention, diagnosis, and management of food allergy, specifically focusing on egg allergy.

The results of a systematic review investigating the relationship between whole foods in the maternal diet during pregnancy and lactation and development of atopic disorders (including egg allergy) in childhood are reported. No widespread or consistent links were identified; however dietary patterns with high Mediterranean diet scores, diets rich in fruits and vegetables, fish, and vitamin D containing foods were suggestive of benefit, requiring further evaluation. From the results of this review, the management of allergy was of particular interest, and this thesis focuses on egg allergy as it the most common food allergy affecting Australian children.

A literature review of skin prick testing (SPT), serum-specific IgE (sIgE) levels and oral food challenge (OFC) protocols used to diagnose and manage egg allergy highlighted heterogeneity in terms of testing reagents, and the type of egg, dosing rates and total dose used for OFCs. Development of standard egg OFC protocols will facilitate consistent clinical care and comparison between studies reporting outcomes of OFCs.

Egg protein is a complex glycoprotein and its structure and allergenicity is affected by heating. OFCs using fresh egg are common; however, to limit the risk of foodborne infection, some allergy units use pasteurised raw egg. Pasteurisation may affect the structure and allergenicity of egg proteins, and this was assessed by comparing binding of serum IgE from egg allergic children to pasteurised whole raw egg powder with fresh whole raw egg. The main egg allergens were present in pasteurised whole raw egg powder, and serum IgE of egg-allergic children bound to them in a similar pattern to those in fresh whole raw egg,

indicating that pasteurised whole raw egg powder is a suitable substitute for raw egg in clinical practice for OFCs.

Extensively heated (baked) egg is tolerated by a majority of egg allergic children before they tolerate less well cooked forms of egg and consumption of baked egg (BE) is associated with immunological changes suggestive of evolving tolerance to all forms of egg. However, there are no RCTs that directly test if the natural history of childhood egg allergy is altered by inclusion of BE in the diet. Studies reporting the effects of BE in the diet of BE tolerant, egg allergic children were not randomised and controlled, and used retrospective comparator groups. The rationale, development, conduct and outcomes of an RCT examining the clinical and immunological effects of ingestion of BE in 1 to 5 year old egg allergic children are reported. The results of this study suggest that tolerance to BE may be indicative of a phenotype of egg allergy that is outgrown, and this may not be influenced by consumption of BE for six months.

Egg white SPT and sIgE levels do not accurately predict BE tolerance, and a BE OFC is recommended to assess tolerance to BE. Using the opportunistic sample of children screened for the trial the utility of whole egg, egg yolk, ovomucoid and ovalbumin SPT, sIgE and whole egg IgG4 testing to predict the outcome of BE OFCs were compared with testing to egg white. The results of this investigation indicated that whole egg and ovalbumin sIgE testing may predict tolerance to BE more accurately than other egg allergens, however no one test was ideal, and a combination of measures may be required. A BE OFC remains the gold standard for determining tolerance to BE.

This thesis strengthens the evidence base for standard protocols for management of IgE mediated egg allergy in children, and provides important information regarding influences of the perinatal maternal diet and atopy development.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I confirm that I completed the work described in this thesis, with the guidance of my supervisors, Professor Maria Makrides, A/Professor Michael Gold, A/Professor Imme Penttila and Dr Patrick Quinn. I was responsible for the study design, management and conduct of the Randomised Controlled Trial (Chapter 6). I completed all of the skin prick testing and supervised the conduct of the oral food challenges described in Chapters 5 and 6. I personally conducted the laboratory analysis described in this thesis (excluding the specific IgE and IgG4 analysis, which was outsourced) with the guidance and assistance of Dr Adaweyah Donato, Irene Kanter and A/Professor Imme Penttila.

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To my family, Angus, Alice and Hamish thank you for your love and support.

LIST OF ABBREVIATIONS

ACTRN	Australian Clinical Trials Registry Number
aOR	Adjusted odds ratio
AUROC	Area under the receiver operator curve
BE	Baked egg
Bis Tris	Bis-tris Methane
BSACI	British Society for Allergy and Clinical Immunology
CI	Confidence Interval
DBPCFC	Double blind, placebo controlled food challenge
DMSO	Dimethyl sulphoxide
EMBASE	Exerpta Medica database
EW	Egg white
EY	Egg yolk
FBS	Foetal Bovine Serum
FOX P3	Forkhead Box P3
FPIES	Food protein induced enterocolitis syndrome
g	gram
Gal d	Gallus domesticus
GALT	Gut-associated lymphoid tissue
HCl	Hydrochloric Acid
HI FBS	Heat Inactivated Foetal Bovine Serum
IgE	Immunoglobulin E
IgG4	Immunoglobulin G4
IL-	Interleukin -
IM	Intramuscular
INF	Interferon gamma
IQR	Interquartile range
kDa	kilo dalton
kUA/L	Kilo Units of Allergen per litre
LR	Likelihood ratio
MeOH	Methanol
mm	millimetre
ml	millilitre

mg	milligram
Min	minutes
n	number
NaHCO3	Sodium Bicarbonate
NaCl	Sodium Chloride
NPV	Negative predictive value
OFC	Oral food challenge
OR	Odds Ratio
OVA	Ovalbumin (Albumin from chicken egg white)
OVM	Ovomucoid
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate Buffered Saline
PHA	Phytohemagglutinin-L
PPV	Positive predictive value
vs	Versus
RCT	Randomised controlled trial
WE	Whole egg
RCT	Randomised controlled trial
ROC	Receiver operating characteristic
RPMI	Roswell Park Memorial Institute Medium (RPMI-1640)
SCORAD	Scoring Atopic Dermatitis
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide
sIgE	Serum-specific Immunoglobulin E
SOTI	Specific Oral Tolerance Induction
SLIT	Sublingual Immunotherapy
SMP	Skim milk powder
SPT	Skin prick test
TGF β	Transforming growth factor beta
T-Regs	Regulatory T-cells
TTBS	Tris-buffered saline and Tween 20
Tris-HCl	Trizma® hydrochloride
Tween 20	Polyethylene glycol sorbitan monolaurate
xg	times gravity
y	years

°F	Degrees Fahrenheit
°C	Degrees Centigrade
<	Less than
>	Greater than
ω-3 LC PUFA	Omega 3 long chain polyunsaturated fatty acids
μg	microgram
μm	micrometer

CONTEXTUAL STATEMENT

In Australia the incidence of food allergy has increased markedly in the last generation, affecting up to 10% of young Australian children, and 1-2 % of adults worldwide[1, 2]. Food allergy imposes an immediate significant burden in terms of general health perception, parental emotional distress and family activities[3]. Furthermore, as individuals with early food allergy may progress to airway hypersensitivity and development of asthma and allergic rhinitis in adulthood, the burden to the individual, family and health care system is magnified. This thesis addresses research questions from the themes of prevention, diagnosis, and management of food allergy, specifically focusing on egg allergy, which affects up to 8.9% of Australian infants[2].

A mother's diet during pregnancy and lactation has potential to influence the development of atopy in her children via multiple mechanisms, either by direct exposure to allergens in utero or via breast milk, or by immunomodulatory effects of dietary components[4]. Avoidance of allergens in the maternal diet was the focus of several randomised trials (RCTs) and many longitudinal cohort studies have investigated correlations between inclusion of certain foods or dietary patterns and atopy in children, but the outcomes of these studies had not been systematically reviewed. The first study in this thesis (Chapter 1), relating to prevention of allergy, is a systematic review investigating the relationship between whole foods in the maternal diet during pregnancy and lactation and development of atopic disorders (including egg allergy) in childhood. The systematic review also identified several studies where elimination of egg in the maternal diet during lactation was used as a treatment for their children's egg allergy. In addition to the maternal diet, early feeding practices may influence later allergy development as many infants display allergic symptoms early in infancy, and exposure to allergens may be important in the development of food allergies[5]. Several large RCTs investigating the optimal timing of introduction of allergens into the weaning diet were in progress or had just concluded when planning this

thesis (LEAP[6]; STAR[7]; STEP, ACTRN 126100003 88011; EAT, ISRCTN14254740; BEAT, ACTRN 12611000535976). The STEP, BEAT and STAR trials specifically address the primary prevention of egg allergy, and so the focus of the other four manuscripts in this thesis was to address key research questions not being actively researched, related to the diagnosis and dietary management of egg allergy as it is the most common food allergy in Australian children.

IgE mediated egg allergy is diagnosed on the basis of clinical history and the presence of antigen specific IgE (sIgE). Skin prick testing (SPT) and sIgE to egg may be used as predictive tests in the diagnosis of an egg allergy, or to predict if an egg allergic child is ready for an oral food challenge (OFC) to determine if they have gained tolerance to egg. The tests are not 100% predictive of allergy, and the 'gold standard' for diagnosis of an allergy remains the double blind, placebo controlled oral food challenge (DBPCFC). However, DBPCFCs are expensive to perform and there are space and resource implications and as such not everyone with a potential egg allergy can be challenged under medical observation. Literature reporting the incidence and management of IgE mediated egg allergy and the use of predictive tests and OFCs in the diagnosis of egg allergy is reviewed and discussed in Chapter 2. Chapter 2 is the literature review component of this thesis and is not presented in publication format.

Raw egg OFCs are commonly used in clinical practice to diagnose egg allergy or to assess if an egg allergic child has gained tolerance to raw egg. The use of fresh whole egg carries some risk in terms of food borne infection so pasteurised raw egg may be used as a challenge vehicle. Hen's egg protein is a complex glycoprotein, and food processing, including heating, disrupts the tertiary structure of the egg protein, affecting IgE binding sites[8] and it is also possible that the heat treatment and desiccation processes used in pasteurisation of egg may affect the allergenicity of the egg protein. The aim of the publication in Chapter 3 was to determine if raw pasteurised egg powder maintains its

capacity to bind IgE, as determined by immunoblotting with serum from egg allergic children, when compared to whole raw fresh egg.

Many egg allergic children tolerate baked egg (BE) even if they do not tolerate less heated forms of egg, and inclusion of BE in the diets of children with egg allergy when tolerated has become accepted clinical practice[9]. Diagnosis of tolerance to BE relies on an OFC as routine egg allergen SPT or sIgE measurements perform poorly when used to predict children who will tolerate baked egg[10]. Anaphylaxis during BE OFC has been reported, including cases where there was negative predictive testing to allergens[11]. As such there is a need to identify which egg allergen, or combinations of allergens, are best utilized by the clinician when predicting when a child is ready for a BE OFC. The utility of egg allergen skin prick, sIgE and egg white IgG4 testing to predict the outcome of BE OFCs in 1 to 5 year old egg allergic children was examined, and the results are reported in Chapter 4.

Consumption of BE by raw egg allergic, BE tolerant individuals is associated with immunological changes suggestive of evolving tolerance development to all forms of egg[12, 13]. It is unclear if this is simply an observation correlated to a phenotype of egg allergy that is outgrown earlier, or if the inclusion of BE in the diet has immunomodulatory effects. There are no RCTs directly testing whether the natural history of egg allergy can be modified by inclusion of BE, and studies investigating immune changes in egg allergic children after consumption of BE were not randomised or controlled. Specific oral tolerance induction using raw proteins is not free of risks and fails to induce the immune changes leading to sustained unresponsiveness to the protein[14] and there is potential for BE to be used as a vehicle for specific oral tolerance induction. The literature regarding the potential of heated food allergens as immunotherapy for children with egg (and milk) allergy was reviewed, and the results are presented in the fourth publication (Chapter 5).

The final manuscript in this thesis (Chapter 6) describes the development, conduct and outcomes of an RCT examining the clinical and immunological effects of ingestion of BE

in 1 to 5 year old children with egg allergy. The aims of this trial were: 1) To determine if raw egg allergy resolves faster in 1 to 5 year old children if they are exposed to BE for 6 months compared with an egg free diet, assessed by a raw egg oral food challenge. 2) To assess changes in sensitisation to common egg allergens, as measured by SPT and egg protein sIgE and whole egg serum specific IgG4 levels in 1 to 5 year old children exposed to baked egg for 6 months compared with an egg free diet. 3) To examine the effect of regular BE exposure on immunity, particularly on patterns of evolving allergen-specific responses, and development of immune memory in 1 to 5 year old children with raw egg allergy exposed to BE for 6 months, compared with an egg free diet.

CHAPTER 1

INFLUENCE OF MATERNAL DIET DURING PREGNANCY AND LACTATION ON DEVELOPMENT OF ATOPY

INTRODUCTION

Chapter 1 examines the effect of maternal diet during pregnancy and lactation (including the avoidance of egg) on atopic outcomes in offspring.

The paper entitled, "Does Maternal Diet during Pregnancy and Lactation Affect Outcomes in Offspring? A Systematic Review of Food-Based Approaches" by Merryn Netting, Philippa Middleton and Maria Makrides was published in the peer reviewed journal, *Nutrition* 30 (2014) 1225–1241. DOI: 10.1016/j.nut.2014.02.015

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Review

Does maternal diet during pregnancy and lactation affect outcomes in offspring? A systematic review of food-based approaches

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ABSTRACT

Objectives: The aim of this study was to investigate the relationship between maternal diet during pregnancy and lactation and development of atopic disorders in childhood.
Methods: We included studies published up to August 2011 that assessed food-based maternal dietary interventions or that examined associations between maternal dietary intake during pregnancy and/or lactation and allergic outcomes (eczema, asthma, hay fever, and sensitization) in their children.
Results: We included 42 studies (>40 000 children): 11 intervention studies (including 7 randomized control trials), 26 prospective cohort studies, 4 retrospective cohort studies, and 1 case–control study. In the randomized control trials, no significant difference was noted overall in the prevalence of eczema and asthma in the offspring of women on diets free from common food allergens during pregnancy. The prospective cohorts investigated a large number of potential associations, but reported few significant associations between maternal dietary intake and development of allergy. Maternal diets rich in fruits and vegetables, fish, and foods containing vitamin D and Mediterranean dietary patterns were among the few consistent associations with lower risk for allergic disease in their children. Foods associated with higher risk included vegetable oils and margarine, nuts, and fast food.
Conclusion: This review did not find widespread or consistent links between mothers' dietary intake and atopic outcomes in their children. However, maternal consumption of Mediterranean dietary patterns, diets rich in fruits and vegetables, fish, and vitamin D-containing foods were suggestive of benefit, requiring further evaluation.

Introduction

The prevalence of allergic diseases in most industrialized countries has increased over the past 20 y and is now estimated to affect one in five individuals [1–3]. Common manifestations of allergic disease include allergic rhinitis or hay fever, asthma, eczema or atopic dermatitis, and food allergies. The risk for allergic disease is increased to about one in three if one first-degree relative (parent or sibling) is atopic and to 70% if both parents are atopic [4]. The pattern of allergy expression differs with age; with the greatest incidence of food allergy and atopic eczema peaking by 1 y of age, whereas asthma and allergic rhinitis continue to increase until around 15 y of age [5]. Many childhood allergies persist, with about 50% of childhood asthma sufferers and 80% of hay fever sufferers continuing to have symptoms into adulthood [6,7]. The cost to the health care system and the burden for the family are high [8,9] and it is estimated that asthma alone costs in excess of $34 billion annually [10].

The increase in allergic disease has occurred too rapidly (within one to two generations) to be a result of population genetic changes, so it is likely related to environmental changes.

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Throughout the industrialized world, strong evidence exists that environmental factors accompanying higher socioeconomic conditions and hygiene standards have contributed to the increased prevalence of allergic disease. Societies with fewer respiratory infections, greater use of antibiotics early in life, fewer older siblings in the household, less contact with farm animals, and general lack of early microbial exposure are repeatedly associated with the greatest burden of allergic disease [11]. The atopic predisposition is believed to arise where the infant has an innate tendency to produce immunoglobulin (Ig)E antibodies (sensitization), which in some individuals progresses to allergic disease. The allergens causing sensitization are nearly always proteins originating from the environment including pollens, house dust mite, or food. Why children are becoming increasingly sensitized to environmental allergens is a matter of debate. Several factors may be involved, including whether the child is breastfed from birth [12], the child's antioxidant status [13], the balance of ω-6 to ω-3 polyunsaturated fatty acids in the diet [14] and environmental influences such as microbial exposure, cigarette smoke, and other pollutants [15].

Because many infants develop allergic symptoms early in infancy, and exposure to allergens may be important in the development of food allergies, there is great interest in maternal dietary strategies during pregnancy and lactation that may prevent childhood allergies, and thus reduce the burden of disease. This systematic review evaluates the effect of food-based approaches in the maternal diet for the prevention of childhood allergies.

Methods

Inclusion criteria

We included studies of any design that either compared a food-based maternal dietary intervention during pregnancy and/or lactation with another intervention or no intervention. We also included studies that examined associations between maternal dietary intake during pregnancy and/or lactation and allergic outcomes in their children from that pregnancy (cohort and case–control studies). Studies with cointerventions, such as timing of introduction of solid foods into the child's diet, use of hydrolyzed formula and non-food–based interventions such as dust mite control were eligible for inclusion.

We prespecified primary outcomes as child eczema, asthma, hay fever, and food allergy and secondary outcomes as allergy symptoms, atopy or atopic disorder, dyspnea, hay fever (allergic rhinitis or allergic rhinoconjunctivitis), wheeze (and recurrent wheeze), cough, food hypersensitivity (IgE-mediated food allergy or food intolerance), and sensitization (e.g., milk, egg, nut, food, inhalant).

We excluded studies assessing infantile colic, as this was not considered an allergic outcome.

As maternal intake needed to be food based, intervention studies designed to assess the effect of dietary supplements were not eligible for inclusion, nor were those where dietary intakes were expressed only in terms of nutrients.

We excluded studies that only investigated maternal nut (including peanut) consumption during pregnancy and/or lactation because nut allergy (particularly peanut) warrants a separate examination and assessment.

Searching

We searched MEDLINE, EMBASE, and the Cochrane Library (last searched end of August 2011) and scanned reference lists of systematic reviews and other relevant retrieved papers for additional studies.

Search terms included prenatal, antenatal, maternal, mother, pregnan*, lactat*, breastf*, intake, consumption, food, diet, wheez*, dermatitis, eczema, atop*, asthma, allerg*, food allergy hypersensitivity. The full search strategy is documented in the appendix.

Study selection and data extraction

Two authors independently assessed search results against study eligibility criteria. Two authors also independently conducted data extraction for each included study.

Data synthesis

Where possible, the results of randomized controlled trials (RCTs) were pooled, using the meta-analysis program RevMan [16]. We used risk ratios with 95% confidence intervals to express dichotomous outcomes. Where statistical heterogeneity was substantial ($I^2 > 40\%$), we used a random-effects model. Differences between subgroups were assessed using interaction tests [17].

The results of non-randomized intervention studies were narratively reported.

The results from cohort and case–control studies were tabulated and narratively summarized. Where available, we reported the adjusted outcomes for these study designs. Results were presented by age of children, with the youngest age first.

Risk for bias

For RCTs, we assessed the risk for bias using the methods outlined in the Cochrane Handbook for Reviews of Interventions [17]. For other study designs we took account of the risk for selection bias, attrition bias, and reporting bias.

Results

This systematic review included 42 studies. Table 1 lists the characteristics of the included studies. Eleven studies were intervention (7 RCTs, 1 participant preference trial, and 3 non-randomized comparisons). These studies examined the effect of eliminating or restricting common allergens from the maternal diet during pregnancy and lactation. Some included cointerventions such as manipulation of the infant's diet, or environmental measures.

The remaining 31 studies were 26 prospective cohorts, 4 retrospective cohorts, and 1 case–control study. These studies looked at the association of different dietary patterns or the frequency of consumption of different foods with atopic outcomes.

One intervention trial and seven other studies were excluded [18–25]. The intervention study reported the outcomes of an intervention based on a supplement rather than food; seven cohort studies reported dietary intakes in terms of nutrients rather than foods.

Results are reported by the major clinical outcomes (eczema, asthma and wheeze, hay fever, sensitization, and food allergy) and study design (intervention or observational).

Risk for bias assessment summary

The risk for bias assessments are described in Table 1 (association studies [26–76]) and in Table 2 (intervention studies [26,29,32,34,36,39,41,42,44,45,78]). Overall, the 11 intervention studies had at least moderate risk for bias. None of the RCTs fully described the methods used to conceal allocations at the time of randomization. Only one study was able to devise a way to blind the intervention and most studies had moderate losses of participants.

The 31 association studies were of reasonable quality: 12 had low risk for bias; 2 had low to moderate risk, and 17 had moderate risk for bias.

Maternal diet and eczema in children

Intervention studies. Five RCTs and two non-randomized comparisons assessed the effects of maternal dietary restriction on eczema in their children (Table 1). The five RCTs examined the effect of avoiding one of more common allergens in the maternal diet during pregnancy and lactation. In a study that randomized 212 women to a diet free of cow's milk and egg during pregnancy and lactation or a normal diet, the development of eczema,

allergic rhinoconjunctivitis, asthma, and food allergy in the offspring up to 5 y of age was tracked [27]. In another study the intervention group was restricted from cow's milk, egg, and peanut consumption and had limited intake of soy and wheat during pregnancy and lactation [34]. Children were followed until 7 y of age and reported any atopic disorder (asthma, food allergy, allergic rhinitis, eczema, and aeroallergen sensitization). In the Isle of Wight Study [36], the 58 women in the intervention group followed a diet that restricted cow's milk, egg, nut, and fish during lactation. This study delayed introduction of allergenic foods in the offspring, and introduced house dust mite control. In one study, women were [39] randomized to a diet without cow's milk during pregnancy and lactation, and in another [32] women followed a diet with a strictly reduced cow's milk and egg intake during late pregnancy. Both trials followed the offspring until 18 mo of age. The first trial reported the development of eczema, and the second reported cord blood IgE, eczema, and asthma. In a non-randomized comparison, [29,30] the children of 65 women who adhered to a diet free of cow's milk, egg, and fish while breast-feeding were compared with the children of 50 women who maintained a normal diet. Solid foods were delayed and the children were followed for eczema and food allergy until 10 y of age. A previous study [41] enrolled 150 pregnant women into a patient preference trial of avoiding cow's milk and egg during pregnancy and lactation. The children were followed until 1 y of age for eczema and sensitization.

In the five RCTs [27,32,34,36,39], there was no significant difference overall in the prevalence of childhood eczema (risk ratio [RR], 0.76; 95% confidence interval [CI], 0.50–1.16; N = 682 children, random effects). These trials showed statistical (I^2 = 44%) and clinical heterogeneity with different dietary restrictions and eczema assessed at different ages. The only RCT showing a significant reduction in childhood eczema included house dust mite control as well as dietary restrictions [36]. Due to the heterogeneity, a pooled result for the five RCTs was not presented (Fig. 1).

Two non-randomized comparative studies also showed no evidence for an effect of dietary restriction. In one non-randomized preference trial, 30 women chose to completely avoid milk and eggs in the last trimester of pregnancy and while breastfeeding exclusively; 33 women also avoided all milk and eggs, but only while breastfeeding; and 41 women chose no dietary restrictions [41]. In this study, six children of mothers in each of the avoidance groups had eczema in the first year of life compared with five children in the group where mothers did not follow dietary restrictions.

In a non-randomized concurrent comparison, when mothers followed a diet free from eggs, cow's milk, or fish in the first 3 mo of their baby's life, the infants were significantly less likely to develop eczema up to 6 mo compared with children of mothers who had no dietary restrictions. This effect was not sustained after 6 mo of age or at follow-up when the children were 10 y old [29,30].

Observational studies. Sixteen prospective cohort studies reported eczema in children as an outcome (Table 1). All used adjusted analyses, unless otherwise stated. A detailed summary of each study may be found in Supplementary Table 1.

3 mo to 1 y. Four prospective cohort studies examined the relationship between maternal diet and eczema in children up to 1 y of age. Physician-diagnosed eczema in 3- to 4-mo-old Japanese children was not associated with their mothers' intake of dairy products, eggs, or fish during pregnancy. However, in this population, a significantly increased risk for eczema was noted with higher levels of meat consumption (>64 g/d) [71]. In two studies, for children up to 1 y of age, daily consumption of about 30 g of fish by their mothers during pregnancy [72] and increased consumption (daily versus less frequently) [53] showed protective effects against eczema. However, neither of these studies adjusted their results for potential confounders. In another unadjusted study [47], increased vitamin C concentration in atopic mothers' breast milk (attributed to dietary intake of vitamin C from fruit and vegetables during lactation) was associated with significantly reduced rates of eczema in their children.

It has been shown that daily maternal fish consumption of about 30 g weakened the otherwise borderline deleterious association between high maternal exposure to pollutants (fine particulate matter measured by environmental air monitors worn by the women and exposure to environmental tobacco smoke) and infant eczema [72].

2 to <3 y. Six prospective cohort studies and one retrospective cohort study examined the relationship between maternal diet and eczema in children from 2 to <3 y of age. Four of the six prospective studies included the same cohort of women and their children, with each study reporting on different maternal dietary exposures.

Two studies compared maternal dietary patterns (healthy, Western, traditional, or processed) [62,75] and two compared consumption of total and individual dairy products [54,68] with childhood eczema at 2 to 3 y of age, and found no significant association. This was also the case for maternal intake of fruit and vegetables in both studies, except that green and yellow vegetables and citrus fruit showed protective associations against eczema in one study [69]. It has previously been noted that there were no significant associations among spinach, celery, cabbage, salad, and citrus fruits [54]. High maternal consumption of margarine and vegetable oils is significantly associated with an increased risk for eczema, but there were no significant associations with eggs, butter, seeds, deep-frying vegetable fat, and nuts [54].

High maternal fish intake (once or twice a week during pregnancy) was associated with significantly lower rates of children's eczema in one study [54]. Another retrospective study with unadjusted analyses [67] failed to find an association between maternal consumption of fish at least once a week during pregnancy and childhood eczema, as found elsewhere [61], where mothers in the highest quartile consumed >70 g of fish a day.

One study [61] failed to find an association between maternal meat intake during pregnancy and childhood eczema.

3 y. Four maternal dietary patterns during pregnancy—a Mediterranean diet, a "healthy" diet, a "prudent" diet and a Western dietary pattern—have been compared [66]. None of these dietary patterns consumed by mothers while pregnant showed significant associations with development of eczema in their children at 3 y of age.

5 y. At least one serving of fish a week by mothers during pregnancy was protective for eczema in their children at 5 y of age in one study, whereas none of the other foods tested (including fruit and vegetables, grains, and fats) showed any consistent associations [58]. One study [60] was unable to find an association between vitamin D from food consumed during pregnancy and development of eczema in children at 5 y of age.

Children's polychlorinated biphenyl (PCB) and mercury levels were measured as markers of maternal dietary intake of fish likely to be contaminated with marine pollutants, and the

Table 1
Characteristics of included studies

Author, Country/Study name	Design	Risk of bias	Number randomized (intervention, control) N (I, C)	Family history of atopy	Type of food intervention (intervention studies) or type of food exposures assessed (cohort studies) Avoidance unless otherwise stated	Timing of intervention or exposure assessment*	Outcomes	Age of child at assessment
Intervention studies								
Falth-Magnusson 1987 [26], 1987 [27], 1992 [28]	RCT	Moderate	212 (104, 108)	Yes	Cow's milk, egg	Pregnancy Lactation	Eczema Allergic rhinoconjunctivitis, asthma, food allergy	≤5 y
Hattevig 1989 [29] 1999 [30] Sweden	Non-randomized comparison	Moderate	115 (65, 50)	Yes	Cow's milk, egg, fish	Lactation Solid foods	Eczema Food allergy	3, 6, 9, 12, & 18 mo; 10 y
Lilja 1989 [31], 1989 [32], 1991 [33]	RCT	Moderate	166 infants (81, 85)	Yes	Cow's milk, egg (allowed traces of cow's milk in margarine and egg wash on breads)	Pregnancy	Cord blood IgE, eczema, asthma	1 y and 18 mo
Zeiger 1989 [34] 1995 [35]	RCT	Moderate	379 recruited (only 288 (103;185) able to be assessed at 4 mo)	Yes	Cow's milk, egg, peanut, limited soy and wheat	Pregnancy Lactation	Any atopic disorder, asthma, food allergy, allergic rhinitis, sensitization (aeroallergens)	4 mo; 1, 2, 4, & 7 y
Arshad 1992 [36]; Hide 1994 [77]; Hide 1996 [37]; Arshad 2007 [38] Isle of Wight study	RCT	Moderate	136 (69, 67)	Yes	Cow's milk, egg, nuts, fish NB: Also dust mite control	Lactation Solid foods	Asthma, eczema, food allergy, Sensitization (aeroallergens and food)	2, 4, & 8 y
Lovegrove 1994 [39] 1996 [40]	RCT	High	26 (12, 14)	Yes Non atopics as control	Cow's milk (dairy food restriction)	Pregnancy Lactation	eczema	6, 12, & 18 mo
Herrmann 1996 [41] Germany	Patient preference trial	High	150 recruited (I: 30: 33: 34 C: 41 able to be assessed)	Yes	Cow's milk, egg	Pregnancy Lactation	Eczema, sensitization (food)	6 & 12 mo
Appelt 2004 [42]	RCT	Unclear	497 (251: 246)	Yes	Cow's milk egg, nuts, fish	Pregnancy Lactation	Sensitization (food)	1, 2, & 7 y

Author, country / Study name	Design	Risk of bias	N	Family history of atopy*	Type of food intervention (intervention studies) or type of food exposures assessed (cohort studies) Avoidance unless otherwise stated	Timing of intervention or exposure assessment*	Outcomes	Age of child at assessment
Intervention studies: eczema management								
Cant 1986 [43]	RCT Crossover intervention	Moderate	19 mother/infant pairs	Yes	Cows milk, egg, chocolate, wheat, nuts, fish, beef, chicken, citrus fruits, colorings, preservatives	Lactation	Eczema	6 wk to 6 mo
Palmer 2008 [44] Australia	Prospective cohort then randomized, blinded maternal food challenge	Low	32 mother/infant pairs (14:16)	Yes	Egg	Lactation	Eczema	0–6mo
Uenishi 2011 [45] Japan	Prospective cohort, then maternal food exclusion challenge	High	92 mother/infant pairs	Yes	Tree-nut related foods (chocolate, coffee), fermented foods (cheese, yogurt, soy sauce, miso soup, fermented soy)	Lactation	Eczema	< 1 y
Cohort or Case Control Studies			INTAKE UNLESS OTHERWISE STATED					
Vance 2004 [46]	Prospective cohort	Moderate (some losses to follow-up)	229 mother/infant pairs	Yes	Egg	Pregnancy Lactation	Atopy	6, 12, & 18mo
Hoppu 2005 [47] Finland	Prospective cohort	Moderate (details only presented for univariate analyses)	34 mother/infant pairs	Yes	Fruit and vegetables	Lactation	Eczema, sensitization (food, aeroallergens)	1 y

Salam 2005 [48]	Nested case–control study	Low–moderate (recall bias likely)	691 (279:412)	Mixed	Fish	Pregnancy	Asthma	≥10y
Calvani 2006 [49] Italy APAL study	Retrospective cohort	Moderate (partial adjustment only; recall bias likely)	988 mother/infant pairs	295 atopic 693 non-atopic mothers	Butter Margarine Fish	Pregnancy	Sensitization (food, aeroallergens)	5 y
Camargo 2007 [50] USA (Project Viva)	Prospective cohort	Low (adjusted, low losses to follow-up	1194 mother/infant pairs	Mixed	Cows milk	Pregnancy	Wheeze	3 y
Devereux 2007 [51] Scotland	Prospective cohort	Moderate (some losses to follow-up)	1924 mother/infant pairs	Mixed	Vitamin D from food	Pregnancy	Wheeze	5 y
Fitzsimon 2007 [52] Ireland	Prospective cohort	Moderate (some losses to follow-up)	631 mother/infant pairs	Unknown	Fruit and vegetables	Pregnancy	Asthma	3 y
Romieu 2007 [53] Spain	Prospective cohort	Low (adjusted , low losses to follow-up)	458 mother/infant pairs	Mixed	Fish	Pregnancy	Eczema sensitization (aeroallergens)	1 y 4 y
Sausenthaler 2007 [54] Germany (LISA)	Prospective cohort	Low (adjusted , low losses to follow-up)	2641 mother/infant pairs	Mixed	Cow"s milk products, eggs, fats and oils, seeds, vegetables, fruit, nuts	Pregnancy	Eczema, sensitization (food, aeroallergens)	1 y 2 y
Willers 2007 [55] Scotland	Prospective cohort	Low (adjusted , low losses to follow-up)	1253 mother/infant pairs	Mixed	Fish, fruit, vegetables, grains, fats	Pregnancy	Eczema, asthma, wheeze, rhinitis	5 y
Chatzi 2008 [56] Menorca	Prospective cohort	Low (adjusted , low losses to follow-up)	482 mother/infant pairs	Mixed	Cow's milk products, cereal, nuts, meat	Pregnancy	Wheeze, sensitization (aeroallergens)	6 y
De Batlle 2008 [57] Mexico	Retrospective	Moderate (recall bias likely)	1476 mother/infant pairs	Mixed	Mediterranean diet score	Pregnancy	Asthma, wheeze, rhinitis	6–7 y
Willers 2008 [58] Netherlands (PIAMA)	Prospective cohort	Low (adjusted , low losses to follow-up)	2832 mother/infant pairs	Mixed	Vegetables, fruit fish, egg, cow's milk and milk products, nuts and nut products	Pregnancy	Asthma, wheeze	8 y
Erkkola 2005 [59] Finland	Prospective cohort	Low (low losses but some baseline differences)	1669 mother/infant pairs	Mixed	Cow's milk	Pregnancy Lactation	IgA, IgG levels	1 y
Erkkola 2009 [60] Finland (DIPP Study)	Prospective cohort	Moderate (some losses to follow-up)	1669 mother/infant pairs	Mixed	Vitamin D from food	Pregnancy	Eczema, asthma, allergic rhinitis	5 y
Miyake 2009 [61] Japan (OMCHS)	Prospective cohort	Moderate (some losses to follow-up)	763 mother/infant pairs	Mixed	Fish, meat	Pregnancy	Eczema, wheeze	1 y
Shaheen 2009 [62] UK (ALSPAC)	Prospective cohort	Moderate (some losses to follow-up)	9516 mother/infant pairs	Mixed	Dietary patterns	Pregnancy	Eczema, wheeze, asthma	2 y 7 y
Venter 2009 [63] UK	Prospective cohort	Moderate-High (Moderate losses to follow-up, not clear if adjusted)	969 mother/infant pairs	Mixed	Cow's milk, wheat, fish, peanut	Pregnancy Lactation	Food hypersensitivity	1 y 3 y
Castro-Rodriguez 2010 [64] Spain (EISL)	Retrospective cohort	Moderate (recall bias possible, missing data and some data discrepancies)	1409 mother/infant pairs	Mixed	Dietary patterns Fruit, vegetables, cereal, legumes fats and oils, fish, meat, nuts, eggs, milk, alcohol	Pregnancy	Wheeze	<1 y
Grandjean 2010 [65] Denmark (Faroe Islands)	Prospective cohort	Low (adjusted, low losses to follow-up)	564 mother/infant pairs	Unknown	Fish	Pregnancy	Eczema	5 y 7 y
Lange 2010 [66] US (Project Viva)	Prospective cohort	Low (adjusted, low losses to follow-up)	1376 mother/infant pairs	Mixed	Dietary patterns	Pregnancy	Eczema, asthma, wheeze	3 y
Oien 2010 [67] Norway (PACT)	Retrospective (during pregnancy) cohort	Moderate (recall bias possible, unadjusted)	3086 mother/infant pairs	Unknown	Fish , vegetables	Pregnancy	Eczema, asthma	1 y 2 y
Miyake 2010 [68] Japan (OMCHS)	Prospective cohort	Moderate (high losses to follow-up)	763 mother/infant pairs	Mixed	Cow's milk products	Pregnancy	Eczema, wheeze	1 y
Miyake 2010 [69] Japan (OMCHS)	Prospective cohort	Moderate (high losses to follow-up)	763 mother/infant pairs	Mixed	Vegetables, fruit	Pregnancy	Eczema	1 y
Nwaru 2010 [70] Finland DIPP Nutrition Study	Prospective cohort	Low (adjusted, low losses to follow-up)	931 mother/infant pairs	Mixed	Cereals, fruits, vegetables	Pregnancy Lactation	Sensitization (food, aeroallergens)	5 y
Saito 2010 [71] Japan (OMCHS)	Prospective cohort	Moderate (high losses to follow-up)	771 mother/infant pairs	Mixed	Cow's milk, eggs, fish, meat	Pregnancy	Eczema	3–4 mo

(Continued on next page)

Table 1 (*Continued*)

Author, Country/Study name	Design	Risk of bias	Number randomized (intervention, control) N (I, C)	Family history of atopy	Type of food intervention (intervention studies) or type of food exposures assessed (cohort studies) Avoidance unless otherwise stated	Timing of intervention or exposure assessment*	Outcomes	Age of child at assessment
Jedrychowski 2011 [72] US/Poland	Prospective cohort	Low -Moderate (losses not stated, smokers excluded)	469 mother/infant pairs	Mixed	Fish	Pregnancy	Eczema	1 y
Lumia 2011 [73] Finland DIPP cohort	Prospective cohort	Low (adjusted, low losses to follow-up)	2680 mother/infant pairs	Mixed	Fats, fish, red meat, cow's milk and milk products	Pregnancy	Asthma	5 y
Miyake 2011 [74] Japan (OMCHS)	Prospective cohort	Moderate (high losses to follow-up)	582 mother/infant pairs	Mixed	fish (mercury)	Pregnancy	Eczema, wheeze	3 y
Miyake 2011 [75] Japan (OMCHS)	Prospective cohort	Moderate (high losses to follow-up)	763 mother/infant pairs	Mixed	Dietary patterns	Pregnancy	Eczema, wheeze	1 y
Nwaru 2011 [76] Finland DIPP Nutrition Study	Prospective cohort	Low (but not clear how much maternal diet data was missing)	652 mother/infant pairs	Mixed	General diet Fats and oils, dairy foods, vegetables, eggs, fish, cereals	Lactation	Sensitization (food , aeroallergens)	5 y

ALSPAC, Avon Longitudinal Study of Parents and Children; APAL, The Lazio Association of Pediatric Allergology; DIPP, Diabetes Prediction and Prevention; EISL, International Study of Wheezing in Infants; LISA, Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood; OMCHS, Osaka Maternal and Child Health Study; PACT, Prevention of Allergy among Children in Trondheim; PIAMA, Prevention and Incidence of Asthma and Mite Allergy; RCT, randomized control trial

* Family history of atopy: Yes/No/Mixed (not selected for atopy, but parents asked about this, and results adjusted accordingly)/Unknown

incidence of atopic eczema in the offspring were compared [65]. At 5 y of age, the children with atopic eczema had lower prenatal PCB levels, indicating lower maternal fish intake, compared with those children without eczema.

7 y. No significant associations were found between childhood eczema at 7 y of age and maternal dietary patterns during pregnancy [62].

Lower prenatal PCB levels were reported in children with atopic eczema than in those without eczema, at 7 y of age, as also reported at 5 y of age [65].

Intervention studies related to management of childhood eczema. Three studies [43–45] considered manipulating the maternal diet to manage existing eczema in breastfed children, as opposed to the effects of the maternal diet on preventing eczema.

A crossover RCT [43] studied the effects of maternal exclusion of cow's milk, egg, chocolate, wheat, nuts, fish, beef, chicken, citrus fruits, artificial food colorings, and preservatives during lactation compared with periods of inclusion of cow's milk and eggs on eczema scores in children with eczema ages 6 wk to 6 mo. At each time point, there were no significant differences in eczema scores.

Eczema and sensitization to egg (on skin-prick testing) was studied in 32 exclusively breastfed babies [44]. All mothers had an egg-free diet, followed by a randomized double-blind crossover challenge with egg. An improvement was seen in the eczema score (Scoring Atopic Dermatitis) with time for both the intervention and control groups, but no statistical difference between the groups related to the egg challenge.

The effect of a non-randomized elimination diet and rechallenge with 92 exclusively breastfed children with eczema has been described [45]. Maternal exclusion of chocolate, coffee, and fermented foods (cheese, yogurt, soy sauce, miso soup, and fermented soy beans) for 8 wk during lactation was associated with improved eczema scores in their babies in 67 of 92 cases. Reintroduction of the foods, predominantly chocolate, yogurt, soy sauce, and miso soup into the maternal diet was associated with deterioration in the eczema score.

Maternal diet and asthma or wheeze in children

Intervention studies. Four RCTs [27,32,34,36] assessed the effects of maternal dietary restriction on asthma in children (Table 1). As described previously, one intervention required avoiding cow's milk and egg during pregnancy [32] and two trials continued this restriction while the mother was breastfeeding [27,34]. One study also restricted peanut and limited soy and wheat intake during pregnancy and lactation. In another study [36], the intervention was initiated postpartum, and in addition to avoiding cow's milk, egg, nuts, and fish, also included household dust mite control measures. Pooling of the four RCTs found no significant differences between maternal restricted and unrestricted diets (RR, 0.95; 95% CI, 0.70–1.30; N = 619 children) on development of asthma in children (Fig. 2).

No RCTs reported wheeze as an outcome.

Observational studies. Eighteen prospective cohort studies reported wheeze or asthma in children as an outcome. All used adjusted analyses, unless otherwise stated. Detailed reports of the study results are available in Supplementary Table 2.

Up to 2 y. A retrospective study with unadjusted analyses [67] failed to find an association between maternal consumption of fish at least once a week during pregnancy and childhood asthma.

Table 2
Risk for bias for intervention studies (N = 11)

	Overall risk for bias	Details
Cant 1986 [78]	Moderate	Randomization not reported, not blinded, moderate losses to follow-up
Falth-Magnusson 1987 [26]	Moderate	Randomization not reported, not blinded, moderate losses to follow-up
Hattevig 1989 [29]	High	Not randomized (matched), not blinded, some non-adherence (crossover)
Lilja 1989 [32]	Moderate	Randomization details not reported, not blinded, low losses to follow-up
Zeiger 1989 [34]	Moderate	Randomization details not reported, not blinded, moderate losses to follow-up
Arshad 1992 [36]	Moderate	Randomization unclear, not blinded, moderate losses to follow-up
Lovegrove 1994 [39]	Moderate	Randomization details not reported, not blinded, moderate losses to follow-up
Hermann 1996 [41]	High	Not randomized (patient preference), not blinded, moderate losses to follow-up
Appelt 2004 [42]	Unclear	No reporting about Randomization, blinding or losses to follow-up (abstract only)
Palmer 2008 [44]	Low	Randomization details not reported, blinded, low losses to follow-up
Uenishi 2011 [45]	High	Not randomized (women acted as own controls), not blinded, high losses to follow-up (most women refused to rechallenge)

Another study [64] reported that antenatal use of olive oil was significantly inversely associated with wheezing in the first year of a child's life.

An inverse relationship was found between a maternal Western dietary pattern and childhood wheeze at 16 to 24 mo, which was no longer apparent when adjusted for maternal α-linolenic acid and vitamin E intake during pregnancy [75]. Neither of the other two dietary patterns in this study (healthy and Japanese) showed any significant associations with childhood wheeze at 16 to 24 mo.

Mercury content of maternal hair samples was measured as an indirect estimate of fish intake during pregnancy; however, no significant difference was found between lower and higher mercury content and childhood wheeze at 29 to 33 mo [74].

3 y. In a retrospective cohort [52], no significant associations were demonstrated between asthma in 3-y-old children and an extensive range of fruits and vegetables eaten by their mothers during pregnancy.

A study involving 3-y-old children [66] failed to find any significant associations between the dietary pattern consumed by the mothers during pregnancy (Mediterranean, Alternate Healthy Eating Index modified for pregnancy, Western, or prudent pattern) and the development of asthma in the children. However, this study found fewer cases of recurrent wheeze in 3-y-old children of women who had a higher Mediterranean diet score compared with those with lower scores. No significant difference in numbers of children with recurrent wheeze at 3 y was observed between the highest and the lowest Alternate Healthy Eating Index modified for pregnancy quartile.

In a U.K. cohort study [62], wheeze in children at 3.5 y (and at 6 mo) was not generally associated with various dietary patterns such as health conscious, traditional, processed, vegetarian, or confectionery.

A moderate to high intake of vitamin D fortified cow's milk during pregnancy has been associated with a lower incidence of wheeze [50].

5 y. No significant associations were noted between consumption of milk and milk products, oils, margarines, butter, other fats, fish, and meat consumed by mothers during pregnancy and occurrence of asthma in their 5-y-old children [73].

No consistent associations was demonstrated between mothers' intake of total fruit, citrus, kiwi fruit, total vegetables, green leafy vegetables, fruit juice, whole grain products, fat from dairy products, or butter versus margarine/low fat spread use during pregnancy and the incidence of asthma in 5-y-old children [58]. However, it was reported that high maternal consumption of apples (>4/wk) appeared to have a protective effect for asthma in children, when assessed as physician-diagnosed asthma or "ever-had" asthma (but not if assessed as asthma and wheeze in the past 12 mo).

In a nested case–control study [48], high maternal intake of oily fish during pregnancy was found to be protective against early persistent asthma in 5-y-old children, but results for any asthma, early transient asthma, or late-onset asthma were not significant. The protective association of oily fish consumption seemed to be more effective when mothers themselves already had asthma compared with those without asthma. In contrast, this study found maternal consumption (at least monthly during pregnancy) of fish fingers (which contained trans fats) was significantly associated with an increased risk for any asthma in the children.

At 6 to 8 y of age. No significant associations were reported between mothers' dietary patterns while pregnant and development of asthma in their children at 7.5 y of age [62]. A retrospective study [57] also failed to show differences for wheeze in the children.

No association was found between the cereal content of mothers' diets and persistent or atopic wheeze in their 6.5 year old children [56].

When maternal diet during pregnancy was reviewed no significant associations were reported between the frequency of consumption of many foods (vegetables, fish, nuts, egg, milk, or milk products) and any wheeze from 1 to 8 y of age in the children [58]. Only daily, versus rare, maternal consumption of nut products such as peanut butter was associated with an increased risk for wheeze in the children.

Children with asthma at 7 y of age had slightly higher PCB exposures than non-allergic children, although this could be attributed to chance. There was no association with mercury levels [65].

Maternal diet and hay fever or rhinitis in children

Intervention studies. Two studies [34,35] examined maternal elimination of allergens from the diet and included allergic rhinitis or hay fever as outcomes.

The studies reported the results of maternal avoidance of cow's milk, egg, and peanuts during the last trimester of pregnancy and breastfeeding, with delayed and staged introduction of solid foods, compared with standard dietary advice during pregnancy and solids introduced from 4 to 6 mo of age. There were no significant differences in the rates of allergic rhinitis between dietary avoidance and control groups at 4, 12, and 24 mo of age; this was maintained at follow-up at 4 and 7 y of age.

Likewise, another study [28] reported the 5-y follow-up of a cohort of 212 children in which 104 mothers (randomized) eliminated cow's milk and egg from their diet from 28 wk

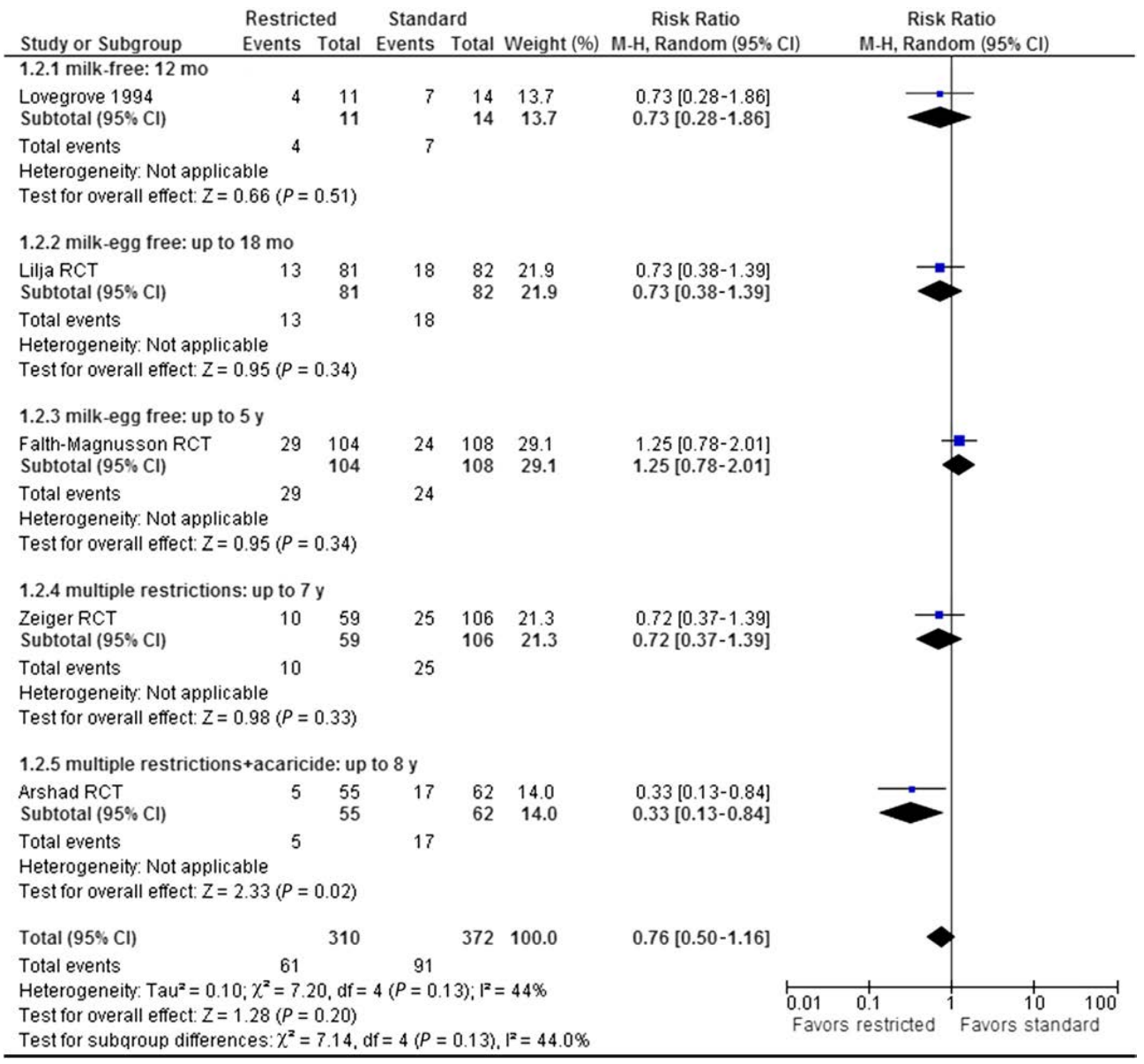

Fig. 1. Forest plot representing the meta-analysis of randomized controlled trials (RCTs) assessing the effect of maternal dietary restriction on childhood eczema.

gestation to birth (and partially during early lactation) versus 108 (randomized) to follow their usual diet (typically 0.5 L milk/d and 3–5 eggs/wk). The elimination group had extra calcium and casein hydrolysate provided. The incidence of allergic rhinitis did not differ between groups.

No meta-analysis was performed as the studies were unsuitable for pooling.

Observational studies. Four cohort studies [50,57,58,62] examined dietary associations that included allergic rhinitis in their outcomes. All studies were prospective cohorts with adjusted analyses, unless otherwise stated.

Dietary associations: diet types. One study [57] compared Mediterranean diet scores for mothers during pregnancy and reported more sneezing at 6 to 7 y of age in offspring of mothers with lower Mediterranean diet scores.

Another study [62] also assessed the intake of processed foods (meat pies, sausages, burgers, fried foods, pizza, chips, crisps, white bread, eggs, and baked beans) in the maternal diet during pregnancy and described more hay fever at 7.5 y of age in children whose mothers had a more highly processed diet, although the difference was lost when the results were adjusted for confounding factors.

Dietary associations: multiple food groups or multiple individual foods

At 5 to 7 y

Vitamin D from food in the maternal diet during the eighth month of pregnancy was looked at [60]. The lowest

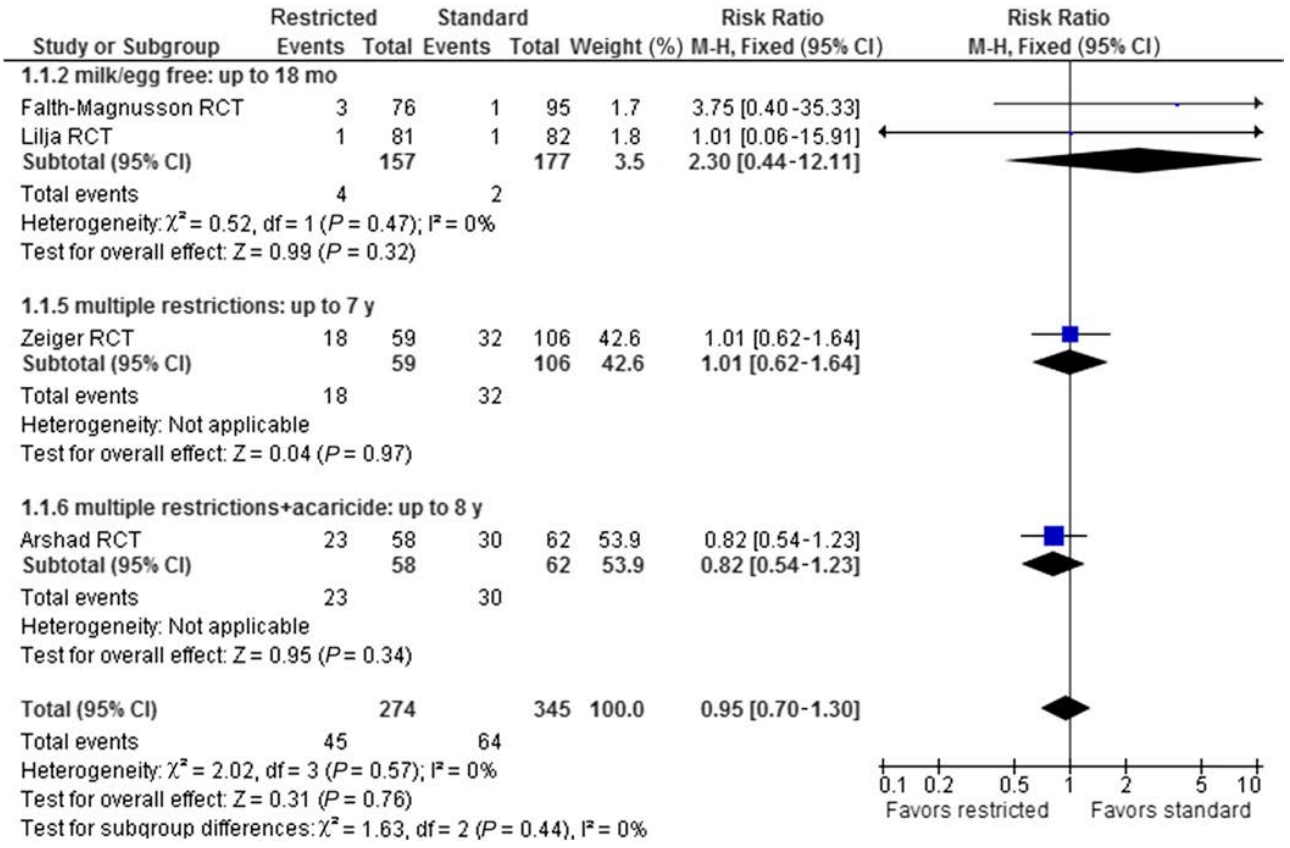

Fig. 2. Forest plot representing the meta-analysis of randomized controlled trials (RCTs) assessing the effect of maternal dietary restriction on childhood asthma.

quartiles of maternal intake of vitamin D were positively associated with allergic rhinitis in children at age 5 y. This association remained significant after adjustment for confounding variables. These results were also corrected for vitamin D supplementation.

In a study of pregnant Scottish women [58], it was reported that the mothers who consumed oily fish more than once a week during the second trimester of pregnancy (N = 1204; OR, 0.37; 95% CI, 0.14–0.98) were less likely to have children with hay fever at 5 y of age. No association was found between maternal cereal intake and hay fever.

Maternal diet and sensitization in children

Intervention studies. The results of three RCTs investigating maternal dietary restriction and sensitization in the offspring are shown in Figure 3. Differing interventions and timings outcome assessment precluded pooling, although each of the multiple restriction studies showed lower sensitization rates for the intervention compared with controls [34–36]. One study [26] randomly allocated women to a normal diet (n = 108) or one without cow's milk and egg (n = 104) from 28 wk gestation to birth and early lactation. No significant difference in sensitization was noted in the offspring up to 5 y of age, although the women on the milk and egg-free diet gained significantly less weight during their pregnancy than did the normal diet group. In 58 women with a family history of atopy, a restricted diet was followed during lactation, induction of allergenic solid foods (cow's milk, soy, egg, wheat, nut and fish) was delayed, and dust mite was controlled. At age 8 y, 37 (59.7%) children had been sensitized to one or more allergens at any time in the control group compared with 14 (25.2%) in the prophylactic group (RR, 0.40; 95% CI, 0.25–0.67) [38]. A significantly reduced rate of definite or probable food allergy in children whose mothers were in the restricted diet group compared with those from the unrestricted group (RR, 0.37; 95% CI, 0.17–0.80) was reported at 2 y, but not at 4- or 8-y follow-up [34,35].

Three other intervention studies also reported the effects of maternal dietary restriction on sensitization [29,41,42,57]. In one, no time point showed significant differences between groups for sensitization to any type of food, except for a significantly higher rate of sensitization to egg at 2 y of age in the intervention group compared with the control group (RR, 1.91; 95% CI, 1.03–3.53) [42]. The other studies [29,41] reported no significant difference in the rates of sensitization between their intervention and control groups at any of the time points measured.

The timing of the maternal dietary intervention varied although no study intervened prior to the third trimester. The type and extent of maternal dietary modifications also varied (details presented in Supplementary Table 5).

If the babies were not breast fed, five studies [26,29,34,41,42] used partly or extensively hydrolyzed formula rather than standard infant formula. The timing of introduction to solid foods and the types of solid foods that were offered also varied. In four studies [29,30,34,35], solid foods were not offered until 6 mo of

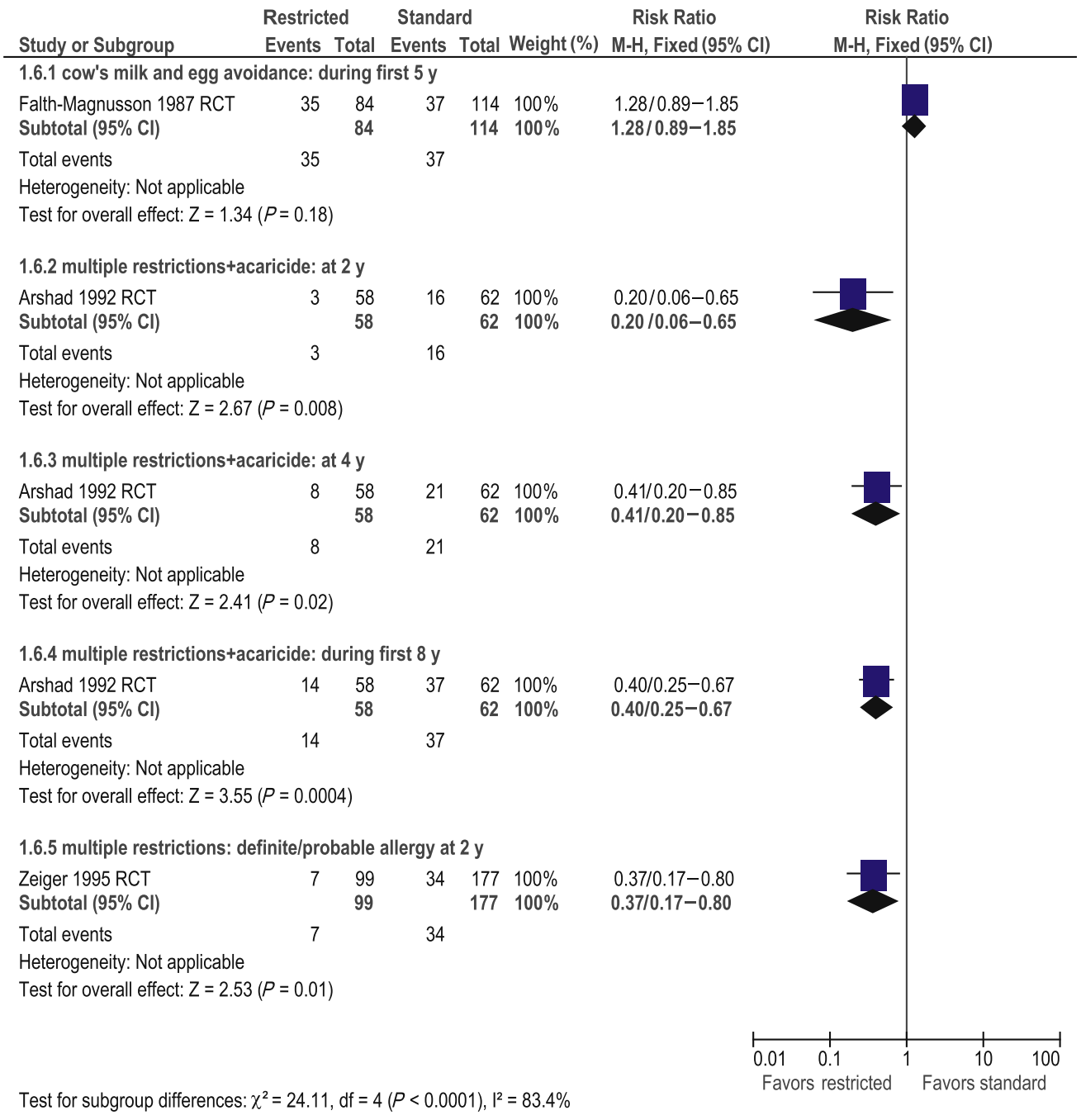

Fig. 3. Forest plot representing the meta-analysis of randomized controlled trials (RCTs) assessing the effect of maternal dietary restriction on childhood sensitization.

age, and introduction of allergenic solid foods was delayed in two of the studies [34,35] group.

Observational studies. Eight cohort studies reported the relationship between maternal food intake and sensitization to foods or aeroallergens in children as an outcome. All studies were prospective cohorts with adjusted analyses, unless otherwise stated.

Dietary associations: diet types, multiple food groups, or multiple individual foods. No studies assessed the relationship between maternal dietary types or patterns and sensitization or allergy outcomes in children younger than age 5 y.

At 5 to 7 y

Two studies [56,62] compared the type of maternal diet or patterns and sensitization or development of allergy in the offspring at 5 to 7 y.

Table 3
Summary of dietary associations by outcome*

Food type or dietary pattern	Outcome				
	Eczema	Asthma	Wheeze	Hay fever/ rhinitis	Sensitization
Fruit and vegetables	Total studies: 1 [47] ↓ risk: 1 [47]	Total studies: 1 [52]	No studies	No studies	Total studies: 2 [47,53] ↓ risk: 1 [47]
Vegetables	Total studies: 3 [54,67,69] ↓ risk: 1 (green & yellow) [69]	Total studies: 2 [58, 67]	Total studies: 2 [58,64]	No studies	Total studies: 3 [54,56,76] ↓ risk: 2 (>8 servings/wk) [56] (potato) [76] ↑ risk: 1 (celery, raw sweet pepper) [54]
Fruit	Total studies: 2 [54,69] ↓ risk: 1 (apple, citrus) [69]	Total studies: 2 [55, 58] ↓ risk: 1 (apple) [55]	Total studies: 2 [58,64] ↓ risk: 1 (fruit juice) [64]	No studies	Total studies: 4 [54,56,70,76] ↑ risk: 2 (citrus; citrus intake vs inhalants [70]; total vs inhalants) [54]
Cereal	No studies	No studies	Total studies: 2 [56,64]	Total studies: 1 [55]	Total studies: 4 [56,63,70,76]
Fish	Total studies: 9 [53–55,61, 65,67,71,72,74] ↓ risk: 5 [53–55,65,72]	Total studies: 5 [48, 58,65,67,73]	Total studies: 3 [58,64,74]	No studies	Total studies: 7 [49,53,54,56,63,65,76] ↓ risk: 1 [49]
Meat	Total studies: 2 [61,71] ↑ risk: 1 [71]	Total studies: 1 [73]	Total studies: 2 [61,64] ↓ risk: 1 [61]	No studies	Total studies: 1 [56]
Eggs	Total studies: 2 [54,71]	Total studies: 1 [58]	Total studies: 2 [58,64]	No studies	Total studies: 1 [54]
Cow's milk and dairy products	Total studies: 3 [54,68,71]	Total studies: 3 [50, 58,73] ↓ risk: 1 [50]	Total studies: 3 [58,64,68] ↓ risk: 1 [68]	No studies	Total studies: 5 [54,56,59,63,76] ↑ risk: 1 (birch) [76]
Butter	Total studies: 1 [54]	Total studies: 1 [73]	Total studies: 2 [55,64]	No studies	Total studies: 3 [49,54,76] ↑ risk: 1 (wheat) [76]
Margarine	Total studies: 1 [54] ↑ risk: 1 [54]	Total studies: 1 [73]	Total studies: 1 [64]	No studies	Total studies: 3 [49,54,76] ↑ risk: 1 (wheat & birch) [76]
Vegetable oil	Total studies: 1 [54] ↑ risk: 1 [54]	Total studies: 1 [73]	No studies	No studies	Total studies: 1 [54]
Seeds	Total studies: 1 [54]	No studies	No studies	No studies	Total studies: 1 [54]
Nuts	Total studies: 1 [54]	Total studies: 1 [58] ↑ risk: 1 (daily vs. rarely) [58]	Total studies: 2 [58,64] ↑ risk: 1 (daily vs. rarely) [58]	No studies	Total studies: 2 [54,56]
Fast food	No studies	No studies	Total studies: 1 [64] ↑ risk: 1 [64]	No studies	No studies
Dietary patterns	Total studies: 3 [62,66,75]	Total studies: 3 [57, 62,66]	Total studies: 5 [57,62,64,66,75] ↓ risk: 2 ↓ risk (Medit & olive oil [64]; Western [75])	Total studies: 2 [57,62] ↓ risk: 1 (Medit diet) [57]	Total studies: 3 [56,62,76] ↓ risk: 2 (Medit [56], traditional [62]) ↑ risk: 1 (health conscious & vegetarian) [62]
Vitamin D from food	Total studies: 1 [60]	Total studies: 1 [60] ↓ risk: 1 [60]	Total studies: 1 [51] ↓ risk: 1 [51]	Total studies: 1 [60] ↓ risk: 1 [60]	No studies

Medit, Mediterranean

* Total number of studies examining each association compared with the total number showing any association. A decreased (↓) risk is one with OR < 1, and an increased (↑) risk is one with OR > 1. Where there is no up or down arrow, no effects were noted, or $P > 0.05$. For studies examining multiple food associations, the food or dietary pattern that was associated with an allergy outcome is noted.

The first study reported that a lower Mediterranean dietary score in pregnant women was associated with more atopy at 6.5 y of age (defined as sensitization or positive skin-prick testing to aeroallergens). The researchers scored the diet of 482 pregnant women by assigning positive scores to beneficial components (vegetables, legumes, fruits, nuts, cereal, fish, and dairy products) if maternal intake was above the median; a detrimental component (meat) was scored positively if intake was below the median. This association remained significant after the child's own diet was adjusted for adherence to a Mediterranean dietary pattern. No subanalysis was reported for the mother's atopic status although adjustment was made for it.

However, different results were reported in the U.K. ALSPAC (Avon Longitudinal Study of Parents and Children) study [62], which compared dietary patterns during pregnancy and two measures of atopy at age 7 y—log total serum IgE and positive skin-prick testing to dust mite, cat, and grass. No association was found between maternal dietary pattern and childhood atopy, but the health conscious and vegetarian dietary patterns were associated with increased total serum IgE compared with a traditional dietary pattern.

Dietary associations: individual foods

At 1 year

Judging by occurrence of atopic eczema and positive skin-prick testing, a decreased risk for atopy at 1 y of age, with increasing vitamin C in breast milk, which reflected maternal intake of fruits and vegetables was described [47].

The Isle of Wight study [63] reported frequency of maternal intake of common allergens during pregnancy and lactation and food hypersensitivity in the offspring at 1 and 3 y of age. However the numbers were too small to detect any differences.

Another study [60] compared a low and high intake of cow's milk and dairy foods during pregnancy and lactation. However, the data from this study could not be used for this review because it reported IgA levels and had no clinical data relating to sensitization in the offspring.

At 2 y

The German LISA (Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood) birth cohort looked at diet during pregnancy and allergic sensitization in 2641 children, defined as specific serum IgE ≥ 0.34 kU/L to egg, cow's milk, wheat, peanut, soy, codfish, house dust, cat dander,

mixed mold, and seasonal pollen allergens [54]. Several associations were found, but it should be noted that the confidence intervals are wide. Increasing maternal intake of celery (odds ratio [OR], 1.61; 95% CI, 1.07–2.41; $P < 0.05$), raw sweet pepper (OR, 1.45; 95% CI, 1.03–2.06; $P < 0.05$), and citrus fruit (OR, 1.82; 95% CI, 1.29–2.56; $P < 0.05$) were associated with increased childhood sensitization to any allergen. Increased maternal intake of deep-frying vegetable fat (OR, 1.61, 95% CI, 1.02–2.54; $P < 0.05$) and citrus (OR, 1.72; 95% CI, 1.02–2.92; $P < 0.05$) also was associated with increased sensitization to aeroallergens. Intake of celery and citrus was associated with increased sensitization to food allergens (OR, 1.85; 95% CI, 1.18–2.89; $P < 0.05$ and OR, 1.73; 95% CI, 1.18–2.53; $P < 0.05$, respectively).

At 4 y

In a study of children at age 4 y, no association with maternal fish intake during pregnancy and specific IgE levels or sensitization to house dust mite was discovered.

At 5 y

Several studies have reported atopy and sensitization in children at 5 y of age.

More sensitization to inhalant allergens was demonstrated in children of mothers who had greater fruit and vegetable intake, particularly citrus fruit during pregnancy and the first 3 mo of lactation [70]. This remained significant after adjustment. No association was found with food allergen sensitization.

As part of the DIPP (Diabetes Prediction and Prevention) Nutrition Study in Finland [76], researchers assessed the associations between maternal intake during lactation in 652 mothers and atopy in their children (measured as serum IgE sensitization to birch, cat, timothy grass, cow's milk, egg, and wheat). After adjustment, none of the dietary variables was significantly associated with sensitization to milk, egg, or timothy grass allergens.

With foods, maternal consumption of butter and butter spreads was associated with increased risk, whereas margarine and low-fat spreads were associated with decreased risk for sensitization for wheat allergen in the children. Maternal consumption of potatoes, milks, margarine, and low-fat spreads was associated with a decreased risk for sensitization to birch. For sensitization to cat allergen, the risk was decreased by maternal consumption of potatoes and increased by consumption of eggs.

In models in which all significant uncorrelated dietary variables were studied together (foods studied separately from nutrients), butters (increased risk), margarine (decreased risk), total and ω-3 polyunsaturated fatty acids, and saturated fatty acids (increased risk) were the most strongly related to wheat allergen. Potatoes (decreased risk), eggs, and vitamin C (both increased risk) remained the most significantly related to cat allergen, whereas potatoes, milks, and margarine and low-fat spreads (all decreased risk) were the most strongly related to birch allergic sensitization. Further adjustment for number of siblings at the time of child's birth, maternal age, maternal education, and pets in the house by 1 y of age did not change any of these results.

Associations between the type of fat in the maternal diet during pregnancy and allergic sensitization in 5 y olds born to allergic and non-allergic mothers were investigated [49]. Less sensitization to common food allergens at 5 y of age with more frequent intake of fish during pregnancy in non-atopic mothers but not in the offspring of atopic mothers was reported. The researchers also reported less sensitization to inhalant allergens in 5 y olds born to atopic mothers who consumed butter once a week (OR, 0.27; 95% CI, 0.10–0.73); however, this association was not maintained for more frequent intakes (OR, 1.59; 95% CI, 0.51–4.79). In non-atopic mothers, consumption of butter once a week, but not for more frequent consumption, was associated with more inhalant sensitization in the children (OR, 1.73; 95% CI, 1.00–2.99 and OR, 0.81; 95% CI, 0.38–1.70).

At 6 and 7 y

Diet during pregnancy was studied in 482 atopic and non-atopic mothers in the Spanish island of Menorca [56]. Skin-prick testing indicated that 70 of the children were sensitized to aeroallergens. A low intake of vegetables ($\leq$8 compared with >8 servings/wk) was associated with a greater rate of sensitization to aeroallergens.

Summary of results

Eleven intervention studies were included in this review. The RCTs noted no significant difference overall in the prevalence of eczema and asthma in the children of women whose diets were free of common food allergens during pregnancy and lactation. One study [36], which had multiple interventions, including restriction of allergens in the maternal diet during lactation, delayed introduction of allergens into the child's diet, and dust mite control reported a lower rate of sensitization in the intervention group at all ages followed up. All of the RCTs testing the relationship between maternal diet and atopy outcomes were judged to be of moderate to high risk for bias, as well as low numbers of participants affecting their statistical power.

A summary of the results from the cohort studies examining the effect of maternal diet during pregnancy and lactation and atopic outcomes in their offspring is presented in Table 3. The results from 27 prospective cohort studies, 4 retrospective cohort studies, and 1 case–control study were included, involving approximately 40 000 children.

Compared with the RCTs, the prospective cohort studies looked at significantly more investigations into relationships between foods and atopic outcomes. Most studies showed no association between maternal food intake and allergy outcomes in their offspring. Maternal dietary patterns associated with less risk for allergic disease in children included Mediterranean dietary patterns, diets rich in fruits and vegetables, fish, and vitamin D-containing foods. Food patterns associated with higher risk for atopy included vegetable oils and margarine, nuts, and fast food.

Discussion

The lack of widespread and consistent influences of maternal diet on children's allergies is perhaps not surprising. Although the development of a predisposition toward allergy may be programmed early in the fetus, possibly in the first or second trimester [79], influences early in the neonatal period such as the mode of birth, feeding type, early infections, introduction to solid foods, and exposure to allergens also influence the developing immune system. Parental atopy, epigenetic factors, gut microbiota, dietary, and other environmental influences (such as pollution, tobacco smoke, and alcohol consumption) have potential to affect this programming. There is interplay between allergen exposure and other dietary components that may have the potential to influence the tendency toward atopy. This systematic review considered foods in a holistic manner rather than individual nutrients alone, aligning with messages from dietary guidelines to emphasize whole foods and dietary patterns. However, it is also useful to reflect how food-based dietary

patterns relate to the investigations of individual nutrients and findings regarding allergy outcomes, in particular related to long-chain PUFAs, antioxidants, vitamin D, and allergen avoidance.

Balance of ω-3 and ω-6 long-chain PUFAs

The types and amounts of long-chain PUFAs present in the diet have known immunomodulatory effects [80]. Diets high in ω-6 fatty acids such as vegetable oils rich in linoleic acid (18:2 ω-6) can enhance the synthesis of T-helper cell type 2 (Th2) cytokines promoting atopic responses [81]. On the other hand, diets rich in ω-3 PUFAs may alter the Th cell balance, inhibiting Th2 cell differentiation, thus moving away from development of allergy [82].

In this review, several cohort studies captured responses that may have reflected effects of differing fatty acid profiles in the diet. These included studies considering margarine, vegetable oil, and fish intake, as well as studies focusing on dietary patterns (Table 3). Four of the 10 studies investigating links between maternal fish intake (high ω-3) and eczema showed a decreased risk for associated childhood eczema; and a decreased risk for childhood asthma was seen in one of six studies. Two of the dietary profiles associated with increased total serum IgE at 7 y of age [62] (health conscious dietary and vegetarian diet patterns) were found to likely be rich in ω-6 fatty acids, particularly linoleic acid, although the diets are not described to this level of detail in the studies.

A number of clinical trials of fish oil intervention during pregnancy have reported modulation of the neonatal immune response toward a less allergenic phenotype and lower rates of eczema and sensitization in the first year of life [81,83–85]. The similarity between the cohort studies and the individual nutrient RCTs highlights the possibility that alterations in fatty acids in the food supply may be an important factor in reducing the rates of allergic disease.

Antioxidants

Foods (particularly fruits and vegetables) contain many antioxidants, including, but not limited to, vitamins C, A, and E; zinc; and selenium. Two opposing theories relate to antioxidant intake and the potential for development of atopy. The first is that dietary antioxidants are protective against oxidant damage and inflammation of airways. Decreased dietary antioxidants have been suggested as one cause of the increased prevalence of atopy in the Western world [69,86]. The opposing theory suggests that increased or excessive antioxidant intake suppresses Th1 differentiation and because of immune-regulatory mechanisms, promotes the development of a Th2 profile (toward atopy) [86].

Several cohort studies focusing on the mother's intake of foods rich in antioxidants showed protective effects against development of eczema and sensitization in their children. However, some cohort studies reported an increased risk for sensitization and others reported null findings (Table 3). None of the cohort studies found any association with maternal intake of breads and cereals (as sources of zinc) and subsequent intake of childhood atopy.

The associations between immune development and maternal fruit and vegetable intake may be partly related to their antioxidant content, although we were unable to find any RCTs using antioxidant vitamin supplements during pregnancy and lactation specifically reporting atopic outcomes in the offspring. Note, however, that in addition to vitamins and minerals with antioxidant potential, fruits and vegetables also contain a range of phytonutrients with the potential to interact with the immune system [87].

Vitamin D

Vitamin D is a hormone with multiple biological roles, including immunoregulation. There is increasing interest in the link between low vitamin D status as a risk factor for the development of atopy, including food allergy [88]. Vitamin D status is derived from exposure to sunlight, natural food sources, vitamin D-fortified foods and dietary supplements. This review includes two studies assessing vitamin D from foods [51,60]. Some studies also showed associations between intake of cow's milk and reduced atopy. As some countries fortify cow's milk with vitamin D, such associations may indirectly reflect vitamin D intake.

One RCT evaluating the effect of vitamin D supplementation in 180 women during late pregnancy reported no difference between children in the supplemented or unsupplemented groups for wheeze, allergic disease, lung function, or allergic inflammation in the first 3 y [89]. The outcomes of other vitamin D supplementation intervention trials currently underway will provide information for future evidence-based dietary guidelines related to vitamin D in the diet and development of atopy.

Allergens in the maternal diet

Several small RCTs tested the effect of maternal avoidance of common dietary allergens during pregnancy and lactation in families with a history of atopic disease and the subsequent development of atopy in their offspring. None of the RCTs found a difference in asthma, hay fever, and rhinitis in children of women who followed restricted diets. Pooling of these RCTs found no difference in asthma and eczema in offspring of women who followed restricted diets. The only RCT to show any difference in maternal restriction of food allergens and the development of eczema in children also reduced exposure to house dust mite [36]. A difference in overall rates of sensitization in the children of the allergy-avoidance group was demonstrated in their first 8 y. One study [34] reported a significantly reduced rate of food allergy at 2 y of age, but not at other ages studied.

Some of the cohort studies compared high and low maternal intakes of common allergens and atopic outcomes in their children. For example, comparing high versus low intakes of common allergens and atopic outcomes (eczema and sensitization to food or aeroallergens) in children at 1 and 2 y of age, no difference was noted between groups for cow's milk, eggs, and nuts but was less eczema was reported in the children at 1 y of age whose mothers had high fish intake [54]. The sensitization rates reported for these groups showed no differences. A similar finding was described with maternal fish intake and eczema in children [53]. Other association studies investigating intake of common allergens failed to show any relationship [71]. Overall, there were no clear patterns, but emerging trends suggest that higher intakes of fish [53,54] and nuts [58] during pregnancy may reduce allergy risk in some populations.

Differences in outcomes between allergy-avoidance intervention studies and observational studies may be related to varying study designs (as well as selected population groups

versus general population studies, and cointerventions), and low numbers leading to lack of statistical power. More work is needed to strengthen recommendations regarding the inclusion of common allergens in the maternal diet during pregnancy and lactation as this continues to cause confusion for health professionals and families. Feeding guidelines for prevention of food allergy have moved to an emphasis on promotion of induction of tolerance by inclusion of allergens early into the child's diet rather than avoidance of allergens [90,91]. Inclusion rather than exclusion of allergens in the maternal diet also may play a role in early immune programming.

Maternal diet during lactation as a treatment for allergies presenting in breastfed babies

Four trials investigating the use of maternal diet during lactation to manage known food allergy in the breastfed infant were also captured in this review. Although this is a separate issue to the etiology of allergy, allergens have been isolated in breast milk and maternal diet adjustment is commonly used to manage food allergies in a breastfed infant. The studies reviewed tested breastfeeding infants with atopic eczema for sensitization to allergens, which were then removed from the maternal diet. Maternal dietary restrictions of known allergens during lactation were associated with reduction in the infant's eczema scores. Our search did not locate any trials that evaluated maternal dietary restriction for non-IgE–mediated allergies in infancy, or for colitis, although this is common practice and an area requiring more research.

Limitations of this review

This is a difficult area to synthesize and so with data from many different sources, there were several limitations. Additionally, the "atopic march" means that children develop different symptoms as they age (eczema and food allergies when young, and then asthma and hay fever as they age). For the cohort studies, the methodology used to gather data on maternal dietary intake varied. Many studies relied on retrospective dietary recall questionnaires that are less accurate than dietary data collected prospectively. The studies were heterogeneous in terms of the atopic potential of the participants. The atopic history of the parents often was not stated, or was adjusted out in the final analysis, making comparisons difficult and possibly masking associations specific to those who have an atopic tendency.

Many studies were subsets of larger cohort studies where atopy was not always a primary consideration (e.g., the DIPP study investigating diabetes). Most of the cohort studies followed children from the same country and differences in local eating patterns may restrict the generalizability of the outcome data (e.g., one Japanese study compared low versus higher dairy food intake, but their highest quartile only consumed 280 g/d of dairy product [68]). The scoring systems used to rate Mediterranean dietary patterns varied [57,66], making direct comparisons difficult.

The primary allergic outcomes in the children were not standardized in terms of diagnostic criteria and age at follow-up or diagnosis. Some studies reported medically diagnosed outcomes using standardized criteria, often ISAAC (International Study of Asthma and Allergies in Children) criteria [2], thus enabling more accurate comparisons between studies. Others reported results of parental questionnaires as the primary outcomes. Many of the cohort studies did not follow children beyond 1 y of age. As the natural progression of atopic disease is that different manifestations are expressed at different ages—eczema, food hypersensitivity, asthma, and then hay fever—studies should plan and fund for long-term follow-up, although it is expensive and participant drop out rates can be high. When sensitization was used as an outcome, the methods of diagnosis varied, with studies reporting outcomes of serum specific IgE measures or skin-prick test results to common allergens. Where sensitization to food allergens is reported as an outcome, it is difficult to draw clear conclusions, as positive tests may not be accompanied by allergic signs when the food is ingested. The gold standard for diagnosis of food hypersensitivity is the clinical outcome on oral challenge to the food. For large studies, this is difficult and costly.

Areas for future research

The results of this systematic review support current policy documents recommending that no specific restrictions in the maternal diet during pregnancy are indicated to prevent the development of atopic disorders in the newborn at this time [90–92].

The influence of maternal diet on childhood allergy continues to be a question addressed by researchers, particularly in the form of large cohort studies such as the Danish National Birth Cohort. We have judged that results from relevant studies published after our search cutoff date would not have substantially changed our findings and conclusions. However, some notable examples are possible attenuation of a link between high maternal fish consumption and reduced eczema risk [93]; alongside a potentially stronger association between high fish intake and decreased risk for asthma [94]. Two subsequent studies have reported links between maternal shellfish intake and increased risk for eczema [93], and also food allergy in children [95]. Associations with maternal intake of dairy products remain unclear, exemplified by one study that reported both risks and protective effects on asthma [96]. A new finding of a link between maternal consumption of artificially sweetened (but not sugar sweetened) soft drink and increased risk for asthma has been reported [97].

A well-balanced diet rich in fruits and vegetables, fish, and vitamin D-containing foods may be beneficial and should be investigated in further studies. Nutrient interactions with the immune system are complex, multifactorial, and may be related to genetic susceptibility, interactions with gut microbiota, and other dietary and environmental factors. As a single simple answer or solution is unlikely, a combination of study designs investigating individual nutrients, foods, and dietary patterns may be most useful in understanding the effect of maternal diet on allergy outcomes in the offspring. The links beginning to appear between maternal fish consumption and ω-3 long-chain PUFAs deserve further investigation in populations at high and normal risk for allergic disease.

The regular inclusion of fresh fruits and vegetables and dietary sources of vitamin D in the maternal diet also warrant further investigation. As food composition varies, data about a combination of foods, nutrients, and dietary patterns may provide the most useful information.

Additionally, any further studies should report parental atopic status, particularly the existence of maternal allergy, as well as relevant environmental variables.

Conclusion

The development of atopic disease is complex and multifactorial, depending on genetic potential along with many environmental influences. This review found no consistent link between maternal dietary intake and atopic outcomes in their children despite considering 11 intervention studies and 31 cohort studies including more than 40 000 children. Dietary patterns more likely to be associated with less risk for allergic disease include Mediterranean dietary patterns, diets rich in fruits and vegetables, fish, and vitamin D. Food patterns associated with higher risk for atopy included vegetables oils and margarine, nuts, and fast food. These findings require further evaluation.

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Supplementary data

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Appendix

Allergy search strategies

MEDLINE (and as adapted for EMBASE): From inception to end of August 2011

1. prenatal or antenatal or lactat* or breastf* or maternal or mother or pregnan*
2. intake or consumption or food or diet or wheez* or dermatitis or eczema or atop* or asthma or allerg* or food allergy sensitivity or IgE
3. 1 and 2

COCHRANE LIBRARY (last searched August 2011)

(prenatal) or (antenatal)
(lactat*) or (breastf*) or (maternal) or (mother) or (pregnan*)
(wheez*) or (dermatitis) or (eczema) or (atop*) or (asthma)
(allerg*)
(#1 OR #2)
(#3 OR #4)
(#5 AND #6)

CHAPTER 2

HEN'S EGG ALLERGY AND ALLERGENICITY A REVIEW OF THE LITERATURE

PREFACE

Chapter 2 reviews current literature related to the prevention, diagnosis and management of egg allergy. This chapter is the formal literature review for this thesis and the only chapter not presented in publication format.

2.1 INTRODUCTION

Food allergies are adverse immunological reactions to a food protein[1] and are classified into two categories - those mediated by immunoglobulin E (IgE) antibodies and those that are mediated by immune cells (non-IgE mediated)[1]. Although it is possible to develop an IgE mediated allergy to any food, most individuals with allergies react to one or a combination of nine common foods: cow's milk, soy, egg, wheat, peanut, tree nuts, sesame, fish and shellfish[15]. The most common symptoms associated with food allergy in children include urticaria, angioedema, eczema, enterocolitis, enteropathy, irritability, vomiting, diarrhea and anaphylaxis[1].

Unlike airborne allergies where the incidence has stablilised in Western countries evidence suggests the incidence of food allergies may be increasing[16] and it is estimated globally that about 220-520 million people may suffer from food allergy[1]. Why young children are becoming increasingly sensitized to food allergens is a matter of debate. Several factors may be involved, including whether the child is breastfed from birth[17], the child's intake of antioxidants[18], the balance of ω-6 to ω-3 polyunsaturated fatty acids in the diet[19] and environmental influences such as microbial exposure, cigarette smoke, and other pollutants[20]. The tendency towards atopy may be programmed in utero and as such the influence of these factors may extend into the uterine environment[4].

Food allergies are one of the first manifestations of allergic disease and significantly impact on general health perception, parental emotional distress and family activities[3]. Young children in particular are at risk of developing food allergy, with up to 10% of toddlers affected compared with 1 to 2 % of adults[1, 2]. Many children outgrow their food allergy, however recent data suggest that some common food allergies persist to late childhood and early adulthood[21, 22] and individuals with early food allergy may go on to have airway hypersensitivity resulting in asthma and allergic rhinitis in adulthood[23] magnifying the burden to the individual, the family and the health care system. Allergic disease poses a

major burden to health care costs in every country[1]. It is estimated that the overall economic cost of food allergy in the USA is $24.8 billion annually[24]. In 2007 allergic disease cost the Australian economy $7.8 billion and the estimated cost due to reduced quality of life (the burden of disease) to Australians who suffer from allergy was $21.5 billion, approximately double the figure for arthritis ($11.7 billion)[25].

Food allergy is diagnosed on the basis of clinical history suggestive of a reaction to the food and in the case of IgE-mediated food allergy demonstration of specific IgE (sIgE). However it is possible for sIgE to be positive even if an individual tolerates the food protein[16]. Diagnosis for non-IgE mediated allergy is more difficult as the history needs to be supported by removal of the potential food allergens from the diet for a period of one to six weeks. If symptoms are improved, then oral food challenges with that food protein should be performed to confirm the diagnosis.

Egg allergy is the most common IgE mediated allergy in Australian children[2] and although avoidance of egg in the diet is not nutritionally compromising it imposes a significant burden. Removal of egg from the diet restricts a range of foods with important textures and may delay achievement of food texture milestones for some young children. The aim of this chapter is to review the natural history of egg allergy, and summarise current knowledge regarding egg allergenicity, and the effect of food processing on egg allergenicity. The prevention, diagnosis and management of egg allergy is reviewed, in particular the use of oral food challenges in the diagnosis and management of children with egg allergy. The use of egg sIgE levels, as determined by skin prick test or serology, to predict suitable candidates for oral food challenges is also reviewed. Current dietary strategies to manage egg allergy are discussed, and the potential for oral immunotherapy as a management strategy is introduced.

2.2 HEN'S EGG ALLERGY

Estimated prevalence figures for IgE mediated egg allergy range from 0.5% to 2.5% of young European children[26] and up to 8.9% of Australian infants[2].

The European incidence figures were based on a meta-analysis performed for EuroPrevall[26], and included six studies reporting children who were both symptomatic and sensitised to egg (on the basis of SPT or positive egg sIgE levels) reporting the prevalence of egg allergy ranging from 0.2% to 7%[27-31] and three studies confirming the diagnosis using double blind placebo controlled oral food challenges to egg allergy reporting a prevalence of 0 to 1.7%[30, 32, 33]. The variation in reported rates highlight the importance of using objective measures of food allergy in combination with measures of sensitisation instead of self-reported outcomes.

The Australian prevalence figures, published by the HealthNuts study group in Melbourne[2], were based on a study of 2848 one year old children, not selected for a family history of food allergy, who had positive skin prick test results and positive open oral food challenge to raw egg white. This incidence is considerably higher than the previously reported rate of 3.2% derived from the Melbourne Atopy Cohort Study, a birth cohort of 620 Australian children at risk of developing food allergy with SPT to egg >6mm[34].

Clinical tolerance to egg, defined as the ability to consume egg without a clinical reaction, is usually gained by two thirds of children with egg allergy by 5 years of age[35], however recent evidence suggests an increasing persistence of egg allergies with children gaining tolerance later in childhood[36-38].

In the clinic setting tolerance to egg may be suspected if a child has had an accidental exposure to a food containing egg without a reaction or the child may be offered a formal oral food challenge to egg to ascertain if they have gained tolerance to egg. A reaction to egg protein is affected by many factors including the form (egg white or egg yolk), dose,

allergenicity of egg protein (in turn affected by food matrix, processing and heating), and augmenting factors (for example co-existent asthma). Because of these multiple factors standardisation of egg challenges within a study context is problematic. This makes it difficult to compare challenge studies which document tolerance to egg as there is no uniformly accepted definition of egg tolerance, for example which specifies the form and dose required to demonstrate tolerance. Clinical tolerance to egg is defined by some allergy clinics as the ability to consume raw egg without an allergic reaction whereas other clinics define tolerance as the ability to consume lightly cooked egg and challenge with cooked egg (boiled, scrambled or fried egg), as raw egg is not commonly consumed in some countries[13]. If a child passes an oral food challenge with raw egg they will tolerate other forms of egg, but if a child passes a cooked egg challenge they may still react to raw egg[37].

When the resolution of egg allergy is studied there may be significant selection bias[38], depending on the cohort. A cohort derived from a general population may outgrow their allergy sooner compared with a cohort from a population referred to an allergy clinic as the general population cohort may have milder phenotypes of egg allergy. Children with comorbidities such as eczema and asthma may take longer to outgrow their allergies than those with egg allergy alone[38].

Three cohort studies examined the development of tolerance to egg with age[36-38]. Savage[36] reported the results of a retrospective chart review of 881 individuals with egg allergy referred to a tertiary medical center. Individuals were included if they had a clear clinical history of an IgE mediated allergic reaction to egg ingestion or an egg IgE >2 kUA/L without known tolerance to egg. Tolerance to 'concentrated egg' (1 whole cooked egg) was achieved in 4% of children by 4 years of age, 12% by 6 years of age, 37% by 10 years of age and 68% by 16 years of age. Children with increased concentrations of egg white specific IgE at all ages, and those with egg white sIgE greater than 50 kUA/L were unlikely to develop tolerance to egg by 18 years of age. The median time to develop tolerance was

consistently greater in children with other atopic disease (asthma, allergic rhinitis, eczema and other food allergies). The children the cohort described by Savage[36] appeared to gain tolerance to egg later than that described by Sicherer et al[38] who studied a cohort of 213 egg allergic children enrolled at 3 to 15 months of age in a multicenter observational study designed to assess the natural history of egg allergy up to the age of 6 years. In Sicherer's cohort the children had likely egg or milk allergy at enrollment and were at risk of peanut allergy, but without current peanut allergy. Approximately one half tolerated cooked egg (scrambled egg or French toast) by 6 years of age, defined as egg tolerance by these authors (as opposed to tolerance of raw egg). Children less likely to have gained tolerance to egg had higher egg sIgE levels, more severe atopic eczema and systemic manifestations when exposed to egg. The third cohort was that reported by Clark et al[37]. This longitudinal study of 95 egg allergic children recruited from a paediatric allergy clinic also included self-referrals from a national patient support group. The children had annual oral food challenges with well-cooked (sponge cake) and raw egg. Consistent with the results of the other cohorts, tolerance was gained more quickly to well-cooked than to uncooked egg (median 5.6 vs 10.3 years). Nearly one third had resolved allergy to well cooked egg at 3 years and two thirds at 6 years of age. Only one half of this cohort demonstrated resolution of allergy to raw egg by ten years of age[37].

In summary, hen's egg allergy is common, with an estimated incidence of up to 9% of children. Tolerance to well cooked egg is usually achieved before tolerance to partially cooked or raw egg. Most children gain tolerance to cooked egg during their primary school years, but may not gain tolerance to raw egg until the late teens or into early adulthood[36, 37]. Children less likely to gain early tolerance to egg are more likely to have other atopic conditions, higher egg sIgE levels, and systemic reactions to egg upon oral food challenge[38]. Understanding of the prognosis of egg allergy in different patients is

important, as this will help clinicians target children who may benefit from potential management strategies such as specific oral induction of tolerance protocols[38].

2.3 CAN EGG ALLERGY BE PREVENTED?

Several strategies, reviewed below, have been investigated to specifically prevent egg allergy in children at risk of developing allergies. The first group of strategies limited the baby's exposure to egg protein, either indirectly, via maternal avoidance during pregnancy and lactation, or directly, by delaying the introduction of egg into the child's own diet. In the last few years the focus has shifted to studies investigating early introduction of egg protein into the baby's diet to develop early tolerance.

2.3.1 EGG AVOIDANCE DURING PREGNANCY AND LACTATION

As reviewed in Chapter 1, eight randomised controlled trials (RCTs) have examined the effect of avoidance of egg in the maternal diet either during pregnancy[39-43]; pregnancy and lactation[44-50] or lactation alone[51-56]. The only RCT to show any difference in maternal restriction of food allergens and the development of eczema in the offspring also reduced exposure to house dust mite[57]. Arshad et al[57] also reported a difference in overall rates of sensitization in the offspring of the allergy avoidance group in their first eight years. Zeiger et al[42] reported a significantly reduced rate of food allergy at two years of age, but not at other ages studied[58].

Four association studies considered maternal intake of egg during pregnancy. Two studies investigated links between maternal egg intake and the development of eczema in their offspring[59, 60]; one investigated the maternal intake of egg and development of asthma[61]; two investigating development of wheeze in children[61, 62]; none considering hay fever or rhinitis and one considering atopic sensitization (to any allergen)[60]. Despite the large number of mother / child pairs investigated when the studies were combined, no trends

were noted between maternal egg intake during pregnancy or lactation and development of atopy in their children at all ages assessed[58].

2.3.2 TIMING OF INTRODUCTION OF EGG INTO A CHILD'S DIET

In babies at risk of developing food allergies (defined as one or two first degree relatives with atopy) delayed introduction of egg into the child's diet until after 12 months was promoted in infant feeding guidelines[63]. However, this approach has no effect on the incidence of food allergies, and guidelines for introduction to solid foods now state that there is no evidence that delayed introduction of any allergen is beneficial in preventing later development of allergy[5, 64-66].

The HealthNuts study[67] reported that delayed introduction of egg until 10 to 12 months of age (adjusted odds ratio, 1.6; 95% CI, 1.0-2.6) or after 12 months of age (adjusted odds ratio, 3.4; 95% CI, 1.8-6.5) was associated with significantly higher risk of egg allergy compared with earlier introduction at 4 to 6 months of age. There is emerging evidence based on RCTs that deliberate early introduction of allergenic food proteins may ameliorate development of allergy. The STAR study (Adelaide) reported that early introduction of egg induces immune tolerance pathways and reduction in egg allergy incidence in infants with a history of eczema[7]. There are at least 3 other RCTs (trial registry details: ACTRN 12610000388011, ACTRN 12611000535976, and JPRN-UMIN000008673) investigating early regular egg exposure to reduce the risk of egg allergy development and the results of these studies are due to be reported in 2015/2016.

The LEAP study[6] investigated the development of peanut allergy in children with early peanut introduction (before 12 months) compared with avoidance of peanut until 5 years of age in two groups of children: a group with negative peanut SPT and a group of children sensitised to peanut with skin prick test weals of 1 to 4 mm. Significantly more peanut allergy was reported in the groups with continued avoidance of peanut (13.7% in the previously unsensitised group and 35.5% in the group with early sensitisation) compared

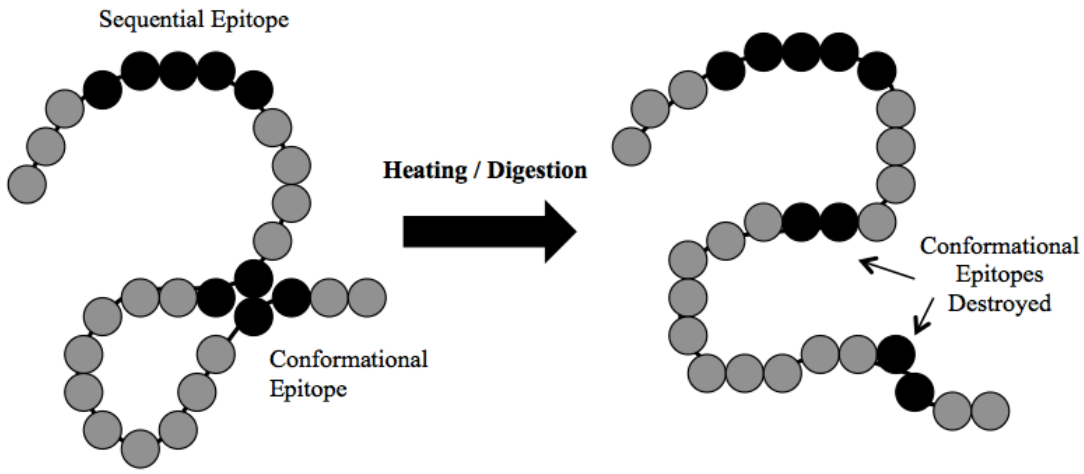
with the early introduction groups (1.9% in the unsensitised group and 10.6% in the sensitised group).

The development of allergy is complex and may be influenced by many factors including genetic potential and environmental factors in utero and during the neonatal period[5]. Early rather than delayed introduction of allergenic proteins, including egg, may promote early tolerance and the results of current RCTs investigating this theory will provide greater evidence to inform guidelines around infant feeding. The influence of whole foods (including, but not limited to egg) in the maternal diet in pregnancy and lactation on the development of atopy in their children was reviewed in Chapter 1.

2.4 HEN'S EGG ALLERGENS

Allergens are proteins or peptides containing IgE binding sites, or epitopes[68]. Epitopes may be formed from several amino acids in a row ('sequential' or 'linear') or formed as part of the protein folds held together by electrostatic charge in the secondary, tertiary or quaternary structure of the protein ('conformational')[69, 70] (see Figure 2.1).

FIGURE 2.1 EFFECT OF HEATING AND DIGESTION ON ALLERGEN EPITOPES



Legend to Figure 2.1 The effect of heating or digestion on sequential and conformational epitopes (modified from Alessandri et al)[69].

Eggs contain 12.7% total protein[71], composed of many different proteins, present in both the egg white and yolk. Although any egg protein has the potential to act as an allergen, six major egg allergens have been identified and are given the prefix "Gal d" *(Gallus domesticus)*. These include ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3), ovomucin, lysozyme (Gal d 4), and the egg yolk proteins chicken serum albumin or α-livetin (Gal d 5)[72] and the vittelogenin-1 precursor, YGP42 protein (Gal d 6)[73]. Hen egg riboflavin binding protein has been identified as a novel IgE binding peptide[74], and it is likely that other potential egg allergens will be identified. Many egg proteins are

glycosylated, making the protein structure complex and difficult to investigate[70]. The structure of the allergen epitopes has been described for ovomucoid and ovalbumin but not for the other egg allergens[8].

2.4.1 EGG IN FOOD AND THE EFFECT OF FOOD PROCESSING ON THE ALLERGENICITY OF EGG PROTEINS

Egg, or purified components of egg may be added to foods for functional or organoleptic reasons and it is for this reason that egg appears in many different food types. For example, lysozyme is used in the food industry to maintain product quality and to prevent spoilage; phosvitin is used for antibacterial and emulsifying properties; ovotransferrin is used as an antioxidant[8, 75]. The IgE reactive peptide in hen egg riboflavin binding protein decreases the perception of bitterness in foods[74, 76].

Common cooking processes including heating, acidulation and mixing can affect the allergenicity of proteins in foods[8]. The differences in allergenicity are, in part, caused by disruption of the hydrogen bonds responsible for the secondary protein structure, which in turn destroys the conformational epitopes as the protein is unfolded when it is heated and thus affecting the ability of the IgE molecule to bind[77]. In the case of egg, heat treatment destroys the conformational epitopes that some individuals form IgE against, thus allowing ingestion of the egg without any adverse reaction. In addition to altering conformational epitopes, heating egg protein with wheat (for example, in a cake) forms a matrix as the disulphide links to gluten proteins during baking. This alters the digestibility of the proteins by affecting their ability to cross intestinal epithelial cells[78] and also causes the IgE binding sites to become less accessible by blocking the IgE binding sites and decreasing the relative allergenicity of the protein[70, 78-80].

Of all the egg proteins ovomucoid is the most stable against heat and digestion[78]. The egg yolk allergen gal d 6 is stable to heat, but not digestion[73]. Ovalbumin and the other major egg allergens ovotransferin and lysozyme are more susceptible to heat treatment[81]. There

is interest in technologically altering egg protein by heating and enzymatic hydrolysis to engineer low egg allergen products for use by the food industry - these may also have potential for use in oral immunotherapy for egg allergy[8, 82].

The characteristics of the major egg proteins and allergens including their protein family, biological function and IgE binding activity when heat treated or exposed to digestive enzymes are summarised in Table 2.1.

TABLE 2.1 CHARACTERISTICS OF MAJOR EGG PROTEINS AND ALLERGENS

Protein	**IUIS Allergen Nomenclature**	**Molecular Weight (kDa)**	**Protein Family**	**Biological Function**	**Allergen epitopes reported**	**IgE Binding Activity**	
						Heat Treated	**Digestive Enzyme Treated**
EGG WHITE PROTEINS							
Ovomucoid	Gal d 1	28	Serine protease inhibitor	Serine protease inhibition	yes	stable	stable
Ovalbumin	Gal d 2	45	Serine protease inhibitor	Storage protein?	yes	unstable	unstable
Ovotransferrin / Conalbumin	Gal d 3	76.6	Transferrin	Iron-binding capacity with antibacterial activity	no	unstable	unstable
Lysozyme	Gal d 4	14.3	Glycoside hydrolase family 22	antibacterial activity	no	unstable	unstable
Ovomucin		165	Contains tripsin inhibitor like domains	antiviral activities	no	unknown	unknown
EGG YOLK PROTEINS							
Phosvitin		35	Transferase?	Metal chelating agent	no	unknown	unknown
Yolk glycoprotein 42	Gal d 6	31	VTG-1 Precursor		no	stable	unstable
α-livetin	Gal d 5	65-70	Serum albumin	Binds ions, fatty acids, hormones	no	unstable	unknown
Apovitellinins I		9.5	Very low density lipoprotein	Lipoprotein lipase inhibitor	no	unknown	unknown
Apovitellinins VI (or Apovitellinins B)		170	Unknown	Lipid binding activity	no	unknown	unknown

Legend to Table 2.1: Characteristics and properties of major egg proteins and allergens. Adapted from Benhamou et al[81], Mine et al[8], Hoffmann- Sommergruber et al[83] and Amo et al[73]. kDa – kilodaltons.

2.4.2 CROSS REACTIVITY BETWEEN BIRD EGG, AND POULTRY MEAT

Cross reactivity describes the phenomenon where sIgE raised to one allergen bind or recognize proteins from another source[84] and occurs in individuals who reacts to multiple foods that share homologous proteins[85]. Cross reactivity exists between egg white and egg yolk proteins[86, 87] and usually an individual who is allergic to egg white is advised to avoid all egg protein. Cross reactivity between proteins from egg from different birds is common, and usually individuals with hen's egg allergy are advised to avoid all other bird egg[86, 88]. Bird-egg syndrome describes the association between respiratory allergy to bird antigens and food allergy to egg yolk, and occasionally poultry meat[88]. This is a rare syndrome in which individuals are usually sensitised to a-livetin (Gal d 5), found in egg yolk, feathers and in poultry meat[89].

2.5 DIAGNOSING EGG ALLERGY

IgE mediated egg allergy is diagnosed on the basis of clinical history and the presence of antigen sIgE. Skin prick testing (SPT) and sIgE to egg (and patch testing to a much lesser extent), may be used as predictive tests in the diagnosis of an egg allergy, or to predict if an egg allergic child is ready for an oral food challenge to determine if they have gained tolerance to egg. The tests are not 100% predictive of allergy, and the 'gold standard' for diagnosis of an allergy remains the double blind, placebo controlled oral food challenge (DBPCFC). However, DBPCFC are expensive to perform and there are space and resource implications and as such not everyone with a potential egg allergy can be challenged under medical observation. Therefore there is a need to rely on testing to predict who is ready to introduce egg into the diet. Clinicians also need to be sure that a child will not react if they advocate a home challenge rather than admitting them to hospital for a medically supervised challenge.

During a SPT a drop of allergen or commercially available allergen extract is placed on the skin, and the top layer of the dermis is pierced. The size of the resulting weal is measured and may be used to predict the likelihood of a reaction to an allergen[90]. The predictive value of the results varies depending on the age of the child, the type of allergen that is used (commercial extract or fresh), the type of device that is used to introduce the allergen into the skin, the site of the test and who performs the test [91]. SPT is relatively safe, inexpensive and quick to perform. For the diagnosis of egg allergy children may be skin prick tested using commercially available standardised extracts of whole egg, egg white or egg yolk. Extracts may be purified from natural allergen or single allergens may be derived using genetically engineered recombinant technology[92]. SPT to fresh egg has been promoted by some researchers in the belief that it mimics real life allergen exposure[93], but is less commonly used.

Antigen specific IgE can be detected by enzyme-linked immunoassay. Results are presented in kUA/L where A is the allergen-specific antibody. Food–specific serum IgE results are usually presented as <0.35kUA/L: 0, absent or undetectable; 0.35 to 0.69 kUA/L: 1, low; 0.71 to 3.49 kUA/L: 2, moderate; 3.50 to 17.49 kUA/L: 3, high; 17.5kUA/L to 49.99 kUA/L: 4, very high; 50 to 100.00 kUA/L: 5, very high; and >100.00 kUA/L: 6, extremely high[94].

Component-resolved diagnostics is the term used for sIgE testing based on purified allergens or recombinant proteins rather than whole egg or egg white proteins[95]. Cut off and positive predictive values for egg allergy on the basis of sIgE levels to different egg allergens have been proposed and these results may be used alone or in combination with SPT to egg to determine if an oral food challenge is required.

During an atopy patch test blotting paper soaked in allergen is left in contact with the skin for 48 hours, removed and the test is evaluated at 72 hours. The resulting erythema, infiltration and papules are scored[96]. Atopy patch testing is not routinely used for evaluation of IgE mediated egg allergy, but may be useful for detecting delayed reactions[96].

Ideally SPT and sIgE testing would predict all children with an egg allergy and would exclude a child from a deliberate challenge if there were likelihood that a reaction would occur. That is, a specific cut-off value would predict all children who would react upon oral food challenge and predict those would not react. However, in reality this is not the case and different studies have variable findings. In part this is because not all studies considering the utility of SPT and sIgE testing for prediction of egg allergy are heterogeneous and the utility of the tests varies with the age of the patient, the allergen being tested and the type of oral food challenge.

2.5.1 SKIN PRICK AND EGG SPECIFIC SERUM IGE TESTING TO PREDICT EGG ALLERGY

The aim of this section of the literature review is, taking this heterogeneity into account, to assess studies reporting skin prick test and egg sIgE levels to predict egg allergy in children with suspected allergy, or to predict tolerance to egg in children with a known egg allergy. In addition this review was also performed to inform patient selection for the randomised trial described in Chapter 6. The PubMed, Exerpta Medica database (EMBASE), Scopus and Web of Knowledge electronic databases were searched. Only studies reporting prediction of egg allergy via SPT or sIgE testing (using the Immuno CAP system) related to outcomes of oral food challenges were included and the search was limited to English language articles. The full search strategy is documented in Appendix 1.

TESTING TO PREDICT EGG ALLERGY: RESULTS OF THE SYSTEMATIC LITERATURE REVIEW

26 studies were identified that developed predictive values for SPT and /or sIgE testing against outcomes of oral food challenges in egg allergy. Five additional studies were excluded; one evaluating the effect of specific oral tolerance induction[97], one reporting average sIgE results[94], one paper related to allergenicity[98], one reporting the development of a diagnostic algorithm[99], and one with inadequate clinical detail related to the type of SPT reagent and oral food challenges used[100].

The results of the systematic review are summarised in three tables: An overview of the allergens measured, the method of testing (SPT or sIgE) and the form of oral food challenge used to assess clinical allergy in each study is presented in Table 2.2. Details related to the clinical population (number, age, clinical history), predictive testing used, oral food challenge protocol and outcomes are reported in Table 2.3 (for SPT) and Table 2.4 (for sIgE levels). Positive predictive values and negative predictive values, sensitivity and specificity are reported, when available.

OVERVIEW OF THE INCLUDED STUDIES

Seven studies considered SPT alone, four discussed sIgE levels alone, and the remaining 15 compared both SPT and sIgE levels (see Table 2.2). 13 out of 25 of the studies were retrospective case note reviews comparing the outcome of oral food challenges with SPT or sIgE results and 12 were prospective studies. Sample sizes (see Tables 2.3 and 2.4) were generally small, ranging from less than 100 participants in 12 studies, 100-250 in 13 studies. One large population based studies included more than 600 participants[101].

CLINICAL HISTORY AND AGE OF THE CHILDREN INVESTIGATED

The clinical history of the children being investigated was used to divide the studies into four groups. Two groups of studies considered the use of SPT or sIgE testing for the prediction of egg allergy, either in children from an unselected population or amongst children referred to allergy clinics with suspected IgE mediated egg allergy. The third group of studies investigated testing to predict tolerance to egg in children with existing IgE mediated egg allergy, and the fourth group of studies considered testing to predict tolerance to baked egg in children with IgE mediated egg allergy.

Most of the studies investigated children 5 years or younger (see Tables 2.3 and 2.4). Four studies investigating tolerance to baked egg enrolled older children with median ages ranging from 4.6 to 7.2 years[102-105].

TYPE OF ORAL FOOD CHALLENGE TO EGG

All of the studies compared the results of SPT or sIgE testing with the outcomes of oral food challenges to egg (summarised in Table 2.2). The oral food challenges varied considerably in terms of the form of the challenge food (eg raw, cooked or baked), total dose and dosing regimen. The egg oral food challenge protocols reported for clinical and research purposes are discussed in detail in the next section of this literature review.

TABLE 2.2 CHARACTERISTICS OF STUDIES USING SKIN PRICK TESTING OR SPECIFIC IGE LEVELS TO PREDICT EGG ALLERGY

Author / Date / Prospective (P) or Retrospective (R)	Test and Egg Allergen Assessed to Predict Outcome of Oral Food Challenge		Oral Food Challenge Vehicle
	Skin Prick Test	Specific IgE	
Sampson and Ho 1997[106] (R)	Egg extract (unspecifed)	Egg (unspecified)	Dehydrated raw egg
Sporik et al 2000[107] (R)	EW	no	Lightly boiled whole egg
Boyano Martinez 2001[35] (P)	EW / EY / OVA / OVM	EW / EY / OVA / OVM	Boiled whole egg / raw egg
Sampson 2001[108] (R)	Egg extract (unspecified)	EW	Dehydrated raw egg
Monti et al 2002[109] (P)	EW (Albumin), EY	EW (Albumin), EY* (*excluded - CAP RAST)	Raw whole egg
Celik-Bilgili et al, 2005[110] (P)	no	egg	Raw whole egg
Verstege et al 2005[93] (R)	Raw whole egg	no	Raw whole egg
Knight et al 2006[111] (R)	EW	EW	Dehydrated raw egg white
Mehl et al 2006[96] (P)	Raw whole egg	egg	Raw whole egg
Tripodi et al, 2009[112] (P)	EW (fresh and extract)	no	Raw whole egg
Ando et al 2008[113] (P)	no	EW / OVA /OVM	Cooked egg white powder / raw egg white powder
Benhamou et al 2008[114] (R)	no	EW	Cooked whole egg / raw whole egg
Dieguez et al 2009[115] (R)	EW/EY/OVA/ OVM/ovotransferrin / lysozyme	EW /EY / OVA / OVM total IgE	Hard boiled / Raw egg white / Lightly cooked whole egg
Montesinos et al 2010[116] (R)	EW / OVA / OVM	EW / OVA / OVM	Hard boiled egg white / raw egg white
Haneda et al 2012[117] (P)	no	EW / OVM	Boiled egg white
Calvani et al 2012[118] (R)	EW / EY	no	Raw egg / heated egg
Vazquez-Ortiz et al 2014 (P)[119]	EW/ OVA / OVM	EW / OVA / OVM	Raw whole egg / Boiled egg white
Marriage et al 2012[103] (P)	EW	EW / OVM	Extensively heated egg / freeze dried or fresh raw egg
Peters et al 2013[101] (P)	EW	EW	Raw egg white / Baked egg
Des Roches et al 2006[120] (P)	WE/EW/EY	no	Baked egg
Caubet et al 2012[105] (P)	no	EW / OVA /OVM	Baked egg
Cortot et al 2012[102] (R)	EW	EW / OVM	Baked egg
Faraj and Kim 2012[11] (R)	Slurry	no	Baked egg
Lieberman et al 2012[104] (R)	EW	EW	Baked egg
Tan et al 2012[121] (P)	Slurry / OVM	no	Baked egg
Bartnikas et al 2013[122] (R)	EW	OVM / total IgE / EW	Baked egg

Legend to Table 2.2: WE (whole egg), EW (egg white), EY (egg yolk), OVA (ovalbumin), OVM (ovomucoid)

ALLERGENS USED AS SKIN PRICK TEST REAGENTS OR FOR SERUM SPECIFIC IGE LEVELS

A variety of SPT reagents were used (see Table 2.3). Two studies used fresh raw whole egg but most used commercially available reagents (12 studies used egg white, two used whole egg, five used egg yolk, and four reported a combination of egg allergens (ovalbumin, ovomucoid, ovotransferrin or lysozyme) and three considered use of testing with slurries of baked egg in the prediction of tolerance to baked egg. Two studies used a commercial egg extract but did not state if this was for whole egg or egg white[106, 108].

Likewise a variety of egg allergen sIgE levels were considered (see Table 2.4), with two studies reporting whole egg sIgE, 13 reporting egg white sIgE levels and the remainder reporting egg yolk, ovalbumin or ovomucoid results.

STATISTICAL METHODOLOGY

The characteristics used to describe and evaluate the performance of the SPT or sIgE levels in predicting outcomes of the oral food challenge tests were[93, 101, 122]:

- Sensitivity - the proportion of true positives detected.
- Specificity – the proportion of true negatives detected.
- Positive predictive value (PPV) - the proportion of symptomatic individuals among test positives (or the level above which a given percentage likely that a patient will react and therefore have a failed oral food challenge).
- Negative predictive value (NPV) – the proportion of non-symptomatic individuals among test negatives (or the level below which it is a given percentage likely that a patient will not react and therefore pass an oral food challenge).
- Efficiency - the fraction of tested individuals correctly classified by the test.
- Likelihood Ratio – calculated using sensitivity and specificity of the test to determine whether a test result changes the probability that an allergy exists.

Most researchers used logistic regression to analyze the relationship between the testing and food challenge outcome and to develop fitted predicted probability curves and to determine a threshold to differentiate allergic from tolerant subjects. Receiver operating characteristic (ROC) curve analysis was used in many cases to evaluate the sensitivity and specificity of the test[122]. Ideally a test will have both a high positive predictive value and a high negative predictive value, along with high specificity and sensitivity to identify children who will tolerate an oral food challenge and to avoid labeling children with an allergy when one does not exist.

TABLE 2.3 USE OF SKIN PRICK TESTING TO PREDICT TOLERANCE TO EGG

Study Country	**Sample Size Median age (range) years Setting**	**OFC**	**Outcome of OFC Allergy prevalence (%)**	**SPT reagent Timing of testing**	**Method**	**SPT cut off (mm)**
Table 2.3 (A) SPT to predict IgE mediated Egg Allergy in Children in an unselected population						
Peters et al 2013[101] Australia	N=656 Mean age 1.1 (+/- 0.69) y Children from community	Raw egg white	445/656 positive Allergy prevalence 68%	Egg white (ALK-Abello) Timing: On the day of the OFC	PPV [95% CI] Sensitivity [95% CI] Specificity[95% CI] ROC Curves	**Egg white 4mm [3.3-5.0]** PPV 95%; NPV 44% 46% [41-50] sensitivity; 93% [89-96] specificity
Table 2.3 (B) SPT to predict IgE mediated Egg Allergy in Children, but in populations of children with suspected or existing IgE mediated egg allergy						
Sampson & Ho 1997[106] USA	N=123 Mean 5.2 (0.6-17.9) y (for all children in trial) Allergy clinic. Eczema -100%	Dehydrated raw egg	92/126 positive Allergy prevalence 73%	Glycerinated food extracts (Greer) Timing: 'at screening'	PPV NPV efficiency sensitivity specificity ROC curves	**Egg 3mm** PPV 85%; NPV 90%; 86% efficiency; 98% sensitivity; 53% specificity For a 'hypothetical, normalized' population with 10% allergy prevalence: 3mm: PPV 19%; NPV 99%
Sporik et al 2000[107] Australia	N=39 < 2 years Allergy clinic. Some with existing egg allergy	Lightly boiled egg white	29/39 positive (subgroup of below) Allergy prevalence 74%	Egg white extract (Dome Hollister Steer) Timing: Within a month pre or post the OFC	specificity ROC curves	**Egg white 5mm** 100% specificity
Sporik et al 2000[107] Australia	N=121 3 (0.1 - 12.8) y Allergy clinic. Some with existing egg allergy	Lightly boiled egg white	93/121 positive Allergy prevalence 77%	Egg white extract (Dome Hollister Steer) Timing: Within a month pre or post the OFC	specificity ROC curves	**Egg white 7mm** 100% specificity

TABLE 2.3 (cont) USE OF SKIN PRICK TESTING TO PREDICT TOLERANCE TO EGG

Study Country	**Sample Size Median age (range) years Setting**	**OFC**	**Outcome of OFC Allergy prevalence (%)**	**SPT reagent Timing of testing**	**Method**	**SPT cut off (mm)**
Table 2.3 (B, cont) SPT to predict IgE mediated Egg Allergy in Children, but in populations of children with suspected or existing IgE mediated egg allergy						
Boyano Martinez et al 2001[35] Spain	N=81 mean 1.3 (0.92-2) y Allergy clinic. Eczema - 43%	Hard-boiled egg white if tolerated progressed to raw egg white 25/81 no OFC	25 / 81 no OFC 18/45 positive to boiled EW 1 positive to boiled EW 20/47 positive to raw EW Allergy prevalence 79%	Glycerinated raw egg white extract (made by clinic) OVA / OVM extracts (Sigma) Egg yolk extract (Stallergenes) Timing: Not stated	PPV ROC Curves	**Raw egg white 3 mm:** PPV 93% NPV 86% 97% [93-100] sensitivity; 71% [49-93] specificity
Monti et al, 2002[109] Italy	N=107 1.25 (1-2) y Dermatology clinic. Eczema -100%	Raw egg white	72/107 positive Allergy prevalence 67%	Albumin (egg white) Egg yolk (Neo-Abello diagnostics, Spain) Timing: In the month prior to the OFC.	PPV	**egg white** 3 mm: PPV 94% 4 mm: PPV 94% 5 mm: PPV 100% **egg yolk** 3 mm: PPV 92% 4 mm: PPV 91% 5 mm: PPV 100%
Verstege et al, 2005,[93] Germany	N=160 1.8 (0.25 – 14.5) y Allergy clinic. Eczema - 87%	Fresh whole raw egg	101/168 positive Allergy prevalence 63%	Fresh egg Timing: Not stated	PPV NPV Efficiency ROC curves Logistic regression	<1yo: 11.2 mm PPV 95% >1yo: 13.3 mm: PPV 95% All children: 13 mm: PPV 95%
Mehl et al, 2006,[96] Germany	N= 193 1.1 (0.25 -14) y Allergy clinic. Eczema - 90%	Fresh whole raw egg	128/193 positive Allergy prevalence 66%	Raw fresh egg white Timing: Not stated	PPV Logistic regression	**Raw Fresh Egg White 9mm:** PPV 95% Reported decision points to use in conjunction with egg atopy patch test results.

TABLE 2.3 (cont) USE OF SKIN PRICK TESTING TO PREDICT TOLERANCE TO EGG

Study Country	**Sample Size Median age (range) years Setting**	**OFC**	**Outcome of OFC Allergy prevalence (%)**	**SPT reagent Timing of testing**	**Method**	**SPT cut off (mm)**
Table 2.3 (C) SPT to predict tolerance to egg in Children with IgE mediated Egg Allergy						
Tripodi et al, 2009[112] Italy & Germany	N=47 Mean 6.2 (1.1-15.7) y Allergy clinic. Eczema - 66%	Fresh raw whole egg	20/47 positive Allergy prevalence 43%	Egg white extract (Stallergenes) Fresh egg white (Serial dilutions of extract 1:1, 1:4, 1:16, 1:64, 1:256, 1:1024, 1:4096 measured after 30min) Timing: Not stated	PPV ROC curves 2x2 tables	**Egg white extract 6 mm**: PPV 100% Also presented PPV etc for fresh EW and SPT using serial dilutions.
Knight et al 2006[111] USA	N=78 Mean 5.1(1.9-14.6) y Children with egg allergy and Se Sp EW IgE <2.5KUA/L	Dehydrated egg white	45/74 positive Allergy prevalence 61%	Egg white extract (Greer) Timing: Prior to OFC	Logistic regression Developed predictive curves for SPT	**Egg white** **3mm** = 50% probability of passing OFC
Dieguez et al 2009[115] Spain	N=157 2.5(1.25-16)y Children with egg allergy	Hard boiled *then* Raw egg white *then* Lightly cooked whole egg	100/157 positive Allergy prevalence 64%	Egg white, egg yolk, ovalbumin, ovomucoid, ovotransferrin and lysozyme extracts (Bial-Aritegui Laboratories) Timing: Not stated	PPV ROC curves	**Egg white: 9mm**: PPV 95% Also presents data for other extracts at a variety of PPV values.
Calvani et al, 2012[118] Italy	N=218 Mean 4.7 (SD3.4) y Children with egg allergy	Raw egg or heated egg	Raw egg 116/192 positive Heated egg 7/ 26 (12 %) positive Allergy prevalence 60%	Extracts from Lofarma, ALK Abello, or Stallergenes. Timing: Not stated	95% PPV 100% PPV 100% PPV Logistic regression	**Egg white: 9mm**: 95% PPV **Egg yolk: 7mm**: PPV 95% **Fresh egg white: 13mm**: PPV 95%

TABLE 2.3 (cont) USE OF SKIN PRICK TESTING TO PREDICT TOLERANCE TO EGG

Study Country	**Sample Size Median age (range) years Setting**	**OFC**	**Outcome of OFC Allergy prevalence (%)**	**SPT reagent Timing of testing**	**Method**	**SPT cut off (mm)**
Table 2.3 (C) SPT to predict tolerance to egg in Children with IgE mediated Egg Allergy						
Marriage et al 2012[103] UK	N=47 4.6 (2-16) y Children with egg allergy	Extensively heated egg or freeze dried powdered egg or raw egg	24/47 positive Allergy prevalence 51%	Egg white extract (ALK) Timing: Not stated	ROC curves	**Egg white ≥3mm** optimal decision point for the prediction of persistent egg allergy
Vazquez-Ortiz et al 2014[119] Spain	N=85 Mean 8.2 (SD 2.4) y 56.2% eczema	DBPC OFC boiled egg white. Then if tolerated; raw egg	Cooked egg: 50/85 positive Raw egg: 64/85 positive	Egg White, OVA, OVM (Alk Abello)	Logistic regression ROC curves PPV Negative and Positive Likelihood Ratios	**For uncooked egg allergy: Egg white** Positive decision point 10mm (94.7% PPV, 32.2% NPV, 27.6% sensitivity 94.7% specificity) **Ovalbumin** Positive decision point 10mm (94.1% PPV, 31.7% NPV, 27.6% sensitivity 94.7% specificity) **Ovomucoid** Positive decision point 8.5 (95.6% PPV, 35% NPV, 37.9% sensitivity 94.7% specificity)
Table 2.3 (D) SPT to predict tolerance to Baked Egg in Children with IgE mediated allergy to egg						
Des Roches 2006[120] Canada	N=60 > 5y	Baked egg	16/60 positive Allergy prevalence 27%	Whole egg, egg white, egg yolk (source of reagent not stated) Timing: Not stated	t-test	**Egg white SPT >10 mm** predictive of reaction to baked egg challenge.
Cortot et al, 2012[102] USA	N=52 7.2 (2.2-18) y	Baked egg	9/52 positive Allergy prevalence 17%	Egg white extract (Greer) Timing: within a year of OFC	Logistic regression ROC curves	**Egg white SPT <10mm** 100% predictive of passing an OFC.
Faraj and Kim, 2012[11] USA	N=84 3.5 (0.5-18) y	Baked Egg	3/44 positive (1/3 anaphylaxis) Allergy prevalence 7%	Egg extract (Omega Laboratories Ltd) Slurry of egg in baked goods Timing: At clinic	Statistical method not described	**Median weal egg extracts was 5mm** (3-9mm) All SPTs with baked egg slurry were negative. NPV 94.8%

TABLE 2.3 (cont) USE OF SKIN PRICK TESTING TO PREDICT TOLERANCE TO EGG

Study Country	**Sample Size Median age (range) years Setting**	**OFC**	**Outcome of OFC Allergy prevalence (%)**	**SPT reagent Timing of testing**	**Method**	**SPT cut off (mm)**
Table 2.3 (D, cont) SPT to predict tolerance to Baked Egg in Children with IgE mediated allergy to egg						
Lieberman et al 2012,[104] USA	N=100 5.9 (1.2-19.9) y Children with egg allergy	Muffin containing 1/3 of a whole egg	31/100 positive 3 challenge inconclusive. Allergy prevalence 31%	Egg white extract (Freer Laboratories) Timing: Not stated	Sensitivity, specificity, PPV, NPV for sIgE levels, but not SPT results.	Median SPT 7mm. No difference in egg white SPT between pass/ fail groups.
Bartnikas et al, 2013[122] USA	N=169 4.7(0.15-23.15) y Children with egg allergy	Baked egg	27/169 positive (5/27 anaphylaxis) Allergy prevalence 16%	Egg white extract (Greer) Timing: Within 1 year before OFC, the most recent after OFC.	PPV NPV ROC curves Logistic regression	**Egg White: 11mm** NPV >90% EW SPT 25mm >95% specificity Unable to establish 95% PPV
Peters et al 2013[101] Australia	N= 185 Mean 1.1 (+/- 0.69 month) Children with egg allergy	Baked Egg	30/185 positive Allergy prevalence 16%	Egg white (ALK-Abello (SPT available for 167) Timing: On the day of OFC.	PPV	**Egg White: 11mm:** PPV 82%
Tan et al, 2013[121] Australia	N=143 3.8 (1.8-6.7) y Children with egg allergy	Baked egg	53/143 positive (8/53 anaphylaxis) Allergy prevalence 37%	Egg white Ovomucoid Fresh Muffin Timing: 'prior to' challenge	PPV ROC curves	**Ovomucoid SPT>11mm** PPV 100% muffin SPT <2mm NPV 100%

Legend to Table 1.3: OFC (oral food challenge), SPT (skin prick test), sIgE (Serum-Specific IgE); EW (egg white), EY (egg yolk), OVA (ovalbumin), OVM (ovomucoid), y (years), PPV (positive predictive value), NPV (negative predictive value)

TABLE 2.4 USE OF SPECIFIC IGE TESTING TO PREDICT TOLERANCE TO EGG

Study Country	**Sample Size Median age (range) years Setting**	**OFC**	**Outcome of OFC Allergy prevalence (%)**	**Allergen tested Timing of testing**	**Method**	**kUA/Lcut offs**
Table 2.4 (A) Serum Specific IgE testing to predict IgE mediated Egg Allergy in Children suspected of allergy						
Sampson & Ho 1997[106]	N=123 Mean 5.2 (0.6-17.9) y (for all children in trial) Allergy clinic. Eczema - 100%	Dehydrated egg	92/126 positive Allergy prevalence 73%	Whole egg Timing: Not stated	PPV NPV ROC curves	**Whole egg** 6 kUA/L: PPV >95%, 72% Sensitivity, 90% specificity. 2 kUA/L: PPV >90%, 89% Sensitivity, 73% Specificity.
Sampson 2001[108] USA	N=75 3.8 (0.25-14) y Allergy clinic. Eczema - 61%	Dehydrated egg	25/75 positive 40% were diagnosed on convincing history & 27% on suggestive history Allergy prevalence 33%	Egg white Timing: 'at screening'	PPV NPV	**Egg white** 6 kUA/L: PPV 96%; NPV 39%; 64% Sensitivity, 90% Specificity. Reactive if ≥ 7 kUA/L Unlikely reactive if < 0.35 kUA/L
Celik-Bilgili et al, 2005[110] Germany	N=227 1.1 (0.08-16.1) y Allergy clinic. Eczema - 88%	Fresh whole raw egg	152/227 Allergy prevalence 67%	Hens egg Timing: Prior to OFC	PPV NPV Sensitivity Specificity Efficiency ROC curves Logistic regression	**Hens egg** < 1 year: 10.9 kUA/L: PPV 95% > 1 year: 13.2 kUA/L: PPV 95% All ages: 12.6 kUA/L: PPV 95%
Mehl et al, 2006,[96] Germany	N= 193 1.1 (0.25-14) y Allergy clinic. Eczema - 90%	Fresh whole raw egg	128 / 193 positive Allergy prevalence 66%	Hens egg Timing: Prior to OFC	PPV Logistic regression	**Hens egg** At 0.35 kUA/L: PPV 79%; NPV 85%; 48% Sensitivity, 96% Specificity. Reported decision points to use in conjunction with egg atopy patch test results.

TABLE 2.4 (cont) USE OF SPECIFIC IGE TESTING TO PREDICT TOLERANCE TO EGG

Study Country	**Sample Size Median age (range) years Setting**	**OFC**	**Outcome of OFC Allergy prevalence (%)**	**Allergen tested Timing of testing**	**Method**	**kUA/Lcut offs**
Table 2.4 (A, cont) Serum Specific IgE testing to predict IgE mediated Egg Allergy in Children suspected of allergy						
Haneda et al 2012[117] Japan	N=100 1.4 (1.0-1.9) y Cohort had eczema / other food allergies or family history of allergy. No prior egg exposure.	Hard-boiled egg white Not given if children had sIgE EW or OVM ≥ 50kUA/L or higher (n=17)	33/83 positive Allergy prevalence 40%	Egg white Ovomucoid Timing: Within previous 3 months.	Logistic regression PPV	**Egg white** 10.0 kUA/L: PPV 30%, 30.0 kUA/L: PPV 65% 61.8 kUA/L: PPV 95% **Ovomucoid** 10.0 kUA/L: PPV54% 30.0 kUA/L: PPV 97% 26.6 kUA/L: PPV 95% Children with undetectable OVM sp IgE, regardless of EW sp IgE to showed an 88% chance of a negative challenge.
Table 2.4 (B) Serum Specific IgE testing to predict tolerance to egg in Children with IgE mediated Egg Allergy						
Boyano Martinez et al 2001[35] Spain	N=81 mean 1.3 (0.92-2) y Allergy clinic. Eczema - 43%	Hard-boiled egg white & if this was tolerated: raw egg white 25 / 81 no OFC	boiled egg white 18/45 positive boiled egg yolk 1 positive raw egg white 20/47 positive Allergy prevalence 79%	Egg white Ovalbumin Ovomucoid Timing: Not stated	PPV ROC Curves	**For any egg allergy: sIgE at 0.35 kUA/L:** **Egg white:** PPV 94%, NPV 68%, 91% sensitivity, 77% specificity **Egg Yolk:** PPV 98%, NPV 37%, 63% sensitivity, 93% specificity **Ovalbumin:** PPV 96%, NPV 36%, 72% sensitivity, 83% specificity **Ovomucoid:** PPV 96%, NPV 35%, 73% sensitivity, 82% specificity
Ando et al 2008[113] Japan	N=108 2.9 (1.2-13) y Allergy clinic. Eczema - 94%	Freeze dried heated egg white: (90C for 60 min). Freeze dried raw egg white	A(N=38/118) positive to heated egg white B (N=29/70) positive raw EW, negative heated EW C (N=41/70) negative to raw & heated EW Allergy prevalence 32%	Total IgE Egg white Ovalbumin Ovomucoid Timing: Not stated	ROC curves Positive and negative decision points. PPV	**For raw egg allergy:** **Egg white** >7 kUA/L PPV 95%, 95% specificity **For heated egg white allergy:** **Ovomucoid** > 11 kUA/L PPV 88%, 96% specificity

TABLE 2.4 (cont) USE OF SPECIFIC IGE TESTING TO PREDICT TOLERANCE TO EGG

Study Country	**Sample Size Median age (range) years Setting**	**OFC**	**Outcome of OFC Allergy prevalence (%)**	**Allergen tested Timing of testing**	**Method**	**kUA/Lcut offs**
Table 2.4 (B, cont) Serum Specific IgE testing to predict tolerance to egg in Children with IgE mediated Egg Allergy						
Haneda et al 2012[117] Japan	N=100 1.4 (1.0-1.9) y no prior egg exposure.	Open OFC hard-boiled egg white Not given if children had sIgE EW or OVM ≥ 50kUA/L	33 positive, including 12 multi system reactions, 3 requiring adrenaline Allergy prevalence 33%	Egg white Ovomucoid Timing: Within previous 3 months.	Logistic regression PPV	**Egg White sIgE** 10.0 kUA/L: 30% PPV, and 30.0 kUA/L 65% PPV. **Ovomucoid sIgE** 10.0 kUA/L: PPV 54% 30.0 kUA/L: PPV 97% sIgE titre indicating a 95% PPV: Egg White - 61.8kUA/L Ovomucoid - 26.6 kUA/L Children with undetectable OVM sIgE, regardless of EW sIgE had an 88% chance of a negative challenge. And those children who did react had milder reaction.
Marriage et al 2012[103] UK	N=47 4.6 (2-16) y	Freeze dried powdered egg or raw egg	24/47 positive Allergy prevalence 51%	Egg white Ovomucoid Timing: At clinic visit	Logistic regression ROC curves	**Egg white and ovomucoid** sIgE levels >0.35 kUA/L optimal decision points for the prediction of persistent egg allergy
Vazquez-Ortiz et al 2014[119] Spain	N=85 Mean 8.2 (=/-2.4) y 56.2% eczema	DBPC OFC Boiled egg white. Then if tolerated: raw egg	Cooked egg: 50/85 positive Raw egg: 64/85 positive	SPT Egg White, OVA, OVM (Alk Abello) sIgE EW,OVA, OVM IgG4 to OVA, OVM	Logistic regression ROC curves PPV Negative and Positive Likelihood Ratios	**For uncooked egg allergy: Egg white** Positive decision point 3.69 (97.5% PPV, 44.2% NPV, 58.6% sensitivity 94.7% specificity) **Ovalbumin:** Positive decision point 2.01 (97.7% PPV, 44.5% NPV, 63.8% sensitivity 94.7% specificity) **Ovomucoid:** Positive decision point 3.7 (97.3% PPV, 41.3% NPV, 52.2% sensitivity 94.7% specificity)

TABLE 2.4 (cont) USE OF SPECIFIC IGE TESTING TO PREDICT TOLERANCE TO EGG

Study Country	**Sample Size Median age (range) years Setting**	**OFC**	**Outcome of OFC Allergy prevalence (%)**	**Allergen tested Timing of testing**	**Method**	**kUA/Lcut offs**
Table 2.4 (C) Serum Specific IgE testing to predict tolerance to Baked Egg in Children with IgE mediated allergy to egg						
Caubet et al 2012[105] USA	N=107 6.9 (1.6-18.6) y Cohort described by Lemon Mule et al[13]	Baked egg	GroupA n=25 (BE reactive) Group B (n=60) (BE tolerant, cooked egg reactive or high EW SPT) Group C n=22 (BE tolerant and cooked egg tolerant)	Egg white OVA OVM OVA and OVM sp IgG_4	Logistic regression model AUROC PPV, NPV sensitivity, specificity 95%CIs Statistical model using IgE and IgG4	**Positive decision point:** **Egg White:** 26.2 kUA/L: PPV 43% NPV: 91% Sensitivity: 12 % Specificity: 95 % **Ovalbumin:** 25.3 kUA/L: PPV 33% NPV: 77% Sensitivity: 8 % Specificity: 95 % **Ovomucoid:** 12.8 kUA/L: PPV 64% NPV: 81% Sensitivity: 28 % Specificity: 95 %
Cortot et al, 2012[102] USA	N=52 7.2 (2.2-18) y	Baked egg	9/52 positive Allergy prevalence 17%	Egg white Ovomucoid Timing: within a year of OFC	Logistic regression ROC curves	**Egg white sIgE** was not a reliable predictive value in this study. Inadequate numbers of subjects had OVM sp IgE measured
Lieberman et al 2012[104] USA	N=100 5.9 (1.2-19.9) y	Muffin containing 1/3 of a whole egg	31/100 positive 3 challenge inconclusive. Allergy prevalence 31%	Egg white Timing: Not stated	Sensitivity, specificity, PPV, NPV	**Egg white sIgE** at 2.5 kUA/L: PPV 44%; NPV 89%, 87% sensitivity; 48% specificity
Marriage et al 2012[103] UK	N=47 4.6 (2-16) y	Extensively heated egg	10/47 positive Allergy prevalence 21%	Egg white Ovomucoid Timing: At clinic visit	Logistic regression ROC curves	**Egg white sIgE** 10. kUA/L: PPV 95% **Ovomucoid sIgE** 6 kUA/L: PPV 95% Se sp IgE to OVM was not superior to Egg White in prediction of allergy to baked egg.

Legend to Table 2.4: OFC (oral food challenge), SPT (skin prick test), EW (egg white), EY (egg yolk), OVA (ovalbumin), OVM (ovomucoid), y (years), PPV (positive predictive value), NPV (negative predictive value

RESULTS OF SYSTEMATIC REVIEW (CONT):

PREDICTION OF EGG ALLERGY IN CHILDREN FROM AN UNSELECTED POPULATION

One study reported the use of SPT results to predict egg allergy in children from an unselected population (see Table 2.3)[101]. The Health Nuts study tested a group of 1 year olds selected from the general population and reported a 4mm weal to egg white as having a 95% PPV and 44% NPV with 46% sensitivity in the prediction of a positive oral food challenge to raw egg[101].

PREDICTION OF EGG ALLERGY IN CHILDREN IN POPULATIONS REFERRED FOR ASSESSMENT OF SUSPECTED ALLERGY

Twelve studies reported SPT to egg allergens[35, 93, 96, 106, 107, 109, 112] and / or egg allergen sIgE levels[96, 106, 108, 110, 117] in children referred to specialist allergy clinics for assessment. Children in these studies ranged from 0.25 to 17.9 years of age and most had eczema. Eight of these studies reported outcomes of oral food challenges to raw egg[93, 96, 106, 108-110, 112, 118].

In the studies using commercial extracts for SPT, reported decision points varied from 3mm for whole egg and 3 to 6mm for egg white. Sampson and Ho[106] estimated that a 3mm weal to egg provides 85% PPV and 90% NPV with 86% efficiency and 98% specificity of a reaction to raw egg. Monti et al[109] reported a 3mm weal to egg white as having 94% PPV in the prediction of reactivity to raw egg white in a population of egg naïve children with eczema. Tripodi[112] reported a 100% PPV for a 6mm weal to egg white in a group of 47 children with an average age of 6.2 years.

Four studies (see Table 2.4) reported predictive values for sIgE levels to egg in children with suspected egg allergy and reported cut off points ranged from whole egg 2kUA/L (>90% PPV)[106] to 12.6 kUA/L (95% PPV)[110].

Sampson[108] reported a 6 kUA/L to egg white to have a 96% PPV, 39% NPV with 64% sensitivity and 90% specificity. However in this study only 33% of the children with suspected egg allergy had oral food challenges. Mehl[96] reported 0.35 kUA/L to hens egg

had a 79% PPV to predict a reaction to fresh whole raw egg, and in addition reported decision points to be used in conjunction with egg atopy patch test results.

Other studies considering prediction of reactivity to egg in children referred to allergy clinics used outcomes of oral food challenges to lightly boiled egg white[107], results of two staged oral food challenges where the children were given hard-boiled egg, progressing to raw egg white if this was tolerated[35] or results to hard-boiled egg challenges[117]. Sporik[107] reported weals to egg white of 4mm (for children under 2 years) and 7 mm (for children under 7 years) having 100% specificity in the prediction of reactivity to lightly boiled egg white. Boyano Martinez et al[35] reported a 3mm weal to raw egg white extract as a 93% PPV in prediction of reactivity to hard-boiled egg and a specific IgE to egg white of 0.35kUA/L (94% PPV) for any egg allergy in 1 year olds.

Rather than using commercially available SPT extracts two studies[93, 96] reported results of SPT to raw fresh egg in the prediction of oral food challenge to fresh whole raw egg. Both authors reported 95% PPV values but much higher weal sizes (9mm[96] and 13mm[93]) than reported in the studies previously discussed.

In summary, for populations at risk of developing food allergy, a 3-6mm weal to egg or egg white provides high PPV of reactions to both raw egg and cooked egg OFC. Egg white sIgE levels of 0.35kUA/L had 94% PPV for any egg allergy in 1 year-olds. Generally, predictive values were lower for younger ages and higher in older children.

PREDICTION OF TOLERANCE TO EGG IN CHILDREN WITH EXISTING EGG ALLERGY

Five studies reported use of SPT to predict development of tolerance in children with existing egg allergy[103, 111, 115, 118, 119] and eight reported egg sIgE levels[35, 103, 113-117, 119]. Most studies reported the outcome of oral food challenges to raw egg, although three[115, 118, 119] challenged with hard-boiled egg initially, and progressed to a raw egg challenge if this was tolerated, and one[117] only challenged with hard-boiled egg white. Mean ages ranged from 1.3 years[35, 116] to 8.2 years[119].

Two studies[115, 118] reported a 9mm weal to egg white extract as having a 95% PPV in both 2 ½ and 4 ½ year old egg allergic children and one study[119] reported a 10mm weal to egg white as having 95% PPV in 8 year olds. Knight et al[111] reported a 3mm weal to egg white as having a 50% probability of tolerating raw egg white in 5 year old egg allergic children if they also had a serum egg white specific IgE level of < 2.5 kUA/L.

The results from the seven studies reporting egg sIgE levels for the children having heated then raw egg challenges ranged from egg white sIgE of 0.35kUA/L (94% PPV) for any egg allergy in 1 year olds[35] and also persistent egg allergy in 2-16 year olds[103] to >7kUA/L (95% PPV) and ovomucoid >11 kUA/L (88% PPV) for a raw egg allergy[113], using data from a slightly older group of children. Montesinos[116] reported egg white sIgE levels predictive of reactivity to raw egg white stratified by age. Vasquez-Ortiz et al[119] reported positive decision points correlating to 95% PPV for sIgE to EW (3.69 kUA/L), OVA (2.01 kUA/L) and OVM (3.7 kUA/L) in prediction of raw egg allergy in 8 year old allergic children)

There is limited data on SPT for children with known egg allergy. Egg white SPT of 9mm are highly predictive of a positive oral food challenge in 2-4 year olds; however it would be unlikely that a child with a known egg allergy would be sent for an oral food challenge with a 9mm weal to egg white. In older children it may be useful to consider both SPT and sIgE testing as suggested by Knight et al[111] and Marriage et al[103]

PREDICTING TOLERANCE TO BAKED EGG

Many children with egg allergy tolerate foods containing baked egg protein, not raw or less well-cooked egg[13]. The inclusion of baked egg in the diets of children with egg allergy is reported to be associated with changes in immune profiles associated with development of tolerance (reviewed in Chapters 5 and 6 of this thesis). Use of SPT for the prediction of tolerance to egg in baked goods in children with existing egg allergy was investigated in eight studies[11, 101-104, 120-122]. Six studies considered sIgE levels to egg white, ovalbumin or ovomucoid to predict tolerance to baked egg[101-105, 122]. Most studies enrolled cohorts of children averaging 3 to 7 years, and one study[2] reported baked egg tolerance in egg allergic children at 1 year of age.

Two studies[102, 120] reported an 10mm weal to egg white as predictive of tolerance to egg in baked goods, and Peters et al[101] reported an 11mm weal to egg white as having an 82% PPV in a group of 1 year olds. Bartnikas[122] also reported an egg white weal of 11mm as having >90% PPV. In contrast with these studies Lieberman[104] reported no difference in the egg white SPT between baked egg tolerant and intolerant children. Tan[121] reported an ovomucoid SPT of >11 mm as having a 100% PPV in predicting failure to tolerate baked egg.

Two studies[11, 121] considered 'prick to prick' testing using a 'slurry' made of the baked egg product used in the oral food challenges, and this technique was reported to have a strong negative predictive value.

Studies considering egg white or ovomucoid sIgE levels to predict tolerance to baked egg varied in their results. Cortot et al[102] were unable to find an egg white sIgE level that had a reliable positive or negative predictive value. Lieberman[104] reported that an egg white sIgE level of 2.5 kUA/L gave 44% PPV, 89% NPV and Marriage et al[103] documented sIgE levels of 10kUA/L for egg white and ovomucoid of 6 kUA/L as having a PPV of 95% for reaction to a baked egg challenge. Caubet et al[105] reported positive decision points for 6.9

year old egg allergic children that were higher than those reported by other researchers, and with lower PPVs; egg white (26.2 kUA/L: PPV 43%), ovalbumin (25.3kUA/L: PPV 33%) and ovomucoid (12.8 kUA/L: PPV 64%).

In summary, an egg white weal of < 10-11mm has been reported by three groups as predictive of tolerance to baked egg. No consistent predictive values for tolerance to baked egg using SPT to ovomucoid or egg sIgE levels have been reported.

IMPLICATIONS FOR PREDICTIVE TESTING FOR EGG ALLERGY

Considerable variation exists in the predictive values for SPT and sIgE levels reported to inform clinicians about reactivity to egg. The aim of this review was to assess studies reporting predictive values for SPT and sIgE testing for consistency to allow comparison of the reported results. There was wide variation in the studies related to the clinical background of populations studied, their age, the SPT reagents used and the oral food challenge regimes used. Some studies selected out children with a prior history of anaphylaxis or those with very high SPT or sIgE results, making the values reported in these studies less reliable.

Accurate predictive tests are important as, along with the clinical history they allow the clinician to make decisions about the diagnosis of egg allergy, and to provide families with advice about the timing of introduction of egg into the diet. Supervised oral food challenges are expensive, and using tests with poor positive predictive value or poor specificity will result in many positive tests. If egg is to be introduced at home, rather than in a supervised manner it is essential that the likelihood of a reaction is minimal. Clinicians interpret the test results alongside clinical history and may consider more than one test result when making a decision regarding an oral food challenge. One study[111] considered the use of SPT to egg alongside egg sIgE levels, particularly for borderline levels to predict timing of oral food challenges for children with existing egg allergy and this approach may provide a more accurate means of predicting which child is ready for an oral food

challenge. The use of egg antigen sIgE: sIgG4 ratios have also been investigated and are worth further consideration[105, 119]. An algorithm to predict suitability for OFC has been developed using routinely collected clinical data (including age, sex, symptoms) along with SPT, sIgE and total IgE, and although this is more complicated it may be an efficient model to use for the selection of candidates for OFC[99].

No clear predictive values were published to inform clinicians of potential tolerance to egg in baked goods. As ovomucoid is resistant to heating and digestion, testing for sensitisation to ovomucoid has been compared to testing for other egg allergens. Overall there was a lack of consistency in the results, and some children still reacted to the baked egg oral food challenge even if they had negative SPT to egg allergens. Four studies considered sIgE levels to egg white and ovomucoid, but no consistent cut-off points to predict tolerance to egg in baked goods were reported[102-105]. The lack of a safe and predictable measure to predict who will tolerate baked egg challenges is worrying as the rate of severe reactions during baked egg oral food challenges was high. Bartnikas[122] reported anaphylaxis requiring adrenalin in 5 of 27 positive challenges, and 1 out of the 3 positive challenges reported by Faraj[11] had anaphylaxis. Several studies considered the use of SPT with products containing baked egg. Overall there was a strong negative predictive value for these tests; however in one study SPT to a slurry of baked egg failed to detect a child who went on to have anaphylaxis during an oral food challenge to baked egg[11]. The lack of a reliable test is concerning as a challenge to baked egg may be perceived as a 'safe' oral food challenge, and perhaps may be more likely to be suggested to be performed at home.

In conclusion, clinicians should be mindful of the background studies used to develop predictive values for SPT or sIgE levels to predict when egg allergic children may be gaining tolerance to egg. The age of the child is important, with lower weal sizes being more significant in younger children than in older children, and the form of egg (raw, lightly cooked or baked) used in the oral food challenge is also important.

2.5.2 ORAL FOOD CHALLENGES TO DIAGNOSE EGG ALLERGY

The 'gold standard' for diagnosis of food allergy is the double blind, placebo controlled (DBPC) oral food challenge. No SPT or blood test is 100% predictive of a reaction to a food and at some stage individuals with an adverse reaction to a food should undergo an oral food challenge. In the review of the literature related to predictive tests for egg allergy it became apparent that many different egg preparations are used in oral food challenges to egg. The aim of this section of the literature review is to summarize the dose, dosing regime and forms of egg used in protocols for oral food challenges to egg reported in the literature to diagnose egg allergy, for ongoing monitoring of egg allergy and to determine tolerance to egg after the use of specific oral induction of tolerance experiments. This is of relevance to this thesis to ensure correct standards and definitions around screening criteria and primary outcomes were adhered to when developing protocols for my primary research project, The CAKE study. Protocols for baked egg challenges and raw egg challenge protocols for the determination of tolerance to egg in egg allergic children were of specific interest.

The full search strategy used was the same as previously described, and is documented in Appendix 1. In addition, reference lists of identified papers were checked for any other studies suitable for inclusion. Studies that described protocols for oral food challenges to egg were included. Protocols from peak allergy and immunology societies were also included. There were no excluded studies. Twenty-four studies reporting oral food challenge protocols for egg were identified and summarised in Tables 2.5 and 2.6. Eight studies described protocols for challenges for more than one form of egg[2, 13, 35, 37, 115, 119, 123-125]. The egg challenges were conducted for several reasons; diagnosis of, or assessment of tolerance development in children with egg allergy or validation of predictive testing[2, 7, 30, 32, 37, 93, 96, 107-110, 112, 114, 115, 126], assessment of tolerance to different forms of egg[11, 13, 35, 102, 104, 120, 122-124] and assessment of the clinical effectiveness of specific oral tolerance induction trials[119].

TYPE OF EGG USED FOR ORAL FOOD CHALLENGES

Eighteen different forms of egg products were used for oral food challenges in the twenty-three studies reviewed (summarised in Table 2.6). The oral food challenges were divided into four groups on the basis of the extent of cooking of the egg: raw egg (egg white or whole egg - using fresh or pasteurised egg liquid or powder), lightly cooked egg (soft boiled, scrambled), well cooked (hard boiled, pancakes, waffles) or baked (cake or muffin).

Fourteen studies reported food challenges using raw egg (white, whole, pasteurised liquid or powder)[2, 7, 30, 35, 37, 96, 108, 109, 112, 115, 119, 123, 124]. Cooked egg was used in 16 studies, and the extent of heat exposure varied from lightly cooked, eg scrambled (in 3 studies)[13, 107, 126] or well-cooked eg hard-boiled egg (4 studies)[114, 115, 117, 119]. Cooking instructions were not described in one study [125]. The use of baked egg was reported in 6 studies[2, 11, 13, 37, 102, 122]. Egg cooked in the form of pancake was not reported in any studies. Most challenge protocols gave one form of egg on the day of the challenge, however three protocols started with a well-cooked form of egg on day 1 and progressed to lightly cooked or raw egg on subsequent days[35, 115, 119].

TABLE 2.5 THE FORM OF EGG, DOSE AND DOSING REGIME USED FOR CHALLENGES IN EGG ALLERGY RESEARCH

Study Author & Location	**Form of Egg**	**Dosing Regimen**	**Total Dose**	**g egg protein**	**Other Comments**
Osborne et al, 2011[2] Australia	Fresh raw egg white	Open Dosing: Drop inside lip, 0.5, 1, 2, 5, 10, 10-13ml Dosing Interval: 15 min Observation Period*: 60 min	28-31.5ml	3.1-3.5g	Egg white from a 60g egg
Jurado-Palomo et al, 2010[123] Spain	Fresh raw egg white	Open Dosing: 1/8, 1/4, 1/2 egg white Intervals: 60 min Observation Period: not stated	7/8 of an egg white Approx. 38g	4.3g	Egg white from 65-70g eggs. Used 43.72 g (as per Escudero et al 2013)[124].
Jurado-Palomo et al, 2010[123] Spain	Pasteurised raw egg white – Liquid	Open Dosing: 4, 8, 16 ml Dosing Interval: 60 min Observation Period: not stated	28ml	3.1g	33ml is equivalent to 1 egg white.
Escudero et al, 2013[124] Spain	Pasteurised raw egg white – Powder	Open 3600mg of egg powder in 43 ml orange juice. Dosing: 9 doses: 0.1, 0.2, 0.5, 1, 2, 4, 6, 9 and 20.2ml Dosing Interval: 30 min Observation Period: 2 hours	3.6g*	4.9g	3.6g powder is equivalent to one fresh egg white
Escudero et al, 2013[124] Spain	Fresh raw egg white	Open Dosing: 0.1, 0.2, 0.5, 1, 2, 4, 6, 9 and 20.2 ml Dosing Interval: 30 min Observation Period: 2 hours	43 ml	4.8g	The average volume of the egg white in a egg is 43 ml.
Boyano Martinez et al, 2001[35] Spain	Cooked egg white (boiled 10 min) Then Fresh raw egg white Egg yolk	Open Dosing: Over 5 days Day 1: 2 doses: 1/8 then ¼ of a boiled EW Day 2: 1 dose: ½ boiled EW Days 3, 4, 5: raw egg white, in similar amounts Dosing Interval: 90 min Observation Period: not stated	3/8 boiled egg white over 2 days, Then ¾ raw egg white over 3 days.	Day 1:1.8g Day 2:2.5g	For patients with history of reaction to EY performed OFC with EY using a similar regime.

TABLE 2.5 (cont) THE FORM OF EGG, DOSE AND DOSING REGIME USED FOR CHALLENGES IN EGG ALLERGY RESEARCH

Study Author & Location	**Form of Egg**	**Dosing Regimen**	**Total Dose**	**g egg protein**	**Other Comments**
Dieguez et al, 2009[115] Spain	Cooked egg yolk (hard boiled) Cooked egg white (hard boiled) Raw egg white Lightly cooked whole egg (2 min)	DBPC, however placebo was given first on each day Dosing: Over 5 days. Day 1: ¼ then 3/4 yolk, (15 min hard boiled) Day 2: ¼, ¾, 1/2 white (15 min hard boiled Day 3 & 4: 1/16 (2g), 1/8 (4g), ¼ (8g) raw egg white Day 4: 1/2 (16g), 1/2 (16g) raw egg white Day 5: 2 min cooked whole egg Dosing Interval: 1 hour (2 hours on day 5) Observation Period: 2 hours	Day 1: 1/4 cooked EY Day2: 1 1/2 cooked EW, Day 3: 14g raw EW. Day 4: 32g raw EW Day 5: 1 whole 2 min cooked whole egg	Day 1: 0.72g Day2: 7.5g Day 3: 1.6g Day 4: 3.6g Day 5: 7.44g	Blinded in potato and carrot soup.
Vazquez-Ortiz et al, 2014[119] Spain	Boiled egg white (10min) Then raw egg white	DBPC. Dosing: Day 1: 1/32 boiled EW (0.12g, 0.24g, 0.48g, 0.96g, 1.9g protein) Day 2: Raw Egg White 0.48g, 0.96g, 1.9g protein Dosing Interval: 1 hour Observation Period: not stated	Day 1: 3.7g boiled egg white protein. Day 2: 3.4g raw egg white protein	Day 1: 3.7g Day 2: 3.4g	Hard-boiled egg blended with vegetable puree. Raw egg white masked in chocolate drink.
Monti et al, 2002[109] Italy	Fresh whole raw egg**	Open Dosing: One egg in 200ml milk or formula. Not graded. Dosing Interval: 1 day. Observation Period: 32 hours in hospital, then 8 days controlled observation at home.	1 whole egg	7.6g	**Mixed with 200ml hot cow's milk or formula. Partly cooked? (60g egg)
Tripodi et al, 2009[112] Italy & Germany	Fresh raw whole egg	Open Dosing: 0.1,0.3,2,3,10,30ml Dosing Interval: 15 min Observation Period: not stated	45.4ml egg	5.6g	
Roehr et al, 2004[30] Germany	Fresh raw whole egg	DBPC Dosing: 0.1, 0.3,1.0,3.0,10,30ml Intervals: 20 min Observation Period: 2 hours. Then 24 & 48 hrs by phone.	44.4ml egg	5.6g	Blinded in formula + orange or chocolate flavour.

TABLE 2.5 (cont) THE FORM OF EGG, DOSE AND DOSING REGIME USED FOR CHALLENGES IN EGG ALLERGY RESEARCH

Study Author & Location	**Form of Egg**	**Dosing Regimen**	**Total Dose**	**g egg protein**	**Other Comments**
Verstege et al, 2005[93] Germany *	Fresh raw whole egg	DBPC or Open (if child was <1yo) Dosing: 7 doses, titrated. Dosing Interval: 30 min Observation Period: 48 hours	1 whole egg	7g	
Celik-Bilgili et al, 2005[110] Germany*	Fresh whole raw egg	DBPC or Open (if child was <1yo & had history of immediate reactions) Dosing: 7 doses; 0.04, 0.11,0.38, 1.14, 3.8, 11.4,38.0ml Dosing Interval: 20 min Observation Period: 48 hours	54.9ml	7g	Mixed in amino acid formula.
Mehl et al, 2006[96] Germany*	Fresh raw whole egg	DBPC or Open (if child was <1yo) Dosing: Successive doses reaching 1 whole egg. Dosing Interval: 30 min Observation Period: 2 hours, then 48hrs later	1 whole egg	7g	* Verstege et al[93], Celik-Bilgili et al [110] and Mehl et al[96] were all from the same clinic.
Osterballe et al, 2005[32] Denmark	Pasteurised whole egg Not stated if liquid or powder	DBPC or Open (if child was <3yo) Dosing: 11, 44, 250, 500, 1000, 2500, 5000 and 40,000mg Dosing Interval: 15 min Observation Period: 2 hours, then 24 hrs later by phone.	49.3g (1 egg)	6.3g	Masked in oat milk, sugar, cocoa & vanilla sugar. In a coloured cup with a lid.
Benhamou et al, 2008[114] Switzerland	Pasteurised egg –not stated if liquid or powder or if whole egg or egg white.	Open or DBPC. Dosing: 2.5, 5, 7.5, 15 and 15g. Dosing Interval: 15 min Observation Period: 2 hours	45g	5.7g (assuming whole egg)	Blinded in 'chocolate tasting preparation'.
Sampson, 2001[108] USA	Dehydrated raw egg (Not stated if whole or white)	DBPC. Active & Placebos on same day 3-4 hours apart. Dosing: Over 90 minute period Dosing Interval: not stated Observation Period: not stated	10g egg powder	?	Dehydrated food camouflaged in juice, infant formula or moist food.
Clarke et al, 2011[37] UK	Pasteurised frozen whole egg nuggets	Open Dosing: 0.5g, 1g, 2g, 6g, 12g Dosing Interval: 10 min intervals. Observation Period: 2 hours	21.5g egg	2.6g #	
Palmer et al, 2013[7] Australia	Pasteurised whole egg - powder	Open Dosing: drop inside lip, 1,2,5,10 & 20ml Dosing Interval: 15 min Observation Period: 2 hours	½ of an egg	3.8g	

TABLE 2.5 (cont) THE FORM OF EGG, DOSE AND DOSING REGIME USED FOR CHALLENGES IN EGG ALLERGY RESEARCH

Study Author & Location	**Form of Egg**	**Dosing Regimen**	**Total Dose**	**g egg protein**	**Other Comments**
Kemp et al, 2009[126] Australia	Cooked whole egg – lightly boiled	Open Dosing: A drop on the buccal mucosa, 1/8, 1/4, 1/2, 1, 2 tsp Dosing Interval: 20-30 min Observation Period: not stated	3 & 7/8th tsp (19.4ml)	2.4g	
Sporik et al, 2000[107] Australia	Cooked egg white - lightly boiled (approx. 2 min)	Open Dosing: Over 3 days Day 1: A drop inside lower lip, 1/8, ¼, ½ & 1 tsp Day 2: 1 tsp, 2 tsp (approx. 1 egg white) Day 3: 1 egg white per day for one week at home Dosing Interval: 30 min Observation Period: not stated	Day 1: 1 & 7/8 tsp Day 2: approx. 1 egg white Day 3-10: 1 egg white	Day 1: approx. 1.6g Day 2-10 approx. 3g	Procedure repeated for egg yolk at home. 1/8 of a level tsp of egg white contains approx. 0.1 g of egg protein.
Pereira et al, 2005 [125] UK	Cooked whole egg	Open Dosing: rub lower mucosa, 1g, 2g, 5g, 10g, 22g, 1 egg* Dosing Interval: 15 min, then 2 hours after 22g dose Observation Period: 2 hours after last dose.	40g + 1 egg	approx. 10.6g (5g + 5.6g if a 45g egg is used for final dose)	Cooking method not stated. Assume whole egg. * a 'small' egg if < 1 yo, a 'medium' egg > 1 year old & an 'extra large' egg if >12 yo.
Benhamou, 2008[114] Switzerland	Cooked whole egg - hard boiled (10 min)	Open Dosing: 3, 6, 10, 15 and 16 g Intervals: 15 min Observation Period: 2 hours	45g	5.6g	
Lemon-Mulé et al, 2008[13] USA	Cooked whole egg - semi cooked (scrambled)	Open Dosing: Increasing doses over 1 hour, to total dose of 1 egg Dosing Interval: not stated (15 min?) Observation Period: 2-4 hours	1 egg	6.5g	
Lemon-Mulé et al, 2008[13] USA	Cooked whole egg - semi cooked (French toast)	Open Dosing: Increasing doses over 1 hour, to total dose of 1 egg Dosing Interval: not stated (15 min?) Observation Period: 2-4 hours	1 egg	6.5g	
Lemon-Mulé et al, 2008[13] USA	Cooked egg – baked (waffle)	Open Dosing: 4 equal portions over 1 hour (2 hours after muffin challenge) Dosing Interval: not stated (15 min?) Observation Period: 2-4 hours	1/3 egg	2.2g #	Waffle maker 500°F for 3 min.

TABLE 2.5 (cont) THE FORM OF EGG, DOSE AND DOSING REGIME USED FOR CHALLENGES IN EGG ALLERGY RESEARCH

Study Author & Location	Form of Egg	Dosing Regimen	Total Dose	g egg protein	Other Comments
Lemon-Mulé et al, 2008[13] USA	Cooked egg – baked (muffin)	Open Dosing: 4 equal portions over 1 hour Dosing Interval: not stated (15 min?) Observation Period: 2-4 hours	1/3 egg	2.2g #	Baked in oven at 350°F for 30 min.
Lieberman et al, 2012[104] USA	Cooked egg – baked (muffin)	Open Dosing: 1/8, ½, ¼, ½ muffin Dosing Interval: 15 min Observation Period: 2 hours	1/3 egg	2.2g #	Baked in oven at 350°F for 30 min. From the same group as Lemon-Mulé et al.
Des Roches, 2006[120] Canada	Cooked egg – baked (cake)	Open Dosing: Not described Dosing Interval: Not described Observation Period: Not described	1 egg	6.8g	1/6 of a cake made with 6 eggs. One cooked egg (personal communication)
Clarke et al, 2011[37] UK	Cooked egg - baked (sponge cake)	Open Dosing: 0.4g, 0.8g, 1.5g, 3g, 6g. Intervals: 10 min Observation Period: 2 hours	11.7 g egg	1g #	
Cortot et al, 2012[102] USA	Cooked egg – baked (muffin)	Open Dosing: graded to a total dose of 1/3 egg. Dosing Interval: not stated. Observation Period: 1 hour, or longer if failed the challenge	1/3 egg	2.2g	2 whole eggs in recipe of 12 small or 6 large baked at 375°F for 30 min.
Bartnikas et al, 2013[122] USA	Cooked egg – baked (muffin)	Open Dosing: 1/8, ¼, 5/8 muffin or cupcake. Dosing Interval: 15 min Observation Period: 30 to 60 min	1/3 egg	2.2g	2 large eggs in recipe for 6 muffins baked at 350°F for 30 min
Faraj and Kim, 2012[11] USA	Cooked egg – baked (muffin)	Open Dosing: 10%, 30%, 30%, 30% muffin Dosing Interval: 30 min Observation Period: 60 min	1/3 egg	2.3g	1/3 egg per muffin baked at 350°F for 30 min.
Osborne et al, 2011[2] Australia	Cooked egg – baked (muffin)	Open Dosing: A crumb, 1/12, 1/6, ¼, ½ muffin Dosing Interval: 15 min Observation Period: 60 min	1/6 egg (10g egg per muffin)	1.24g	2x 60g eggs in 12 muffins.

Legend to Table 2.5: *Lemon-Mule study uses muffin + waffle for heated egg challenges, and scrambled egg or french toast for 'regular egg' challenge.
cited from paper. Other figures are derived from the dose reported from the food challenge. **after final dose or after any reaction.

TABLE 2.6 DIFFERENT FORMS OF EGG USED FOR ORAL FOOD CHALLENGES

TYPE OF EGG PRODUCT USED FOR OFC	REFERENCE
RAW EGG	
Fresh raw whole egg	(30, 96, 109, 110, 112)
Fresh raw egg white	(2, 35, 115, 119, 123, 124)
Dehydrated raw egg	(108)
Pasteurised whole egg	(7, 32)
Pasteurised whole egg – frozen nuggets	(37)
Pasteurised egg white - liquid	(123)
Pasteurised egg white - powder	(124)
COOKED EGG	
Cooked egg – lightly boiled	(126)
Cooked egg – lightly boiled (2 min)	(107)
Cooked egg – semi cooked / scrambled	(13)
Cooked egg – French toast	(13)
Cooked egg – hard boiled (10 min)	(114)
Cooked egg white - hard boiled (10 min)	(115, 119)
Cooked egg white - hard boiled (20 min)	(117)
Cooked egg yolk - hard boiled (10 min)	(115)
BAKED EGG	
Baked egg – muffin (30 min at 180°C)	(2, 11, 13, 102, 122)
Baked egg – waffle (3 min at 500°F)	(13)
Baked egg – sponge cake	(37)

DOSING

The cumulative dose of egg protein used for oral food challenges varied. Most raw and cooked egg challenges delivered one whole egg or a whole white or whole yolk. These challenges provide 3 to 4g protein for an egg white challenge and 6 to 7g of protein for a whole egg challenge (depending on the size of the egg used for the challenge).

The dose of egg protein in the baked egg challenges was the most variable, with some clinics giving 1/6 egg (1.1g protein)[2, 37], 1/3 egg (2.2g protein)[11, 102, 122], up to one whole egg (6 g protein) per challenge[120]. There is no obvious reason for this variability, although it may be related to regional differences in baked products that would be consumed by the child at home.

TIME BETWEEN DOSES

Dosing intervals varied from 15 to 90 minutes[2, 35]. Most of the challenge protocols were given over a single day with all of the challenge doses observed by clinic staff. However several protocols extended over more than one day, requiring several admissions to the challenge unit, or overnight admissions. The extended protocols were food challenge protocols developed in the early 2000's when protocols were not as well researched and clearly documented in the scientific literature[35]. Some lengthy protocols were developed specifically to investigate food allergies in atopic eczema and as such had an extended period of follow up[115, 119]. Modern challenge protocols are usually limited to a half or, in some occasions, a full day to fit into staffing hours if the challenge is performed as a day case in a medical day ward or supervised in an office setting.

POST CHALLENGE OBSERVATION

Children were observed for one or two hours after their last dose in the oral food challenge, or for the same period of time after their last symptom of a reaction. Some of the original food challenge protocols did not specify the post challenge observation period, or had extended observation times as the children were admitted to hospital overnight or were attending the allergy clinic on a daily basis.

2.5.3 SUMMARY OF RESULTS – EGG ORAL FOOD CHALLENGE PROTOCOLS

The results of this systematic review indicate that a large degree of heterogeneity exists related to the preparation of egg given for the oral food challenges, the total dose, dosing regimen and the post challenge observation period.

Some of the differences in food challenges are cultural and relate to the form of egg that is usually consumed in the community. In the USA, for example, raw egg is not often consumed, and so food challenges to raw egg are uncommon[13]. In other countries (eg France, Spain, Australia) uncooked or raw egg may be consumed in the form of mayonnaise, in gelato and egg-nog and as a result allergy units routinely offer oral food challenges to raw egg[2, 123]. The form of egg given for a food challenge also informs the subsequent dietary restrictions placed on an individual with the egg allergy who goes on to pass the challenge. A raw egg challenge gives a definitive answer as to the existence of egg allergy; however a cooked egg challenge is reflective of what is usually consumed in the diet at home. If a child tolerates a raw egg challenge they will tolerate all forms of egg in their diet, but a child who passes a baked egg challenge will not necessarily tolerate raw egg[37]. There is a small but real risk of food borne infection when raw egg products are used for oral food challenges and some allergy clinics use pasteurised egg products for oral food challenges. The effect of pasteurisation and drying on the allergenicity of whole raw egg will be discussed in Chapter 3.

Different regional terminology was used to describe the form of egg offered during an oral food challenge, for example the term 'regular egg' was used by some groups to describe a challenge with lightly cooked egg (eg scrambled egg or French toast)[13], however the term 'regular egg' could be misinterpreted to mean raw, uncooked egg. As discussed previously, differing degrees of heat treatment affect the relative allergenicity of egg protein and as such the type of egg offered during the oral food challenge must be kept in mind when

comparing studies reporting food challenge outcomes and when determining rates of resolution of food allergy.

The rate of false positive reactions to oral food challenges is estimated to be as high as 30%, and ideally all food challenges would be placebo controlled[127, 128]. However, oral food challenges are time consuming and costly to perform in hospital. Blinding of a food for an oral food challenge requires time, planning and dedicated food service staff and because of this many allergy clinics perform open food challenges where a child is given an undisguised dose of a challenge food. In this review nine studies (summarised in Table 2.5) used double blinded placebo controlled protocols[30, 32, 93, 96, 108, 110, 114, 115, 119].

Standard protocols for egg challenges have been developed by allergy societies in the USA[129], Europe[130], Japan[131] and Australasia[132] specifying the form of egg, the dose, timing between dosing and the timing of the post challenge observation period (see Table 2.7). The AAAAI / EAACI PRACTALL consensus report provides a standard approach to the conduct and reporting of DBPCFC for researchers and clinicians [133]. Although there is still some variation between protocols, the use of standard protocols is recommended and will facilitate direct comparisons of outcomes of oral food challenges.

TABLE 2.7 STANDARD EGG CHALLENGE PROTOCOLS

Study Author & Location	**Form of Egg**	**Dosing Regimen**	**Total Dose**	**g egg protein**	**Other Comments**
American Academy of Allergy, Asthma and Immunology (AAAAI) Nowak-Wegrzyn et al, 2009[129]	Egg, whole, dried	Open, single blind or double blind based on clinical assessment of potential for bias in interpretation of result. Dosing: For IgE mediated allergy: Initial dose: 0.1-1% of total challenge food. (Lower than expected threshold dose, if known.) Higher risk patients should be challenged with a low initial dose. Or a simplified open OFC, divide into 3 equal portions. Dosing Interval: 10-30 min Observation Period*: 60 to120 min	10g	4.8g	Total quantity of food tested should approximate the regular, age-appropriate serving size of the food.
	Egg, white, dried		10g	7.5g	
	1 slice of French toast (1 egg per 1 slice of bread) 1 hard boiled or scrambled egg		1 egg	3g -7.5g	
European Academy of Allergy and Clinical Immunology (EAACI) Bindslev-Jensen et al, 2004[130]		Open, single blind or double blind Dosing: 1mg, doubling dose until top dose is reached or incremental dosing using logarithmic mean. Dosing Interval: 15-30 min Observation Period*: not stated			Top dose should normally be the normal daily intake in a serving of the food in question, adjusted for the patient's age
Japanese Society of Pediatric Allergy and Clinical Immunology Ito and Urisu 2009[131]	Step 1*: boiled Egg Yolk Step 2: boiled Egg White **	Open, single blind or double blind Dosing: 3-6 incremental doubling doses eg, Yolk: 1g, 2g, 4g, 8g. White: 0.1g, 0.2g, 0.5g, 1g, 2g. Dosing Interval: 15-30 min Observation Period*: ≥ 2 hours	15g (1 egg yolk) or 3.8 g egg white	2.4 g (egg yolk) 0.4g (egg white)	*step wise protocol for high risk patients **"or processed foods such as cookies"
AAAAI / EAACI PRACTALL Consensus Sampson et al 2012 [133]	Pasteurised liquid egg (12.8% protein) or egg powder (47% protein)	Double blinded is preferable Dosing: 3mg, 10mg, 30mg, 100mg, 300mg, 1000mg, 3000mg egg protein Dosing interval: at least 20min Observation Period: 2 hours		4.4g	
Australasian Society for Clinical Immunology and Allergy (ASCIA) www.allergy.org (accessed 22 Dec 2014) [132]	Cooked Egg	Open Dosing: Cooked egg: touch on lip, 0.6g, 1.25g, 2.5g, 5g, 10g, 13.1g (rest of egg) Muffin: 1/16, 1/8, ¼, ¼, remainder of muffin Dosing Interval: 20 min Observation Period: 60 to120 min	45g (1 large egg)	5.7g	
	Baked Egg		8.33g baked egg	1.9g	2x 50g eggs in 12 muffins

2.6 TRADITIONAL AND EMERGING APPROACHES TO DIETARY MANAGEMENT OF EGG ALLERGY

Management of a food allergy consists of removal of the food from the diet and provision of an action plan for management of any accidental exposure to the food protein.

2.6.1 DIETARY MANAGEMENT OF BREAST FED BABIES WITH EGG ALLERGY

Egg protein consumed in the maternal diet can be isolated from their breast milk[134]. In egg allergic, eczematous breast-fed babies exacerbations in eczema may be triggered by egg in the maternal diet. Palmer et al[135] studied eczema and sensitization to egg (on SPT) in 32 exclusively breast-fed babies. All mothers had an egg free diet, followed by a randomised double blind crossover challenge with egg. There was an improvement in the eczema score (SCORAD) with time for both the intervention and control groups, but no statistical difference between the groups related to the egg challenge. In a cross-over RCT, Cant et al[136] studied the effect of maternal exclusion of egg, (and also cow's milk, chocolate, wheat, nuts, fish, beef, chicken, citrus fruits, artificial food colourings and preservatives) during lactation compared with periods of inclusion of cow's milk and eggs on eczema scores in children with eczema aged 6 weeks to 6 months. At each time point, there were no significant differences in eczema scores.

2.6.2 THE EGG FREE DIET

Removal of egg from the diet includes avoidance of all forms of egg including egg used as an ingredient in home cooked and commercially prepared foods. Current Australian legislation requires mandatory labelling to identify the presence of egg in a commercial food as well as ingredients derived from egg[137].

2.6.3 THE BAKED EGG CONTAINING DIET

More recently complete exclusion of known allergen from a food allergic individual has been questioned, and it is possible that including the allergenic food in the diet has

potential to increase the likelihood of a child outgrowing their allergy more quickly, or to allow a certain amount of the allergen to be tolerated[138]. Complete and absolute avoidance of a food allergen may not be necessary in a child's diet if they show tolerance to that food in an alternative format. Many children with egg allergies tolerate foods containing baked egg protein[13], and the hypothesis that this might be associated with tolerance development has moved dietary management strategies away from strict avoidance to allowing the inclusion of the baked protein in the diet if it is tolerated[9, 12, 139]. This is reviewed in Chapter 5 of this thesis and is the basis of the RCT discussed in Chapter 6.

Protocols used for baked egg OFCs have been discussed earlier in this chapter. Once a child has demonstrated that they can tolerate baked egg via a supervised OFC many allergy clinics use the concept of an egg "ladder" as an education tool to indicate the forms of egg (classified by degree of cooking) that may be included in their child's diet[9]. Figure 2.2 is example of an egg "ladder" developed for an Australian population.

2.6.4 SPECIFIC ORAL IMMUNOTHERAPY TO TREAT EGG ALLERGY

With the rising incidence in food allergies there has been interest in improving their management, with the aim of offering a treatment strategy that does not solely rely on avoidance of food proteins in the diet. Immunotherapy is used successfully for managing venom and pollen allergies and thus, there has been increasing research into the use of immunotherapy to manage common food allergies[140]. Current research related to oral immunotherapy for management of IgE mediated cow's milk and egg allergy, and in particular the potential for heated proteins to be used as vehicles for oral immunotherapy is reviewed in Chapter 5 of this thesis.

FIGURE 2.2 EXAMPLE 'EGG LADDER'

Women's & Children's Hospital

The egg tolerance ladder

Some children with an egg allergy can tolerate eggs in well baked items, but not in other forms. Other children can tolerate most cooked forms of egg, but not undercooked or raw egg.

Your child has passed an egg challenge, and the table below indicates the types of egg/egg containing foods that are likely to be tolerated. This will be different for different children.

Only allow your child to eat foods from the column(s) ticked, and it is recommended that you always read food labels. If a food is not on this list, check with your doctor, clinic nurse or dietitian before offering it to your child.

If your child has a reaction to any food containing egg, follow your action plan and call your doctor for further advice. Do not give this food again until you have discussed this with your doctor or allergy clinic staff.

Direction of tolerance →

☐ Baked egg	☐ Whole cooked egg	☐ Nearly raw egg
> Cakes/Muffins/Cupcakes/Biscuits Avoid cakes and biscuits with custard or cream centres, or 'gooey' centres as these may contain raw egg. The hospital challenge muffins have 2 eggs per dozen muffins (approximately 1/6th of an egg per serve). Most cakes and muffin have similar amounts of egg and are likely to be tolerated. Some cakes/muffins may have more than 2 eggs in a batch and may not be tolerated. If your child is reacting to a baked item discuss this with your doctor or the allergy clinic staff before giving it to your child.	> Poached egg > Soft boiled egg > Scrambled egg > Fried egg > Hard-boiled egg > Omelette > Frittata > Egg in fried rice > Quiche > Pancakes/pikelets > Waffles > Fresh or dried egg pasta > Chicken nuggets > Egg used as binder ie. rissoles or meatloaf > Crumbed meats ie. schnitzel	> Dried egg powder > Raw egg in cake mix > Some mayonnaise and salad dressings > Tartar/e sauce > Hollandaise sauce > Chocolate mousse > Some fresh ice-cream > Some sorbets > Royal icing > Marzipan > Crème egg > Some chocolates with nougat or soft centres > Baked custards eg. crème caramel, crème brulee, egg custard tart > Cakes containing custard ie. éclairs > Egg custards > Egg glaze eg. on sticky buns > Pavlova > Meringue

For more information

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2.7 SUMMARY AND CONCLUSION

Allergy to hen's egg allergy is the most common food allergy in Australian children[2] and for this reason has been chosen as the focus of this thesis. Although egg allergy frequently resolves during childhood, individuals with a history of egg allergy are more likely to develop longer-term atopic disorders[23].

Opportunities exist to develop strategies to prevent the development of allergies, in particular egg allergy and the outcome of RCTs investigating early introduction to egg protein in infants will inform the direction that this will take.

Diagnosis of egg allergy relies on clinical history and the use of predictive and diagnostic testing using SPT, sIgE and OFC. The PPV of diagnostic testing changes as children get older. This literature review highlights the heterogeneity in the egg allergens used for SPT and sIgE testing, as well as the form of egg used for OFC protocols. The use of standardised OFC protocols will facilitate greater comparison between studies reporting outcomes of egg OFCs.

For those with egg allergy allowing diets that contain baked egg, if tolerated, decrease the burden of disease and improve the quality of life[141]. However, predictive tools used in clinical practice (SPT and egg sIgE levels) do not accurately predict candidates for baked egg OFC and as such candidates for these challenges should be selected carefully.

Early trials investigating specific oral tolerance induction for the treatment of food allergies, including egg, are promising; however SOTI is not yet ready for use in mainstream clinical practice[142]. More information is required about identification of suitable candidates, appropriate treatment foods and dosing regimens and if adjunct therapy is required[143].

CHAPTER 3

ALLERGENICITY OF PASTEURIZED WHOLE RAW HEN'S EGG COMPARED WITH FRESH WHOLE RAW HEN'S EGG

INTRODUCTION

Chapter 3 compares the binding of serum IgE from egg allergic children to *in vitro* digested and undigested pasteurised whole raw egg powder with unpasteurised fresh whole raw egg.

The paper entitled, "Allergenicity of Pasteurized Whole Raw Hen's Egg compared with Fresh Whole Raw Hen's Egg" by Merryn Netting, Adaweyah Donato, Maria Makrides, Michael Gold, Patrick Quinn, and Irmelli Penttila was published in the peer reviewed journal, Pediatric Allergy and Immunology 02/2015; DOI:10.1111/pai.12365

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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Merryn Netting		
Contribution to the Paper	Responsible for conception, design and conduct of the study including acquisition of data, analysis and interpretation of data. Prepared first draft of the manuscript and subsequent revisions.		
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Contribution to the Paper	Supervised study design, analysis and interpretation. Critical revision of the manuscript. Final approval of version to be submitted.		
Signature		Date	19/8/15

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Signature		Date	

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Pediatric Allergy and Immunology, *26*(3), 234-238

CHAPTER 4

DOES SKIN PRICK TESTING AND SERUM SPECIFIC IgE LEVELS TO COMMON EGG ALLERGENS PREDICT THE OUTCOME OF BAKED EGG CHALLENGES IN YOUNG EGG ALLERGIC CHILDREN?

INTRODUCTION

Chapter 4 evaluates the utility of SPT and sIgE to common egg allergens (whole egg, egg white, egg yolk, ovalbumin and ovomucoid) in addition to whole egg IgG4 levels and egg allergen specific IgE to whole egg IgG4 ratios in the prediction of the outcome of baked egg oral food challenges in 1 to 5-year old egg allergic children.

The paper entitled, "Does Skin Prick Testing and Serum Specific IgE Levels to Common Egg Allergens Predict the Outcome of Baked Egg Challenges in Young Egg Allergic Children?" by Merryn Netting, Jennie Louise, Michael Gold, Patrick Quinn and Maria Makrides will be submitted for publication in the peer reviewed journal, Current Opinion in Allergy and Clinical Immunology in November 2015.

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PREDICTING BAKED EGG TOLERANCE IN EGG ALLERGIC CHILDREN

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Running Title: Predicting Baked Egg Tolerance in Egg Allergic Children.

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Material in the electronic repository:

E-Table 1 Clinical History of Children with Anaphylaxis to Baked Egg Oral Food Challenge

E-Fig 1: Skin Prick Test Results by Outcome of Baked Egg Oral Food Challenge

E- Fig 2: Egg Allergen Specific IgE Results by Outcome of Baked Egg Oral Food Challenge

E- Fig 3: Egg Allergen Specific IgE/IgE4 Ratios by Outcome of Baked Egg Oral Food Challenge

Abbreviations: Ig (Immunoglobulin); SPT (Skin Prick Testing); sIgE (serum-specific IgE); EW (Egg White); EY (Egg Yolk); WE (Whole Egg); OVA (Ovalbumin), OVM (Ovomucoid); PPV (Positive Predictive Value); NPV (Negative Predictive Value): ROC (Receiver Operator Curve); AUROC (Area Under ROC curve); BE (Baked Egg); OFC (Oral Food Challenge).

DECLARATION

The study was supported by grant funding from the Women's and Children's Hospital Foundation and the Ilhan Food Allergy Foundation. The Link Group / Stallergenes donated the skin prick test reagents and the IgE/IgG4 analysis was supported by Phadia. MN was supported by a MS McLeod Research Trust PhD scholarship and a Center for Food and Allergy Research NHMRC CRE top up scholarship. MM is an NHMRC Principal Research Fellow. The funding bodies had no role in the design of the study or interpretation of the data.

TITLE: Predicting Baked Egg Tolerance in Egg Allergic Children.

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ABSTRACT

Background: Egg allergic children usually tolerate baked egg (BE) before raw egg. This study evaluated the utility of egg allergen skin prick testing (SPT), serum-specific IgE (sIgE), and sIgE/whole egg specific IgG4 ratios to predict BE tolerance in egg allergic children.

Methods: 83 egg allergic children (aged 2.08 years, IQR 1.25-3.17) had open BE oral food challenges (10 grams egg). Egg allergen SPT (egg white, egg yolk, whole egg, ovalbumin and ovomucoid), sIgE (egg white, whole egg, ovalbumin and ovomucoid) and whole egg specific IgG4 were quantified. The result with the highest PPV ≤95% was determined using logistic regression analysis, and negative predictive value (NPV), sensitivity, specificity, positive and negative likelihood ratios calculated.

Results: 51.8% (43/83) were BE tolerant and egg allergic. BE tolerant children had lower SPT wheals and sIgE for all egg allergens, apart from ovomucoid, compared with BE intolerant. 95% PPVs to predict BE tolerance could not be calculated for any SPT or sIgE level, however 80% PPVs were calculated for whole egg and egg white sIgE/sIgG4 ratios. On ROC curve analysis all SPT regents performed poorly (AUROCS < 0.69). Whole egg (AUROC 0.75, p=0.01) and egg white sIgE (AUROC 0.77, p=0.04) performed better than ovomucoid sIgE (AUROC 0.57). Whole egg, egg white and ovalbumin sIgE/IgG4 ratios performed significantly better than ovomucoid sIgE /IgG4.

Conclusion: Egg allergen SPT and sIgE testing does not accurately predict BE tolerance in young egg allergic children. The use of sIgE/ sIgG4 ratios is promising, however a BE oral food challenge remains the gold standard.

Key Words: egg allergy, baked egg, tolerance.

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INTRODUCTION

Children with IgE mediated egg allergies usually tolerate baked egg (BE) before raw egg (1, 2). A number of factors facilitate tolerance to BE including disruption of allergenic epitope sites through heating, the formation of disulphide links with wheat that block IgE access to allergenic epitopes, and reduced gastrointestinal absorption of egg (3-6). Some egg allergens epitopes, including ovomucoid (OVM) (Gal-d-1) and the egg yolk (EY) allergen Gal-d-6 maintain their structure when heated (7, 8).

To demonstrate tolerance a BE oral food challenge (OFC) is required, however, there is a risk of adverse events including anaphylaxis during BE OFCs (9-11). As such there is the need for accurate testing to predict which children are likely to tolerate a BE OFC to avoid unnecessary OFCs, and also to know which children can be safely challenged in low risk settings.

While 95% positive predictive values (PPV) have been published to predict the likelihood of raw egg allergy no consistent decision points predicting outcomes of BE OFC with 95% PPV, and associated high negative predictive value (NPV), specificity and sensitivity are reported for egg allergen IgE as determined by skin prick testing (SPT) or serology (Table 1). Studies reporting immunologic predictors of BE tolerance are heterogeneous with regard to the age of participants, the egg allergen tested and the dose of BE in the OFC. SPT wheal size and serum-specific IgE (sIgE) titres predicting the probability of reacting to a BE OFC have been reported for egg white (EW) SPT (12, 13), EW sIgE (13, 14), OVM SPT (11) and OVM sIgE (15, 16 Marriage, 2012 #209). SPT to slurries of BE containing muffin has strong negative predictive values (9, 11). We are not aware of any studies reporting the use of WE or EY SPT or sIgE to predict BE tolerance.

Egg protein specific IgE/IgG4 ratios may also predict tolerance to BE (17) because as tolerance to an allergen develops, decreased allergen specific IgE and increased allergen specific IgG4 is observed, among other immune changes. IgG4 blocks antigen binding

sites, reducing access by IgE antibodies (18). Caubet et al (17) reported a statistical model including interactions between sIgE and IgG4 to ovalbumin (OVA) and OVM that although not ready for the clinic setting, predicts BE reactivity.

The aims of this study were to evaluate the utility of egg allergen SPT and sIgE levels in the prediction of BE tolerance in 1 to 5 year old egg allergic children. We also sought to compare the utility of egg allergen IgE/WE IgG4 ratios with egg allergen SPT and sIgE levels. The results of this study will allow comparison with similar studies and inform clinical decisions related to the timing of BE OFC for young egg allergic children.

TABLE 1 REPORTED EGG ALLERGEN SPT AND SPECIFIC IGE DECISION POINTS FOR BAKED EGG ORAL FOOD CHALLENGES

Study	Sample size	Median Age (range) (yrs)	Allergen	Decision point	PPV	NPV	Sensitivity	Specificity	AUROC
SKIN PRICK TESTING (WHEAL SIZE)									
Cortot et al, 2012(15)	52	7.2 (2.2-18)	Egg White	10mm	-	100	-	-	0.6406
Peters et al 2013(12)	185	Mean 1.1 (SD 0.69)	Egg White	11mm	82	82	0	99	0.624
Bartnikas et al, 2013 (10)	169	4.7 (0.15-23.15)	Egg White	11mm	23	90	69	56	0.624
Tan et al, 2013 (11)	143	3.8 (1.8-6.7)	Ovomucoid	10mm	73	65	32	91	0.67
SERUM SPECIFIC IgE									
Lieberman et al 2012 (13)	100	5.9 (1.2-19.9)	Egg white	10 kUA/L	60	71	20	94	-
Peters et al 2013(12)	143	Mean 1.1 (SD 0.69)	Egg White:	10 kUA/L	88	85	9	100	0.624
Marriage et al 2012(14)	47	4.6 (2-16)	Egg White	10 kUA/L	95	-	-	-	-
			Ovomucoid	6 kUA/L	95	-	-	-	-
Bartnikas et al, 2013 (10)	169	4.7 (0.15-23.15)	Egg White	9.65 kUA/L	58.8	88.7	37	95	0.721
			Ovomucoid	9.74 kUA/L	66.7	84.9	7.41	99.3	0.645
			Ovomucoid	6 kUA/L	95	-	-	-	-
Caubet et al 2012(16)	107	6.9 (1.6-18.6)	Egg White	26.2 kUA/L	43	91	12	95	0.759
			Ovalbumin	25.3 kUA/L	33	77	8	95	0.799
			Ovomucoid	12.8 kUA/L	64	81	28	95	0.667

Legend to Table 1: - not reported; PPV (Positive Predictive Value), NPV (Negative Predictive Value), AUROC (Area under Receiver Operator Curve), Sensitivity and Specificity are reported as percentages. SD (standard deviation)

METHODS

STUDY DESIGN

This study, approved by the local institutional review board (Human Research Ethics Committee) of the Women's and Children's Health Network (WCHN), Adelaide, Australia, was a cross sectional comparison of SPT and serology with outcomes of a BE OFC, and used the opportunistic sample of children screened for participation in the CAKE study (a randomised double blind placebo controlled trial comparing clinical and immunological outcomes after consumption of BE with an egg free diet in 1 to 5 year old BE tolerant, egg allergic children; trial registry number ACTRN 12612000173897). Six month to 5 year old children with IgE mediated egg allergy were recruited from Women's and Children's Hospital Allergy Clinic. Written informed consent was obtained prior to trial participation. Children were excluded if they were consuming BE; had parents or caregivers unable to provide informed consent; had non IgE mediated egg 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.

CLINIC APPOINTMENT

Children attended an appointment for SPT, blood sampling and an open, medically supervised BE OFC (muffin, containing 10 grams egg) according to standard protocol (19). All children had SPT according to standard methods (20). The allergens assessed were EW (Alyostal, Stallergenes, Antony, France), EY (Alyostal), WE (ALK-Abello, Texas, USA), OVA (ALK–Abello, Madrid, Spain), and OVM (ALK-Abello Spain). The negative control used was 50% (w/v) glycerin / saline (Holister-Steir Laboratories, WA, USA) and the positive control was histamine phosphate (10mg/ml). A positive SPT to an allergen was defined as a mean of two perpendicular wheal diameters of 3mm or greater in size than the mean wheal of the negative control site at 15 minutes.

A 5 ml peripheral blood sample was taken for quantification of WE (f245), EW (f1), OVA (f232) and OVM (f233) sIgE and WE (f245) sIgG4 using the Phadia ImmunoCAP system

by the Department of Immunopathology, at the WCHN. For sIgE, the lower limit of detection was 0.1kUA/L and the upper limit of detection was 100kUA/L. For WE sIgG4, the lower limit of detection was 0.07mgA/L and the upper limit of detection was 30mgA/L. For analysis, values below the lower limit of detection were replaced by half the lower limit of detection.

The challenge food was prepared in the WCH special diet kitchen according to standard recipe. BE tolerant children with EW SPT < 95% PPV for whole egg allergy [<5mm (6 months to 2yo) or <8mm (2 to 5yo)], with no clinical reactions to egg in the previous 12 months had a raw egg OFC (21) to confirm the existence of an egg allergy.

Clinical outcome data of the OFCs was collected including the maximum dose of challenge food, and allergic symptoms of any positive challenge. A positive reaction to an OFC was defined by the development of allergic symptoms within 2 hours of the egg challenge and included at least 3 concurrent non-contact urticarial lesions persisting for at least 5 minutes, generalised skin erythema, vomiting and/or anaphylaxis (as defined by multi-system involvement which included circulatory and/or respiratory involvement) (22).

Background information collected included sociodemographic information (parental age, education, employment, child gestational age, birth weight, birth order and sex) and health information related to atopy risk (parental smoking, parental and sibling history of allergic disease). Information related to the diagnosis of egg allergy and clinical details of any prior reaction to egg was also collected. For children with eczema the clinical severity of the child's eczema was scored using a SCORAD assessment (23).

STATISTICS

Analyses were performed with STATA 13.1 (StataCorp, Texas, USA) and the InStat program v 6.05 (Graph Pad software, USA). Statistical significance was assessed at the 0.05 level. Mann-Whitney *U* tests were used to test differences between BE tolerant and BE intolerant children. Univariate logistic regression was used to derive predicted probabilities

of BE allergy, and the diagnostic capacity of the testing was assessed using Receiver Operating Characteristic (ROC) curves; the area under the ROC curve (AUROC) was calculated to quantify the accuracy of the test. The cutoff of predicted probability giving a PPV closest to (but not greater than) 95% was determined and this cut off was used to calculate the corresponding PPV, NPV, sensitivity, specificity and likelihood ratios. 95% exact binomial confidence intervals were calculated for these measures. IgE/IgG4 ratios were calculated using direct ratios of kUA/L to mgA/L to allow comparison with studies reporting similar results.

RESULTS

PARTICIPATION

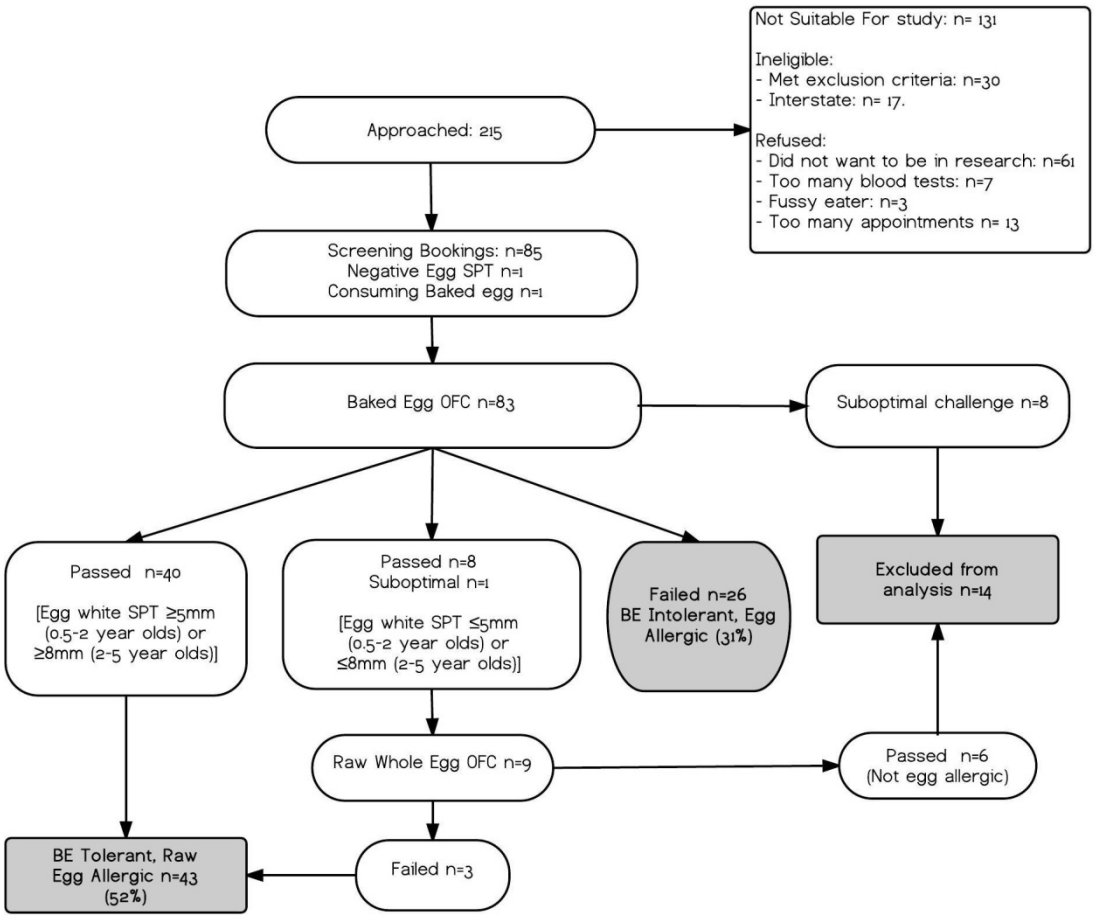
85 children were screened between 22nd May 2012 and 16th January 2014. Two children were excluded (one child had negative SPT to egg, and the other was consuming BE at home) and so in total 83 children had a BE OFC (see Fig 1).

OUTCOMES OF THE BAKED EGG ORAL FOOD CHALLENGES

51.8% (43/83) of the children were determined to be BE tolerant and egg allergic (Table 2). 58% (48/83) passed and 31% (26/83) children failed the BE OFC. 11% (9/83) had suboptimal challenges defined as failure to eat adequate amounts of the challenge food. Eight of the 48 (17%) children passing the BE OFC and one child with a suboptimal BE OFC had EW SPT ≤ 5mm (1-2 year olds) or ≤ 8mm (2-5 year olds) and were given a raw egg OFC (scheduled on a different day). 33% (6/9) children passed the raw egg OFC (including the child with a suboptimal baked egg challenge) and were determined not to have an egg allergy and were excluded from the final analysis. The family background and allergy history of the 77 children included in the final analysis is outlined in Table 3.

Three children had anaphylaxis to the BE OFC and their clinical background, SPT results, clinical reactions and treatment are summarised in E-Table 1.

FIGURE 1 STUDY FLOW



Legend to Figure 1: SPT (Skin Prick Test), OFC (Oral Food Challenge), BE (Baked egg).

TABLE 2 OUTCOME OF BAKED EGG ORAL FOOD CHALLENGES

OUTCOME OF ORAL FOOD CHALLENGE	N (%)
Pass	48 (57.8%)*
Fail	26 (31.3%)
Suboptimal test	9 (10.8%)**
Average quantity of baked egg consumed before ceasing the OFC (grams)***	
Passed OFCs	9.0 (1.4) g
Failed OFCs	4.4 (2.8) g
Suboptimal OFCs	1.6 (1.3) g
STOP CRITERIA	
Number of children reaching >1 stop criterion	7 (26.9%)
I. Skin	**12 (46.2%)**
A: Erythematous Rash	0 (0.0%)
B: Pruritus	0 (0.0%)
C: Urticaria / Angioedema	10 (38.5%)
D: Appearance of new skin rash	2 (7.7%)
II. Upper Respiratory	**8 (34.8%)**
A: Sneezing / Itching	0 (0.0%)
B: Nasal Congestion	1 (3.8%)
C: Rhinorrhoea	0 (0.0%)
D: Laryngeal	3 (11.5%)
III. Lower Respiratory	**0 (0.0%)**
IV. Gastrointestinal	**15 (57.7%)**
A: Subjective complaints (>18 months old)	7 (26.9%)
B: Objective complaints	9 (34.6%)
V. Cardiovascular	**0 (0.0%)**
TREATMENT	
None	2 (7.7%)
Cetirizine	24 (92.3%)
Adrenaline	2 (7.7%)
Prednisolone	2 (7.7%)

Comments regarding Table 2: *51.8% (43/83) were BE tolerant, egg allergic (8/48 BE tolerant children had raw egg OFC, 6/8 tolerated raw egg and were excluded from final analysis). **1/9 with suboptimal BE OFC tolerated a raw egg OFC. 'Stop criterion' results do not add to 100% as 7/27 children failing the BE OFC met > one criterion.

***1/8 of a muffin contained 1.25g of baked egg (0.2g egg protein), mean (SD)

TABLE 3 CHARACTERISTICS OF STUDY PARTICIPANTS

CHARACTERISTIC	N=77
Maternal age (years)*	34.23 (4.40)
Mother of Caucasian ethnicity †	64 (82.35%)
First degree relative with atopy †	66 (79.52%)
Male Sex †	47 (60.24 %)
Age (years) ‡	2.08 (1.25-3.17)
Birth weight (grams) *	3451 (516)
Gestational age at birth (weeks)*	38.95 (1.4)
Ever breastfed? †	73 (94.80%)
Still breastfed? †	7 (9.09%)
Age at diagnosis of egg allergy (months)*	8.4 (4.5)
Clinical reaction to egg? †	39 (50.65%)
History of anaphylaxis to egg? †	13 (16.9%)
Sensitised to egg, but no known prior ingestion? †	38 (50.1%)
Other IgE mediated food allergies †	61 (79.22%)
Eczema †	63 (81.82%)
Eczema severity (Objective SCORAD score) ‡	3.9 (0-11.75)
Asthma (Doctor diagnosed) †	15 (19.5%)

Legend to Table 3: Characteristics of study participants. Values are presented as follows: *mean (SD), †number (percentages) or ‡median (IQRs).

SKIN PRICK TEST RESULTS COMPARED WITH OUTCOME OF BAKED EGG ORAL FOOD CHALLENGE

The SPT results compared with the outcome of the BE OFC are presented in Table 4 and E-Fig 1. SPT wheal sizes for all allergens were lower for children who passed the BE OFC compared with those who failed, although this difference was only significant for WE (p=0.02) and EY (p=0.005). Eighteen children had negative or very low (≤ 3mm) OVM SPT wheals, including six who failed the BE OFC.

TABLE 4 SUMMARY OF SKIN PRICK TEST, SPECIFIC IgE, IgG4 RESULTS, IgE/IgG4 RATIOS AND OUTCOME OF BAKED EGG ORAL FOOD CHALLENGE

Allergen	**Passed BE OFC**	**Failed BE OFC**	**P value****
SKIN PRICK TEST WHEAL SIZE (MM)			
Whole Egg	5.0 (3.0-6.5)	6.0 (4.5-8.5)	0.02
Egg White	10.0 (8.0-12.0)	12.0 (8.3-14.0)	0.20
Egg Yolk	7.5 (4.6-9.0)	10.0 (7.0-11.0)	0.005
Ovalbumin	6.3 (4.1-8.0)	7.0 (6.0-9.9)	0.06
Ovomucoid	5.3 (0.0-9.4)	8.5 (3.3-12.0)	0.18
SERUM SPECIFIC IgE (kUA/L)			
Whole Egg	1.7 (0.48-8.6)	9.1 (4.4-29.0)	<0.001
Egg White	2.1 (0.55-11)	8.2 (4.0-20)	0.004
Ovalbumin	1.4 (0.4-5.4)	5.0 (2.0-8.9)	0.001
Ovomucoid	1.0 (0.05-4.1)	2.0 (0.02-13.0)	0.31
SERUM SPECIFIC IgG4 mgA/L			
Whole Egg	0.16 (0.01-0.90)	0.10 (0.06-0.6)	0.75
SERUM SPECIFIC IgE/ IgG4 RATIO			
Whole Egg / Whole Egg	17.50 (4.17-17.50)	92.40 (36.37-170.2)	<0.001
Egg White / Whole Egg	17.75 (4.13-58.82)	91.90 (36.33-166.0)	<0.001
Ovalbumin / Whole Egg	8.39 (3.20-38.50)	50.20 (21.41-140.0)	0.001
Ovomucoid / Whole Egg	4.30 (1.67-20.63)	5.45 (0.14-83.44)	0.63

Legend to Table 4: BE OFC: Baked egg oral food challenge. Results are reported as median (IQR)

SPECIFIC IGE AND IGG 4 RESULTS COMPARED WITH OUTCOME OF BAKED EGG ORAL FOOD CHALLENGE

Serum was available for 71 of the 77 children. Median allergen specific IgE levels for WE (p<0.001), EW (p=0.004), OVA (p=0.001), but not OVM were significantly lower for BE tolerant children compared with those who failed the BE OFC (see Table 4 and E-Fig 2). 16 children (n=8 passed BE OFC and n=8 failed BE OFC) had OVM sIgE 0.00 to 0.05mg

kUA/ml. There was no significant difference in IgG4 levels between the children who passed compared with those failing the BE OFC.

ALLERGEN SPECIFIC IgE TO IgG4 RATIOS COMPARED WITH OUTCOME OF BAKED EGG ORAL FOOD CHALLENGE

Ratios for WE, EW and OVA sIgE to WE sIgG4 were significantly lower for BE tolerant children compared with those intolerant to BE (Table 4 and E-Fig 2).

LOGISTIC REGRESSION AND ROC CURVE ANALYSIS OF SKIN PRICK TEST AND SPECIFIC IgE RESULTS

The results of the logistic regression analysis of the egg allergen SPT and sIgE results are presented in Table 5. The 95% PPV predicting tolerance to BE could not be calculated for any SPT reagent or specific IgE allergen and so the value with the highest PPV less than or equal to 95% was determined along with the associated NPV, sensitivity, specificity and positive and negative likelihood ratios. The relationship between sensitivity and specificity was analysed using ROC curves (Fig 2 A and B) and the area under the ROC curve (AUROC) calculated. All SPT regents performed poorly (AUROCs < 0.69) with no significant difference between AUROCs for any SPT reagent. For specific IgE, WE sIgE had the highest AUROC (0.7500, 95% CI: 0.6302-0.8698), however, the WE AUROC was not significantly higher than any other AUROC except that for OVM sIgE (p value for difference in AUROC = 0.012).

The results of the logistic regression analysis for the egg allergen sIgE/WE IgG4 ratios are presented in Table 6. 80% PPVs could be calculated for both WE and EW sIgE/WE IgG4 ratios. When assessed by ROC curve analysis all of the ratios performed significantly better than OVM sIgE/WE IgG4 (p value for difference in AUROCs: WE/WE p=0.009; EW/WE p=0.010; OVA/WE p=0.045) (Fig 2 C).

TABLE 5 CAPACITY OF SKIN PRICK TEST AND SPECIFIC IgE TO COMMON EGG ALLERGENS TO PREDICT ALLERGY TO BAKED EGG

Allergen	Result*	PPV % (95% CI)	NPV % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	LR+ (95% CI)	LR- (95% CI)	AUROC (95% CI)
SKIN PRICK TEST WHEAL SIZE (mm)								
Whole Egg	8.5	75.00 (42.81-94.51)	61.54 (48.64 -73.35)	26.47 (12.88-44.36)	93.02 (80.94-98.54)	3.79 (3.42-4.21)	0.79 (0.78-0.81)	0.6219 (0.4948-0.7490
Egg White	17	85.71 (42.13-99.64)	60.00 (47.59-71.53)	17.65 (6.76-34.53)	97.67 (87.71-99.94)	7.59 (6.38-9.03)	0.84 (0.83-0.85)	0.5673 (0.4338-0.7008)
Egg Yolk	14.5	80.00 (28.36-99.49)	58.33 (46.11-69.85)	11.76 (3.30-27.45)	97.67 (87.71-99.94)	5.06 (4.22 -6.06)	0.90 (0.89-0.91)	0.6850 (0.5647-0.8053)
Ovalbumin	15	66.67 (9.43-99.16)	57.53 (45.41-69.03)	6.06 (0.74-20.23)	97.67 (87.71-99.94)	2.61 (2.14-3.18)	0.96 (0.95-0.97)	0.6061 (0.4788-0.7333)
Ovomucoid	15	55.56 (21.20-86.30)	57.35 (44.77-69.28)	14.71 (4.95-31.06)	90.70 (77.86-97.41)	1.58 (1.42 -1.75)	0.94 (0.93-0.95)	0.5920 (0.4627- 0.7212)
SERUM SPECIFIC IGE (KUA/L)								
Whole Egg	29.30	75.00 (34.91-96.81)	70.18 (56.60-81.57)	26.09 (10.23-48.41)	95.24 (83.84-99.42)	5.48 (4.82- 6.22)	0.78 (0.76-0.79)	0.7500 (0.6302-0.8698)
Egg White	52.40	75.00 (19.4 -99.37)	66.10 (52.61-77.92)	13.04 (2.78-33.59)	97.50 (86.84-99.94)	5.22 (4.33-6.28)	0.89 (0.88-0.90)	0.7174 (0.5914-0.8434)
Ovalbumin	39.90	66.67 (9.43-99.16)	66.13 (52.99-77.67)	8.70 (1.07-28.04)	97.62 (87.43-99.94)	3.65 (3.00-4.45)	0.96 (0.92-0.95)	0.7293 (0.6066-0.8521)
Ovomucoid	22.00	83.33 (35.88-99.58)	69.49 (56.13-80.81)	21.74 (7.46-43.70)	97.62 (87.43-99.94)	9.13 (7.66-10.88)	0.80 (0.79-0.82)	0.5663 (0.3984-0.7342)

Legend to Table 5: *Result with the highest PPV ≤ 95%. Positive Predictive Value (PPV), Negative Predictive Value (NPV), Likelihood Ratio (LR), Area Under the ROC Curve (AUROC)

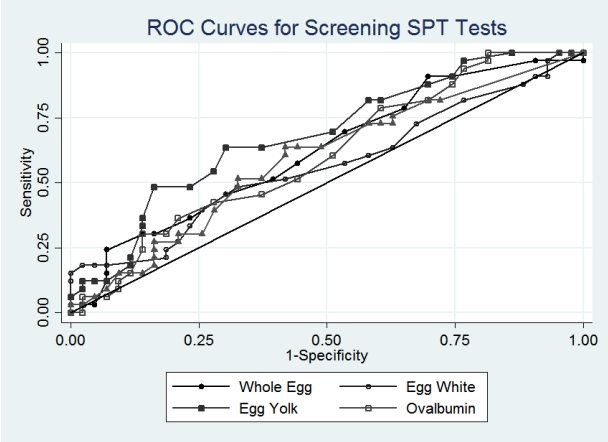
TABLE 6 CAPACITY OF RATIO OF EGG ALLERGEN SPECIFIC IgE TO WHOLE EGG IgG4 TO PREDICT ALLERGY TO BAKED EGG

IgE:IgG4 Ratio	Ratio	PPV (95% CI)	NPV (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	AUROC (95% CI)
Whole Egg / Whole Egg	350	80.00 (28.34-99.49)	67.80 (54.36-79.38)	17.39 (4.95-38.78)	97.56 (87.14-99.94)	0.7734 (0.6464-0.9004)
Egg White / Whole Egg	327	80.00 (28.36-99.49)	67.24 (53.66-78.99)	17.39 (4.95-38.78)	97.50 (86.84-99.94)	0.7707 (0.6416-0.8997)
Ovalbumin / Whole Egg	336	66.67 (9.42-99.16)	65.57 (52.31-77.27)	8.70 (1.07-28.04)	97.56 (87.14-99.94)	0.7370 (0.6048-0.8691)
Ovomucoid / Whole Egg	220	75.00 (19.41-99.37)	66.67 (53.31-78.31)	13.04 (2.76-33.59)	97.56 (87.14-99.94)	0.5359 (0.3680-0.7038)

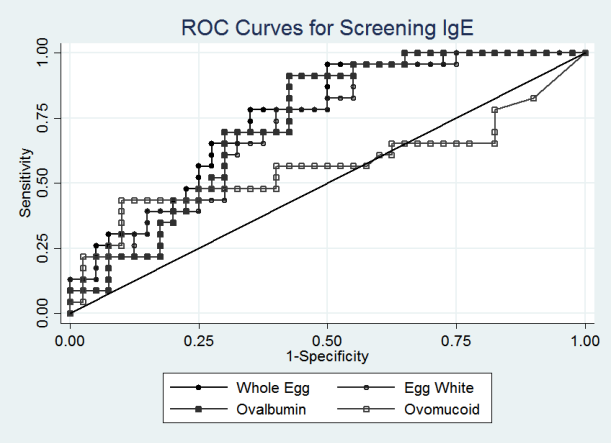
Legend to Table 6: **Result with the highest PPV ≤ 95%. Positive Predictive Value (PPV), Negative Predictive Value (NPV), Likelihood Ratio (LR), Area Under the ROC Curve (AUROC)

FIGURE 2 ROC CURVE ANALYSIS OF EGG ALLERGEN SKIN PRICK TEST, SPECIFIC IgE AND IgE/WHOLE EGG IgG4 RATIOS TO PREDICT OUTCOMES OF BAKED EGG ORAL FOOD CHALLENGE

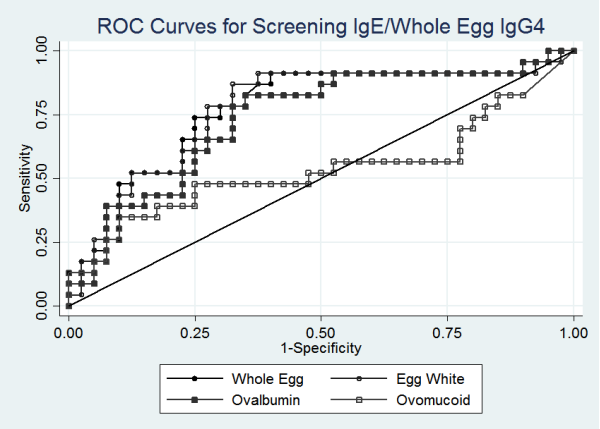
A



B



C



Legend to Figure 2: ROC Curve analysis for predictive testing to egg allergens and outcomes of baked egg oral food challenges: **A**. Skin Prick Test; **B** Specific IgE; **C** Specific IgE/Whole Egg IgG4 Ratios

DISCUSSION

Children with egg allergy usually gain tolerance to BE prior to raw egg, and the age that this is achieved may relate to differing phenotypic presentations of egg allergy (24). In our cohort of 1 to 5 year olds (median age 2.08yrs) 51.8% were confirmed egg allergic with BE tolerance. This is consistent with other Australian cohorts of similar age, reported by Peters et al (24) who challenged 185 egg allergic 2 year olds and Tan et al (11) who reported BE tolerance in 63% of egg allergic children aged 3.8 (1.8-6.7) years. Our cohort appears to have developed BE tolerance at a younger age compared with the cohort of egg allergic English children reported by Clark et al (1) where 14% were BE tolerant at 2 years of age, 44% at 5 years of age, and 60% at 6 years of age.

We were able to achieve higher PPVs for egg allergen sIgE levels compared with the same egg allergen SPT indicating that sIgE measures may be more accurate in predicting the outcome of a BE OFC; however when ROC curves were used to assess the accuracy of the tests, AUROCS for all SPT reagents and egg allergen sIgE were below 0.8, indicating acceptable but not excellent tests (25). When AUROCs were compared, WE sIgE testing performed as well as EW and OVA sIgE. Egg yolk contains a combination of heat stable and heat labile egg allergens (8), and for this reason whole egg extracts which contain both egg white and egg yolk allergens may be useful reagents to predict tolerance to BE. We previously reported sensitisation to egg yolk allergens in egg allergic children intolerant to BE but the clinical significance of this is unclear (26).

In our cohort OVM SPT and OVM sIgE could not reliably be used to predict the outcomes of BE OFC, and 50% (8/16) of the BE intolerant children had absent or very low OVM sIgE titres. Absent OVM sIgE has been reported in 20-30% BE intolerant children (10, 17). OVM sIgE has been promoted as predictive of tolerance to BE, however the commonly cited reference (27) reported use of OVM sIgE to predict the outcome of

OFC to heat treated liquid egg white (heated to 90C for 60 minutes then dried), as opposed to a BE OFC.

Ratios of egg sIgE to WE IgG4 performed better than egg allergen sIgE levels alone in predicting the outcomes of a BE OFC. As single SPT or sIgE measures perform poorly more than one measure may be required to reliably predict tolerance to BE. Caubet et al (17) report an accurate logistic regression model with an AUROC of 0.87 incorporating specific IgE and IgG4 to OVA and OVM for prediction of outcomes of BE OFC. A highly accurate model incorporating clinical and immunological parameters has been proposed for prediction of raw egg allergy but does not predict tolerance to BE (28).

Strengths of this study include a well-defined cohort with an age range of 11 months to 5 years compared with other studies with wider age ranges (up to 18 years) (2, 15). All children had a BE OFC regardless of SPT wheal size, sIgE titre and allergy history. The SPT and blood draw were performed on the same day ensuring a direct correlation of results with the outcome of the BE OFC. Consistent protocols were used for the BE OFC and reporting of clinical outcomes of the OFC.

Limitations of this study include the cross sectional sampling and the relatively small sample size. The result of an OFC relies on consumption of adequate challenge food to provoke a reaction, and detection (or not) of reactions that reach pre-determined 'stop criteria' (22). In our study, 10.8% (9/83) of the children had suboptimal BE OFC due to refusal to eat the challenge food. Refusal to consume challenge foods may be related to neophobic behavior as the child is asked to consume an unfamiliar food during an OFC (in our study one child who had a suboptimal BE OFC subsequently passed a raw egg OFC), or may be related to early oral signs (itchy mouth or tingling lips) preventing the child from consuming the food (24, 29). As these symptoms are subjective in nature a challenge that is ceased because of an itchy mouth is still deemed to be suboptimal as an objective stop criterion is not achieved, even though refusal to eat the challenge food may be a sign of an

allergic symptom. A double-blinded OFC may have discriminated between this, however for resource reasons we only performed open OFCs.

Anaphylaxis to BE OFC has been described (9-11), which has safety implications for clinicians deciding the setting for an OFC. Guidelines for considering home BE OFC have been published by the British Society for Allergy and Clinical Immunology (BSACI) (30) and using these guidelines the three children in our cohort who had anaphylaxis to BE would not have been considered for home BE OFC due to their SPT results.

CONCLUSION

We report egg allergen SPT and sIgE cut off values and associated PPVs, NPV and likelihood ratios to predict BE tolerance in 1 to 5 year old egg allergic children. OVM SPT and sIgE performed poorly compared with other egg allergens in predicting BE tolerance, as many of the children were not sensitised to OVM. Egg allergen sIgE to WE sIgG4 ratios may be useful measures for clinicians selecting the optimal time for an OFC, but require further investigation. For the present a BE OFC remains the most reliable means of determining tolerance to BE in an egg allergic individual.

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APPENDIX

E-TABLE 1 CLINICAL HISTORY OF CHILDREN WITH ANAPHYLAXIS TO BAKED EGG ORAL FOOD CHALLENGE

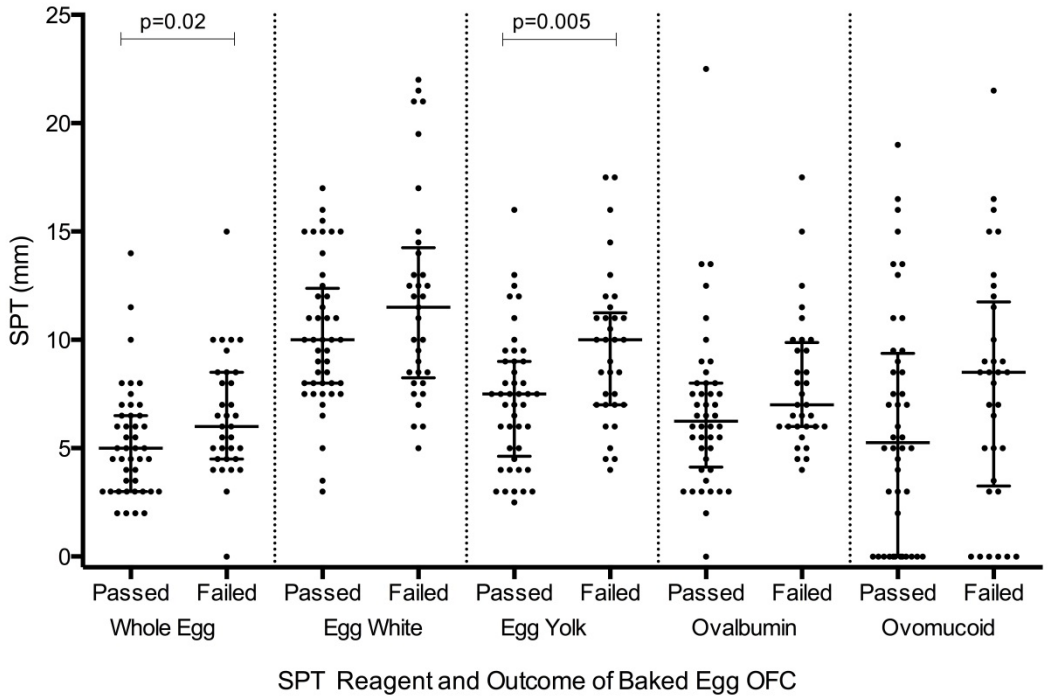
Age*	Prior history	SPT results					Amount consumed	Stop criteria	Treatment
		WE	EW	EY	OVA	OVM			
4.9 yrs	1 tsp scrambled egg at 10 months of age causing urticaria, angioedema, rash, wheeze IgE mediated peanut, cashew shell fish allergies eczema	4	19.5	10	9.5	15	7/8 muffin	II.D:IV.A	adrenaline cetirizine
4.9 yrs	Sensitised to egg IgE mediated peanut and cashew allergies eczema	5.5	22	10	15	15	1/8 muffin	II.D	cetirizine
2.2 yrs	Sensitised to egg IgE mediated cow's milk, peanut and cashew allergies eczema	10	21.5	14.5	10	0	3/8 muffin	1.B; ID;IIA; IIB; II.D:IV.A	cetirizine adrenaline

Legend to E-Table 1: * age at challenge. WE (Whole egg); EW (egg white); EY (egg yolk); OVA (Ovalbumin); OVM (Ovomucoid). See Table 2 for definitions of 'stop criteria'.

CHILDREN WITH ANAPHYLAXIS TO THE BAKED EGG ORAL FOOD CHALLENGE

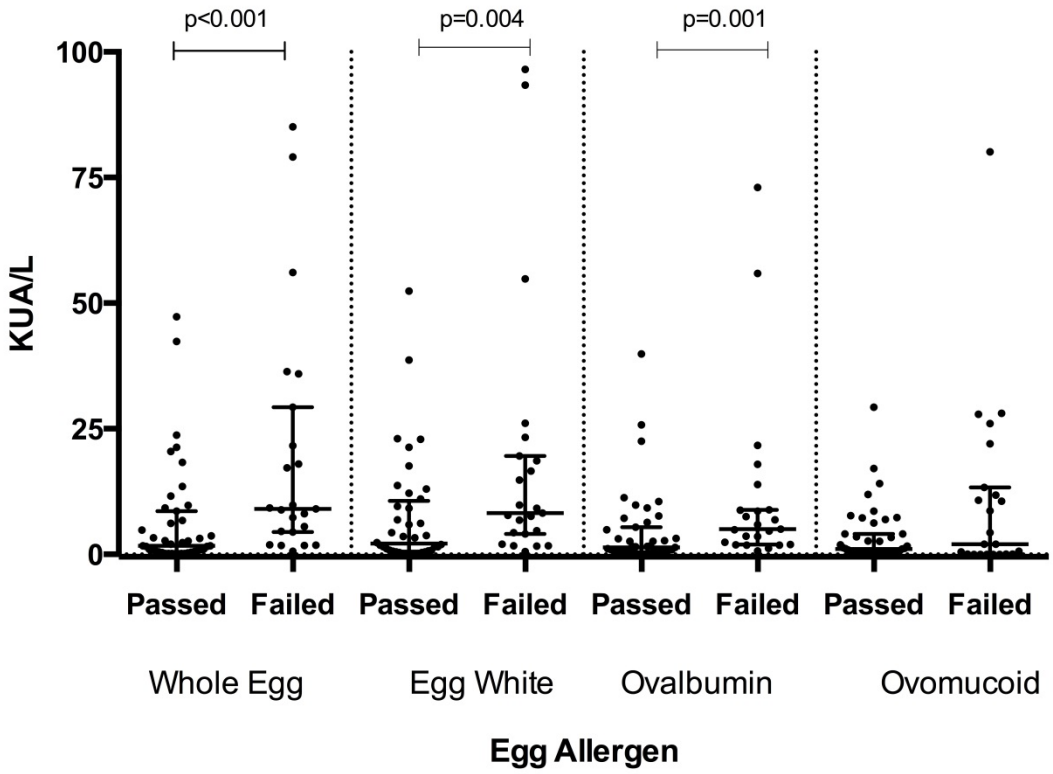
Subject 004 (4.9 years of age) had eczema, and a previous history of anaphylaxis to scrambled egg at 10 months of age. The SPT results were 4mm (whole egg), 19.5mm (egg white), 10mm (egg yolk), 9.5mm (ovalbumin) and 15mm (ovomucoid). After consumption of 7/8 of a muffin the child developed abdominal pain and cough, and was treated with cetirizine and IM adrenaline. Subject 031 (also 4.9 years of age) had no prior documented reaction after consumption to egg, but was highly sensitised on SPT; 5.5mm (whole egg), 22mm (egg white), 10mm (egg yolk), 15mm (ovalbumin) and 15mm (ovomucoid). The child developed cough after consumption of 1/8 of a muffin, and was treated with cetirizine alone. Subject 071 (2.2 years of age) also had eczema and sensitization to egg without prior exposure. SPT results were 10mm (whole egg), 21.5mm (egg white), 14.5 mm (egg yolk), 10mm (ovalbumin), 0mm (ovomucoid). The child consumed 3/8 of a muffin and developed itch, increased eczema, upper respiratory symptoms and abdominal pain, managed by cetirizine and IM adrenaline.

E-FIGURE 1 SKIN PRICK TEST RESULTS BY OUTCOME OF BAKED EGG ORAL FOOD CHALLENGE



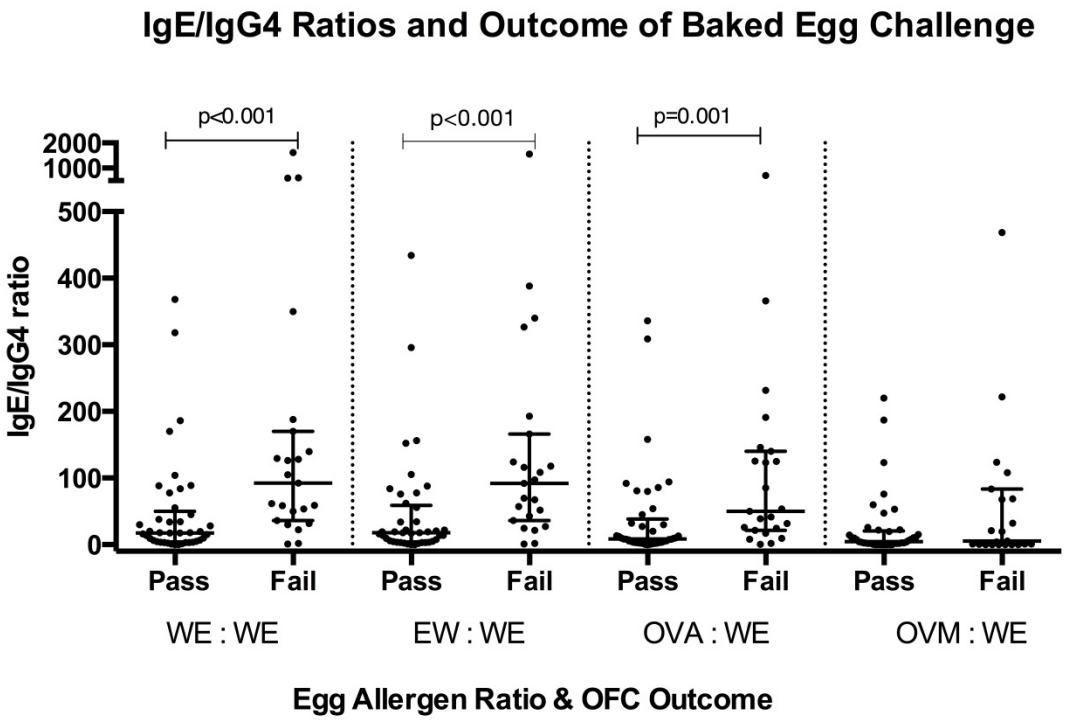
Legend to E-Figure 1: Outcome of BE OFC compared with outcome of Skin Prick Testing to Egg Allergens. Whole Egg (WE); Egg White (EW); Egg Yolk (EY); Ovalbumin (OVA) and Ovomucoid (OVM). The line and bar indicate the median and IQR.

E-FIGURE 2 EGG ALLERGEN SPECIFIC IGE RESULTS BY OUTCOME OF BAKED EGG ORAL FOOD CHALLENGE



Legend to E-Figure 2: Serum specific IgE levels to Egg Allergens and outcome of Baked Egg Oral Food Challenge. Whole Egg (WE); Egg White (EW); Ovalbumin (OVA) and Ovomucoid (OVM). The line and bar indicates the median and IQR.

E-FIGURE 3 EGG ALLERGEN SPECIFIC IgE/IgG4 RESULTS BY OUTCOME OF BAKED EGG ORAL FOOD CHALLENGE



Legend to E-Figure 3: Outcome of Baked Egg oral food challenge compared with sIgE to whole egg IgG4 ratio. Whole Egg (WE); Egg White (EW); Ovalbumin (OVA) and Ovomucoid (OVM). The line and bar indicates the median and IQR.

CHAPTER 5

HEATED ALLERGENS AND INDUCTION OF TOLERANCE IN FOOD ALLERGIC CHILDREN

INTRODUCTION

Chapter 5 reviews the use of specific oral tolerance induction as treatment for food allergic children, and the potential for heated allergens, specifically egg and milk for use as vehicles for specific oral tolerance induction. The article reviewed both heated egg and milk as there were limited publications related to egg alone.

The paper entitled, "Heated Allergens and Induction of Tolerance in Food Allergic Children" by Merryn Netting, Michael Gold, Patrick Quinn, Maria Makrides and Irmelli Penttila was published in the peer reviewed journal, *Nutrients* 2013, 5, 2028-2046; doi:10.3390/nu5062028.

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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Merryn Netting		
Contribution to the Paper	Responsible for conception, design and conduct of the study. Responsible for acquisition of data, analysis and interpretation of data. Prepared first draft of the manuscript and all subsequent revisions.		
Signature		Date	19/8/15.

Name of Co-Author	Prof Maria Makrides		
Contribution to the Paper	Input into the design. Interpretation of data. Critical revisions of the manuscript.		
Signature		Date	19/8/15

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Contribution to the Paper	Contributed to the design of the project. Interpretation of data and critical revisions of the manuscript.		
Signature		Date	19-8-15

Name of Co-Author	Dr Patrick Quinn		
Contribution to the Paper	Contributed to the design of the project. Interpretation of data and critical revisions of the manuscript.		
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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

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Contribution to the Paper	see page 1 of statement		
Signature		Date	

Name of Co-Author	A/Prof Imme Penttila		
Contribution to the Paper	Input into conception and design of study. Academic supervision of analysis of data and interpretation of results. Final approval of version of manuscript that was submitted.		
Signature		Date	19/8/15

Name of Co-Author			
Contribution to the Paper			
Signature		Date	

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Review

Heated Allergens and Induction of Tolerance in Food Allergic Children

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Abstract: Food allergies are one of the first manifestations of allergic disease and have been shown to significantly impact on general health perception, parental emotional distress and family activities. It is estimated that in the Western world, almost one in ten children have an IgE-mediated allergy. Cow's milk and egg allergy are common childhood allergies. Until recently, children with food allergy were advised to avoid all dietary exposure to the allergen to which they were sensitive, in the thought that consumption would exacerbate their allergy. However, recent publications indicate that up to 70% of children with egg allergy can tolerate egg baked in a cake or muffin without apparent reaction. Likewise, up to 75% of children can tolerate baked goods containing cow's milk, and these children demonstrate IgE and IgG4 profiles indicative of tolerance development. This article will review the current literature regarding the use of heated food allergens as immunotherapy for children with cow's milk and egg allergy.

Keywords: egg; milk; allergy; heated allergens; tolerance; oral; immunotherapy

1. Introduction

Food allergies have become increasingly common around the world, and it is estimated that globally, about 220–520 million people may suffer from food allergy [1]. Although it is possible to develop an IgE-mediated allergy to any food, most individuals with allergies react to one or a combination of nine common foods: cow's milk, soy, egg, wheat, peanut, tree nuts, sesame, fish and shellfish [2].

Food allergies are one of the first manifestations of allergic disease and have been shown to significantly impact on general health perception, parental emotional distress and family activities [3]. Young children, in particular, are at risk of developing food allergy, and it is estimated that 5% to 8% of toddlers are affected, compared with 1% to 2% of adults [1]. The incidence of allergic diseases is increasing globally, and this poses a major burden to healthcare costs in every country around the world [1].

Many children outgrow their food allergy; however, recent data suggest that the natural history of food allergy has changed, and it is now common for allergies to persist to late childhood and early adulthood [4,5].

In an affected patient with a food allergy, management consists of removal of the food from the diet and provision of an action plan for management of any accidental exposure to the food protein. More recently, complete exclusion of the food from a food allergic individual has been questioned. It is possible that including the allergenic food in the diet has potential to increase the likelihood of a child outgrowing the allergy more quickly or to allow a certain amount of the food to be tolerated [6]. Complete and absolute avoidance of a food allergen may not be necessary in a child's diet if they show tolerance to that food in an alternative format. Many children with cow's milk or egg allergies tolerate foods containing baked milk [7] or egg protein [8], and the hypothesis that this might be associated with a move towards tolerance of the protein has moved dietary management strategies away from strict avoidance to allowing the inclusion of the baked protein in the diet if it is tolerated [9–11].

With the rising incidence in food allergies there has been interest in improving their management, with the aim of offering a treatment strategy that does not solely rely on avoidance of food proteins in the diet. Immunotherapy is used successfully for managing venom and pollen allergies, and thus, there has been increasing research into the use of immunotherapy to manage common food allergies [12]. This review will summarize current research related to oral immunotherapy for management of IgE-mediated cow's milk and egg allergy and, in particular, the potential for heated proteins to be used as vehicles for oral immunotherapy.

2. Development of Oral Tolerance

The intestine is the largest immune organ in the body, and it works to protect the body from a constant onslaught of pathogens and ingested proteins derived from the outside environment by secreting Immunoglobulin A (IgA) and activating immune cells to fight pathogens or to act as regulatory cells to maintain gut homeostasis. Gut-associated lymphoid tissue (GALT), in particular, Peyer's Patches, are involved in the induction of immunity and maintenance of gut homeostasis [13,14]. The gut epithelium, along with dendritic cells, plays an active role in this process by continually

sampling the gut luminal contents [15]. The interactions between microbes, epithelium and GALT are involved in developing memory of the immune system and are essential for the development of oral tolerance [16].

Our understanding of the exact mechanisms involved in the development of tolerance to an allergen is evolving; however, the precise mechanism(s) by which the normal intestinal immune system responds to food and its involvement in the development of allergy remains essentially unresolved [17,18]. Development of oral tolerance is dependent on a number of early events, including allergen exposure (age, dose and timing), gut colonisation and the influence of maternal milk [19–25]. The resulting regulated immune response is an antigen driven process involving repeated exposure to the antigen and establishment of healthy gut colonization with commensal bacteria [25].

Development of oral tolerance to a food allergen involves early changes at the level of the gut mucosa [26]. Initially, there is a need to establish a local immunosuppressive gut milieu dominated by immunomodulatory cytokines, such as IL-10 and TGFβ, which control inflammation, non-specifically [27]. This environment is then conducive to the development of a highly regulated systemic response and differentiation of antigen-specific ($CD4^+CD25^+FOXP3^+$) regulatory T-cells (T-regs), essential for immune homeostasis in the intestine [23,24,28–31]. However, the role of these regulatory T-cells in food allergic disease and regulation of immune response development to allergens in the gut remains unclear. Studies have shown that transcription factor Foxp3 is critical for the development and function of T-regs. A variant of IPEX syndrome, which is characterised by autoimmune and severe allergic symptoms, including severe enteropathy, food allergies, atopic dermatitis, hyper-IgE and eosinophilia, is associated with a mutation within an upstream non-coding region of Foxp3 that affects its function [32]. Recent studies have also shown that in the intestine retinoic acid producing dendritic cells and $Foxp3^+$ T-regs interact to induce oral tolerance [33,34]. With regard to food allergy, the frequency of $Foxp3^+$ cells in the periphery is low in individuals with food allergies, and children with food allergy have reduced T-regulatory cell function [35]. Other studies have shown that a higher frequency of food allergen-specific T-regulatory cells correlates with a milder clinical phenotype and favourable prognosis [36].

3. Immunotherapy for Food Allergies

Given the potential of exposure to a small amount of allergen to promote tolerance to that allergen and the successful use of immunotherapy for management of venom and pollen allergies, there has been increasing research into the use of immunotherapy to manage common food allergies [12]. Early use of subcutaneous immunotherapy for food allergy resulted in severe reactions, and so, recent focus has been on oral immunotherapy [37]. Forms of oral immunotherapy to treat food allergy include specific oral tolerance induction (SOTI) [12], where the allergen is ingested, or sublingual immunotherapy (SLIT), where a minute amount (picograms) of allergen, in the form of allergen extract or diluted allergen, is placed under the tongue [12,38]. Both approaches start with a ‘build up phase’, initially starting with very small, diluted doses of the food protein with the amount of protein included in the dose slowly increased in a stepwise manner. This is followed by a “maintenance phase” with a consistent dose of allergen given at regular intervals. The regimes are followed with a “discontinuation phase” and a “tolerance food challenge” to test the effectiveness of the

immunotherapy treatment. The entire process may take 12 months or longer to complete. Adverse events are common during oral immunotherapy—this risk of an adverse event increases with illness, exercise, menses and every time the dose of the food is increased [39,40]. If regular consumption of the allergen is discontinued during the maintenance phase, there is a risk that tolerance will be broken, and an allergic reaction may follow exposure [38]. For food allergy, the endpoint of immunotherapy may not always be tolerance—the therapy may result in *desensitization*, an increase in the antigen dose required to elicit symptoms arising from a transient altered immune response and dependent on regular (daily) exposure to the allergen, or the therapy may result in true *tolerance* [41]—the ability to ingest the allergen without any symptoms once regular exposure has ceased, owing to persistent changes in the immune response. Desensitization still has a benefit in that larger doses of the allergen can be tolerated before eliciting a hypersensitivity reaction, thus lessening the potential for an anaphylactic reaction from accidental exposure.

Oral immunotherapy has been attempted for many food proteins, but we have specifically chosen to discuss cow's milk and egg oral immunotherapy, because of the interest in using baked forms of these foods as a treatment strategy. There is one Cochrane review from 2012 [42] on oral immunotherapy for cow's milk allergy, but none for egg. The Cochrane review included 16 studies, representing five trials and concluded that milk oral immunotherapy can lead to desensitization in the majority of individuals, although development of long term-tolerance has not been established.

Key trials reporting cow's milk oral immunotherapy and egg oral immunotherapy in the last 10 years are summarized in Tables 1 and 2. Nine studies reported outcomes of cow's milk oral immunotherapy and also nine for egg oral immunotherapy. These include three randomized controlled trials (RCTs) for cow's milk SOTI [38,43,44] and three RCTs investigating egg oral immunotherapy [45–47], but most of the studies report on open, uncontrolled prospective trials. We have added one trial [38] that was not included in the cows' milk immunotherapy Cochrane review. These studies have limitations. The studies utilized highly variable selection criteria, dosing regimens and maintenance doses, making them difficult to compare. The individual studies reported have limited statistical power, as only a small number of children were included in the treatment group.

All of the 18 studies reported SOTI, and one study reported results of a SLIT/SOTI combination protocol for cow's milk immunotherapy. Keet *et al*. [38] compared the outcome of children with cow's milk allergy who were given a SLIT protocol initially and then randomized to a low or high dose SOTI protocol or SLIT alone. More children in the SLIT followed by the high dose SOTI group passed the final oral food challenge after six weeks compared to those in SLIT alone or the lower SOTI dose.

Dosing regimens varied from 15 mL to 200 mL cow's milk and 0.3 g to 60 g of egg for the maintenance phase. Furthermore the allergen used for the reported regimes varied—for example fresh raw whole egg, fresh raw egg white, pasteurized egg white, pasteurized whole egg, lightly cooked egg have all been used for the egg oral immunotherapy studies. Most of the cow's milk immunotherapy studies used fresh cow's milk, but some reported use of dried non fat milk powder. Some of the studies asked participants to consume the maintenance dose on a daily basis, and some required the dose to be consumed every 2–3 days.

Table 1. Summary of key egg-specific oral immunotherapy trials.

Study	Study Design	Subjects *N*, age (range) in active groups	Protocol	Clinical Outcomes	Clinical Results: if no endpoint challenge, tolerance was determined by tolerance of maintenance dose	Immunological Outcomes
Burks, 2012 [46]	RCT	*N* = 40 7 years (5–11)	SOTI Egg white powder *vs.* corn starch Blinded until 10 month challenge	***Maintenance*:** up to 2 g egg white powder daily (1/3 egg) ***Endpoint challenge*:** OFC at 10 and 22 months (5 g EW powder) If child passed 22 month OFC, OIT ceased and egg-free diet for 4–6 weeks. At 24 months, these children had an OFC with 10 g egg white powder and one cooked egg. Evaluations at 30 and 36 months per telephone.	**At 10 month OFC:** 22/35 tolerant 1/35 withdrew **At 22 month OFC** 30/34 tolerant **At 24 months OFC**: 11/29 tolerant **30 months phone call:** 11/11 tolerant **36 months phone call:** 10/10 tolerant	SPT ↓ IgE ↓ IgG ↑ Egg-specific basophil activation ↓
Meglio 2013 [45]	RCT	*N* = 10, median age 8.4 years	SOTI Adjunct Therapy Raw fresh egg *vs.* egg-free diet	***Maintenance*:** 25 mL raw egg three-times/week, alternatively could consume foods corresponding to about three eggs per week. ***Endpoint challenge*:** No	**At six months:** 8/10 tolerant 1/10 partially tolerant 1/10 withdrew (2/10 in control group achieved tolerance of raw egg in same time period.)	SPT ↓ SPT ↓ IgG4 ovomucoid ↔ IgG4 ovalbumin ↑ Cytokines and transforming growth factors (IL-5 ↑)
Dello Iacono 2013 [47]	RCT	*N* = 10 7 years, 7 months (5–11)	SOTI Raw fresh egg *vs.* egg free diet	***Maintenance:*** 40 mL raw egg (one small egg). ***Endpoint challenge*:** No	**At six months:** 9/10 partially tolerant 1/10 no tolerance	SPT ↓ IgE ↓
Patriarca, *et al.* 2007 [48]	Prospective, open study with reference group	*N* = 14 3–16 years	Sublingual initially, then higher doses orally. Adjunct therapy Raw fresh egg	***Maintenance*:** one egg 2–3 times/week. ***Endpoint challenge*:** No	10/14 tolerant 1/14 partially tolerant 1/14 failed 2/14 withdrew	SPT ↓ IgE ↓ IgG4 ↑

Table 1. *Cont.*

Fuentes-Aparico 2012 [49]	Prospective, open study with reference group	*N* = 19 mean 9.2 years (4–14)	SOTI Pasteurised egg powder	***Maintenance*:** 10 g powdered egg (equivalent to one egg) ***Endpoint challenge*:** No Follow up at six and 12 months	16/19 tolerant 2/19 withdrew 1/19 partially tolerant to cooked egg	IgE ⇔ IgG4 ↑ T-cell subtypes: B-cells, NK cells, NKT cells, CD8+T-cells: ⇔ Effector memory CD4+T-cells (TEM): ↓ CD4+ recent thymic emigrants (RTEs): ↑ CD38/RO neg T-cells: ↑ Cytokines: IL-12, IL4, IL-13 & IL-β: ⇔ Th1 cytokines (IL-2, TNF-α and IFNγ): ↓ Th2 cytokines (IL-5 and IL-10) & Th9 (IL-9), Th22 (IL-22) and Th17 cytokines (IL-17A): ↓ IL-13 ↑(trend NS)
Patriarca *et al.* 2003 [50]	Prospective, open study	*N* = 12 <16 years	SOTI Adjunct therapy Raw fresh egg	***Maintenance*:** one egg (50 mL) 2-3 times/week. 3-8 months ***Endpoint challenge*:** No	10/12 tolerant 2/12 withdrew	SPT ↓ ⇔ IgE ↓ IgG4 ↑
Buchanan *et al.* 2007 [51]	Prospective, open study	*N* = 7, 4 years (14–84 months)	SOTI Egg white powder	***Maintenance*:** 300 mg daily for 24 months. ***Endpoint challenge*:** DBPCFC at 24 months (10 g egg white). If passed, followed by open scrambled egg challenge.	**24 month DBPCFC** 4/7 tolerant. 3/7 failed, but tolerated more egg	IgE ↓ (NS) IgG4 ↑
Itoh, 2010 [52]	Prospective, open study	*N* = 6, mean 9.7 years (7–12)	SOTI Raw powdered egg white and lightly cooked (whole) egg	***Maintenance*:** one whole egg (60 g) At least two times/week. ***Endpoint challenge*:** OFC to powdered egg at 9–12 months after starting maintenance.	**9–12 months OFC:** 3/6 tolerant 3/6 failed At 16 months: 6/6 tolerated >60 g heated egg.	SPT ⇔ IgE ↑ then ↓ IgG4 ↑ Histamine release test ↓; Th1/Th2 ratio ↓ at six months, not at 12 months; IL-4, IFNγ ⇔; IL-10 ↓, TGFβ1 ↑
Garcia Rodriguez, 2011 [53] **Urra, 2012** [54]	Prospective, open study	*N* = 23 mean 8.1 years (5–17)	SOTI Pasteurised raw egg white and whole cooked egg	***Maintenance*:** one cooked egg daily for three months. Exposure then extended to once in 48 h and, at six months once, in 72 h. ***Endpoint challenge*:** No	20/23 tolerant 1/23 withdrew 2/23 switched to slow protocol and achieved tolerance.	SPT ↓ IgE ↓ IgG4 ↑ CD4 + FoxP3 + cells: ↑

N, number; RCT, randomized controlled trial; SOTI, specific oral tolerance induction; OFC, Oral Food Challenge; DBPCFC, Double Blind, Placebo Controlled Food Challenge; EPSPT, Endpoint titration skin prick testing; SPT, skin prick test; NS, not significant.

Table 2. Summary of key cow's milk specific oral immunotherapy trials.

Study and Study Type	**Study Design**	**Subjects *N*, age (range) in active groups**	**Protocol**	**Outcomes**	**Clinical Results: if no endpoint challenge, tolerance was determined by tolerance of maintenance dose.**	**Immunological Outcomes**
Skripak 2008 [43]	RCT	*N* = 13 9 years (6–21)	SOTI Dried powdered milk *vs.* Profree	***Maintenance*:** 500 mg milk protein (15 mL milk) ***Endpoint challenge*:** DBPCFC up to 2000 mg, cumulative dose of 8 g milk protein	12/13 tolerant 1/13 withdrew	IgE ↑ IgG4 ↑
Martorell 2011 [44]	RCT	*N* = 30 * 2 years (2–3)	SOTI Fresh cow's milk *vs.* milk-free diet	***Maintenance*:** 200 mL daily plus unlimited dairy products. ***Endpoint challenge*:** only in two children who did not tolerate SOTI.	**At 16 weeks:** 27/30 tolerant 1/30 partially tolerant 1/30—no tolerance 1/30—withdrew	IgE ↓
Keet 2012 [38]	RCT	*N* = 30 6–16 years SLIT group *n* = 10 median age: 8 year (6–11), OITB group *n* = 10 9 year (6–15), OITA group *n* = 10 8 years (6–16)	SLIT alone, *vs.* SLIT followed by SOTI (high or low dose). SLIT, target dose 7 mg. OITA, target dose of 2 g. OITB, target dose of 1 g. SLIT, CM allergenic extract SOTI, dried non-fat milk powder	***Maintenance*:** SLIT, target dose 7 mg. OITA = target dose of 2 g. OITB = target dose of 1 g. ***Endpoint challenge*:** OFC to 8 g cow's milk protein at 32 and 80 weeks (on therapy) *and* at 81 and 86 weeks (off therapy)	28/30 completed protocol 2/30 withdrew **Overall at end of protocol** SLIT/SLIT: 1/10 tolerant SLIT/OITB: 3/10 tolerant SLIT/OITA: 5/10 tolerant **Challenge threshold at 32 weeks (12 weeks of maintenance)** SLIT: 7× increase OITB: 64× increase OITA: 79× increase **Desensitization *vs.* tolerance: 81 week challenge (one week off therapy):** 2/10 (SLIT/OITB group) failed, both requiring adrenalin. **86 week challenge:** SLIT/OITB group 1/10 failed SLIT/OITA group 3/10 failed	EPSPT IgE ↓ (in OIT, but not SLIT) IgG4 ↑ basophil histamine release ↓ (NS), constitutive CD63 expression ↓ (SLIT/SLIT group), CD203c expression ↓, intracellular spleen tyrosine kinase (Syk) levels ↔

Table 2. *Cont.*

Patriarca 2003 [50]	Prospective, open study with reference group	*N* = 16 <16 years	SOTI adjunct therapy fresh cow's milk	***Maintenance*:** 120 mL milk at least 2–3 times/week ***Endpoint challenge*:** No	10/16 tolerant 3/16 interrupted schedule 3/16 withdrew	SPT ↓ ⇔ IgE ↓ IgG4 ↑
Patriarca 2007 [48]	Prospective, open study with reference group	*N* = 14 3–16 years	SLIT, then SOTI. adjunct therapy fresh cow's milk	***Maintenance*:** 130 mL milk at least 2–3 times a week ***Endpoint challenge*:** No	10/14 tolerant 1/14 partially tolerant 1/14 failed 2/14 withdrew	SPT ↓ ⇔ IgE ↓ IgG4 ↑
Meglio 2004 [55] **& 2008** [56]	Prospective, open study	*N* = 21 6 years (5–10)	SOTI Adjunct Therapy Fresh cow's milk	***Maintenance*:** 200 mL milk per day for six months ***Endpoint challenge*:** No	15/21 tolerant (8/15 asymptomatic 7/15 symptomatic and managed with adjunct therapy) 3/21 withdrew **After four years** 13/20 tolerant 1/20 partially tolerant	SPT EPST ↓ IgE ⇔ Faecal blood: no occult blood reported.
Narisety 2009 [57] Follow up of [43]	Prospective, open study	*N* = 15 6–16 years, tolerating 75 mL cow's milk.	Tolerant children increased dose of dried powdered milk by 50% every two weeks.	***Maintenance*:** median dose 7000 mg milk protein (≈200 mL milk) ***Endpoint challenge*:** OFC at 13–75 weeks cumulative milk dose of 16 mg milk protein (480 mL)	**At 13–75 weeks** 6/16 tolerant 7/16 partially tolerant 2/16 ongoing symptoms with maintenance, so no OFC	EPSPT ↓ IgE ↓ IgG4 ↑
Alvaro 2012 [58]	Prospective, open study	*N* = 66 mean 8 years (44/66 with anaphylaxis to cow's milk)	SOTI fresh cow's milk	***Maintenance*:** 200 mL daily ***Endpoint challenge*:** No	**non anaphylactic group:** 16/22 tolerant 6/22 partially tolerant **anaphylactic group:** 35/44 tolerant. 7/44 partially tolerant 1/44 tolerant to 1 mL 1/44 withdrew	IgE ↓
Bedoret 2012 [59]	Prospective, open study	*N* = 10 8years (7–17)	SOTI adjunct therapy fresh cow's milk	***Maintenance*:** 2000 mg cow's milk protein (60 mL) ***Endpoint challenge*:** DBPCFC at week 24 Cumulative dose of 7250 mg, then open challenge of 120–240 mL milk.	**At week 24** 9/10 tolerant 1/10 withdrew	SPT ↓ IgE ↓ IgG4 ↑ CD4 T-cell proliferation ↓ T-reg cells ⇔ IFNγ /IL-4 ratio ↑ Basophil activation ↓

SLIT, sublingual immunotherapy.

Not all of the studies assessed actual tolerance to cow's milk or egg at the end of the intervention. Some used a double blinded, placebo controlled food challenge, the recognized "gold standard" for diagnosis of food allergy and some of the trials used open oral food challenges. The end point challenges varied in terms of the type of food used for challenge, with some testing the raw cow's milk or egg and some testing cooked egg for the case of egg immunotherapy. The volume of cow's milk or egg given in the final challenge varied.

Many children who underwent immune therapy were able to reach tolerance to cow's milk or egg in these studies however overall there was a high incidence of adverse reactions and a risk of loss of clinical desensitization after small periods off therapy [38]. Keet *et al.* reported loss of tolerance in as little as one week off SOTI [38]. The longest follow up period after the oral immunotherapy reported was three years for egg allergy [46] and four years for cow's milk allergy [56]. There are no safety data available testing the efficacy of oral immunotherapy beyond this length of time.

Many of the human intervention studies investigating clinical outcomes of individuals undergoing oral immunotherapy to cow's milk and egg have used skin prick testing as a marker of IgE levels or have measured serum specific IgE / IgG4 levels against the specific allergens to look for changes in the immune system towards tolerance (Tables 1 and 2). Only the more recent studies have begun to investigate changes at the cellular level [38,46,49,53]. Production of cytokines (IL10, TGFß and IFNγ) by T-cells influences the production of protective antibody responses by B cells including secretion of allergen-specific IgG4 and IgA and later inhibition of IgE. Immunotherapy has also been shown to lead to 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 implying that oral tolerance is being established [41].

Oral immunotherapy to egg and cow's milk shows potential as a management strategy for children with cow's milk and egg allergies and is able to induce desensitization and promote immune changes indicative of moves towards tolerance. However, SOTI carries a risk of adverse events both during the initiation phase and after periods off therapy, and the ideal treatment protocol is still to be identified. For researchers, a strategy that would facilitate this is the development of harmonized protocols, or, at least, harmonization of reporting of studies into SOTI to enable reporting, so that they can be clearly compared.

4. Use of Baked Proteins for Oral Immunotherapy—An Alternative Approach?

The form in which an oral allergen is delivered can influence the development of tolerance and the subsequent immune response profile generated. The studies summarized in Tables 1 and 2 all used raw proteins for the initial immunotherapy. If tolerance is achieved, in an individual, to raw uncooked proteins, then other forms of the food will also be tolerated. This has probably been the reason why centres have chosen to use raw foods as the vehicle for SOTI in the above reported clinical trials. Liquid or powders are also easy to measure, making the doses easy to deliver. However, SOTI using raw proteins is associated with a high rate of adverse events and often does not lead to permanent tolerance.

It is recognized that some individuals with cow's milk and egg allergies are able to tolerate the proteins they are allergic to in the form of baked goods. Traditionally, allergic individuals were advised to avoid all forms of the allergenic protein in their diet, even if the individual was including the allergen in cooked food, for example, without any adverse events, in the belief that this could delay the resolution of the allergy [6]. However, it is now thought that achievement of tolerance to a heat treated protein may be the first step towards "outgrowing" the allergy and inducing tolerance [10]. In a longitudinal study of British children [60], tolerance to well-cooked egg (baked in a sponge cake) was gained at a median of 67 months (5.7 years) compared to 127 months (10.6 years) for raw egg. Resolution still continued to occur to cooked egg up to the age of 158 months (13.2 years) and for raw egg up to 182 months (15.2 years). There are no similar longitudinal studies that assess the natural history of resolution to products containing baked milk proteins; however, Wood *et al*. [61] recently published data on a multicentre cohort of children with cow's milk allergy. Of 293 participants, the median age of resolution of cow's milk allergy was 63 months (5.25 years). Of the 155 children with unresolved allergy, 32 (20.6%) were able to tolerate products containing baked milk at the five year time point.

5. Why Are Baked Proteins Different?

Inclusion of baked milk or baked egg in the diet enables many foods that were previously avoided to be included in the diet, and this liberalization results in an improved quality of life for the child with food allergies. It is possible that regular inclusion of products containing the baked protein reduces the risk of reactions, due to accidental exposure to products containing egg or milk.

Proteins are three-dimensional molecules held together by electrostatic charge. IgE binding sites (or epitopes) may be sequential (several amino acids in a row) or conformational (part of the shape of the protein) [62]. Differences in allergenicity are, in part, due to changes in the structure of the proteins when heated, which affect the specific conformational IgE binding sites on the protein molecule. It is recognised that heat treatment of a protein can result in conformational changes in epitopes by affecting hydrogen bonds within the protein and, thus, affecting the ability of the IgE molecule to bind [63]. In the case of egg, heat treatment destroys the conformational epitopes that some individuals form IgE against, thus allowing ingestion of the egg without any adverse reaction. Some proteins are more susceptible to heat treatment than others, for example, the egg protein ovalbumin is more susceptible to heat treatment than ovomucoid [64]. In addition to altering the epitopes, heating the egg protein with wheat (for example, in a cake) forms a food matrix, also acting to reduce the allergenicity of the protein by affecting the digestibility of the proteins or making the IgE binding sites less accessible [64–66]. Other means of altering the allergenicity of a food protein include hydrolysis, acidification and other forms of food processing [67].

6. Studies Assessing Tolerance to Baked Proteins in Individuals with Egg Allergy and Cow's Milk Allergy

In 2008, Konstantinou [9] described the retrospective evaluation of 94 children (median age 24 months range 12–48 months) with egg allergy (n = 55) or IgE sensitization to egg (n = 39). Ninety percent of the children tolerated an open oral food challenge to baked egg (containing 1.5 g baked egg

protein). The 87 children who tolerated baked egg were then allowed to freely consume baked egg in their diet for six months and, then, were given a whole egg challenge. Only four out of 87 (4.6%) children reacted to this challenge.

Lemon Mule *et al*. [8] were the first to study immune changes associated with consumption of baked egg in a wheat matrix by individuals with egg allergy. They enrolled 117 children with diagnosed egg allergy (mean age 6.6, range 1.6–18.6 years) and challenged them with heated (baked) egg in the form of a muffin or a waffle—70% of the study group tolerated the baked egg and were then advised to include baked egg in their diet. The group was followed up at three, six and 12 months. Regular consumption of heated egg was associated with decreasing skin prick test (SPT) weal sizes to egg white and increasing ovalbumin- and ovomucoid-specific IgG4 levels. Intestinal permeability assessed by measurement of urinary clearance of non-metabolized sugars was no different between children consuming baked egg and those not consuming baked egg, and the children continued to grow well.

The study group was followed for six years, and the results of subsequent food challenges were reported by Nowak-Wegrzyn [68] and Leonard [69]. After incorporating heated egg into their diet, 58% of children eventually tolerated regular egg (French toast or scrambled egg) in their diets after a median of 16.6 months (interquartile range (IQR) 12.9–37.1 months). Children in the treatment group (consuming baked egg) were 14.6-times more likely to develop regular egg tolerance than children in the retrospective comparison group, and the children consuming baked egg developed tolerance earlier (median 50.0 *vs.* 78.7 months; $p < 0.0001$) compared with those who did not.

Nowak-Wegrzyn *et al*. [70] challenged 100 cow's milk allergic children (average age 7.5 years, range 2.1–17.3 years) with cow's milk baked in waffles or muffins. Seventy-five percent of the cow's milk allergic children tolerated baked milk. Those who tolerated baked milk were asked to consume products containing baked milk at home for three months and, then, were re-evaluated. Immune response (serum-specific IgE and IgG4), growth and intestinal permeability were also monitored. After three months, children consuming baked milk products had significantly smaller SPT and higher casein—IgG4 compared with baseline. On repeat challenges with uncooked cow's milk, the group of children who tolerated heated cow's milk outgrew their milk allergy quicker than the group who did not tolerate heated cow's milk in their diet [7].

Kim [7] reported the results of the long-term follow-up of the group initially described by Nowak-Wegrzyn. The initial baked milk challenge was tolerated by 65 of 88 children. Compared with the children strictly avoiding milk, baked milk-tolerant children were more likely to become tolerant to unheated milk than baked milk-reactive children (Odds Ratio 2.8 (95% Confidence Interval, 4.8–162.7); $p < 0.01$). No difference was noted in the milk-specific IgE levels between groups; however, both the casein IgE and β-lactoglobulin IgE values in the baked milk tolerant group decreased significantly over time. There was a significant increase in the median casein IgG4 value, but not the β-lactoglobulin IgG4 level from base line to final visit in the baked milk tolerant group. Eosinophilic esophagitis, which has been identified as a potential complication of SOTI [51], was reported in the baked milk study, but not the baked egg study. Two subjects in the active group and five in the comparison group had eosinophilic esophagitis, but this was not related to the intervention.

The baked egg and milk studies by Konstantinou, Lemon Mule *et al*. and Nowak-Wegrzyn *et al*. [8,9,70] are promising, but need substantiation, as the studies were not randomized controlled trials, and the

results may be due to comparisons of different phenotypes of milk or egg allergy, changes with time or other unidentified confounders. Neither of the studies tested tolerance to the raw protein after a period of avoidance of the baked products, so it is unknown whether the effect was permanent.

There are several advantages of using baked products as a mode of inducing tolerance to egg or cow's milk protein. Once tolerance to a baked product is proven, the child is able to include it in their diet on a regular basis. Compared with traditional SOTI protocols, this means that there is less cost in terms of hospital admission times and inconvenience to the family, as there is no intense "up dosing" phase required. This approach is potentially safer compared with protocols, which rely on parents making up and administering the appropriate diluted dose of the allergen at home. The ability to consume baked products also moves the child towards a more normal, less restricted diet.

At present, there are no tests that predict tolerance to baked egg or baked milk proteins. Foods containing baked egg are reported to elicit anaphylaxis in egg allergic individuals, and as such, individuals chosen for this therapy should be carefully screened [9].

Furthermore, to date, there are few studies reporting immune mechanisms after oral immunotherapy, and this is one area that requires further research to help guide development of immunotherapy protocols. There is one study by Shreffler *et al*. that has shown a significantly higher percentage of proliferating casein-specific CD25/CD271 T-cells (regulatory T-cells involved in tolerance induction) from casein-induced peripheral blood mononuclear cell cultures taken from extensively heated milk-tolerant children compared with cells from extensively heated milk-reactive children. They showed no significant difference between the groups in the frequency of polyclonal T-cells or casein-specific effector T-cells. However, casein-specific regulatory T-cells correlated with the phenotype of having a mild transient milk allergy and favourable prognosis [36]. Induction of these regulatory T-cells is critical for the development or oral tolerance, and therefore, the study supports the concept of using heated allergens for tolerance induction.

While allergen specific oral immunotherapy has potential, there are significant questions regarding effectiveness and safety [71]. We have focused our review on tolerance induction to egg and milk; however, peanut allergy is also a significant health issue. Unfortunately, using heated peanut as a potential immunotherapeutic may not be feasible, as some forms of heat treatment (roasting) enables exposure of new epitopes to form, rendering this form of peanut more allergenic than boiled or fried peanut [72].

7. Animal Studies Supporting the Use of Heated Proteins as Immunotherapeutic Agents

There has been some work in animal models of allergy describing the effect on ingestion of heated egg proteins, but little about heated milk proteins. Martos [64] demonstrated that heat treatment of ovalbumin and ovomucoid reduces the allergenicity partly by changing the digestibility of the protein. Egg-sensitized C3H/HeJ mice were challenged with either untreated or heated ovalbumin or ovomucoid. Oral challenge with the unheated protein induced anaphylaxis in all of the mice, but not when challenged with the heated proteins. Even when challenged intraperitoneally, the majority of the mice only displayed mild reactions. In a separate experiment, mice fed heated ovalbumin had minimal T-cell proliferation in the mesenteric lymph nodes, or Peyer's Patches, compared with those consuming unheated ovalbumin, suggesting that heating ovalbumin affects the intestinal absorption of

the intact form capable of triggering effector cells and T-cells. In this same paper, Martos [64] also reported that heated ovalbumin and ovomucoid still retained the ability to bind to serum from egg allergic children on immunoblotting, indicating that the epitopes were still intact.

Leonard [73] used a mouse model of SOTI to show that heated egg protein (ovomucoid) is able to desensitize egg allergic mice as effectively as unheated egg protein. C3H/HeJ mice sensitized to egg were treated with increasing doses of egg white or heated or native ovomucoid. Mice were challenged with ovalbumin or ovomucoid either one day or two weeks after the end of the desensitization period. At day 1, significantly fewer of the SOTI treated mice experienced anaphylaxis when compared with controls; however, when the mice were re-challenged at two weeks after ceasing SOTI, the previously desensitized mice were no longer protected from anaphylaxis. Mice given heated ovomucoid were completely protected against anaphylaxis compared with controls, as were mice given a higher dose egg white treatment compared with a low dose treatment. Antigen specific IL-13, IL-10 and IFNγ responses were all significantly lower in SOTI treated mice compared with controls, and this difference remained two weeks post treatment. Whole genome microarray analysis of jejunum of sensitized control mice compared with those who were treated with SOTI indicated that administration of SOTI may lead to the regulation of a subset of genes in the intestine, including some expressed by intestinal epithelial cells. They postulated that the reduction in both the IL-10 and IL-13 after the SOTI indicated that the effect was related to an overall suppression rather than a shift in TH1/Th2 response.

As discussed, human studies investigating the ingestion of heated cow's milk and heated egg in allergic individuals demonstrated that this was associated with decreased skin test weal diameters and allergen-specific IgE levels and increased allergen-specific IgG4 levels [8,74]. Further studies are required to further quantify the immune changes that occur with ingestion of heated proteins to enable us to understand if ingestion of heated egg or heated cow's milk affects the natural history of egg and cow's milk allergies when compared with strict avoidance. Other important information that is required includes identification of diagnostic tests to accurately predict reactions to heat treated proteins and target potential candidates for this form of therapy, development of optimal protocols and evaluation of their efficacy and safety.

8. Conclusions

Oral induction of tolerance is a promising option for the management of food allergy, particularly with the increasing numbers of children being diagnosed with food allergies worldwide. However, SOTI with raw proteins is not free of risks, and there is much work to be done before it can be safely recommended as routine. The inclusion of baked cow's milk and egg proteins in the diet of children with IgE-mediated cow's milk and egg allergies appears to move the children towards a more tolerant immune profile, and the use of baked proteins is appealing, as it is safe, improves quality of life as the child moves towards a more normal diet and mimics the natural history of development of tolerance.

Areas for future research include unravelling the underlying mechanisms in the development of tolerance to a food. This will aid in the understanding and identification of the optimal route and protocols for dosing during the desensitization phase and also help to identify markers showing tolerance development as opposed to desensitization. Diagnostic tests to correctly identify candidates to approach regarding SOTI are required as are steps taken to optimize the safety of protocols.

Harmonized study protocols or at least harmonization of reporting of studies into SOTI to enable reporting, so that they can be compared, is essential to the progression of this important area of research. Longer-term trials comparing children of like age groups and allergy phenotypes are required. Quality of life data should also be gathered from the individuals undergoing SOTI, as this has been largely absent from the studies reported, so far. Much more research is required to investigate if this will help children outgrow their allergies quicker, thus lessening the burden of managing food allergic disease.

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Conflict of Interest

MN and MG serve on a scientific advisory board for Nutricia. MM serves on scientific advisory boards for Nestle, Fonterra and Nutricia. Associated honoraria are paid to the institution to support conference travel and continuing education for post-graduate students and early career researchers. No other disclosures were reported.

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CHAPTER 6

RANDOMISED CONTROLLED TRIAL OF A BAKED EGG INTERVENTION IN YOUNG EGG ALLERGIC CHILDREN

INTRODUCTION

Chapter 6 reports on the conduct and outcomes of randomised controlled trial which aimed to compare clinical and immunological outcomes after 6 months consumption of baked egg (BE) with an egg free diet in 1-5 year old BE tolerant, but raw egg allergic children.

The paper entitled, "Randomised Controlled Trial of a Baked Egg intervention in Young Egg Allergic Children" by Merryn Netting, Adaweyah El-Merhibi, Michael Gold, Patrick Quinn, Irmeli Penttila and Maria Makrides will be submitted for publication in the peer reviewed journal, Pediatric Allergy and Immunology in November 2015.

The simplified title for the trial was: "Can Egg Allergic Kids Eat Baked Egg? The CAKE Study."

Supplementary material related to the trial is located in the following Appendices: Appendix 4 - Study Protocol; Appendix 5 - Study Information Sheet; Appendix 6 - Consent Form; Appendix 7 - Materials and Methods; Appendix 8 - Development of Intervention Products.

Statement of Authorship

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Author Contributions

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Name of Principal Author (Candidate)	Merryn Netting		
Contribution to the Paper	Responsible for conception, design and conduct of the study including acquisition of data, analysis and interpretation of data. Prepared first draft of the manuscript and subsequent revisions.		
Signature		Date	19/8/15

Name of Co-Author	Dr Adaweyah El-Merhibi		
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Contribution to the Paper	Input into study design. Clinical supervision of the trial. Interpretation of results. Critical revision of the manuscript.		
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Contribution to the Paper	Input into study design. Clinical supervision of the trial. Interpretation of results. Critical revision of the manuscript.		
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Author Contributions

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RANDOMIZED CONTROLLED TRIAL OF A BAKED EGG INTERVENTION IN YOUNG EGG ALLERGIC CHILDREN

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ABSTRACT

Background: Consumption of baked egg by egg allergic children is associated with immune changes suggesting developing tolerance however causation has not been tested using a double blind randomized controlled trial.
Objective: We aimed to compare clinical and immunological outcomes after consumption of baked egg (BE) with an egg free diet in 1 to 5 year old BE tolerant egg allergic children.
Methods: In a double blind, randomized controlled trial BE tolerant, egg allergic children were randomized to consume 10 g BE two to three times per week for six months (n=21 intervention group) or similar egg free products (n=22 control group) while maintaining an egg free diet. The final assessment was a raw egg oral food challenge (OFC) one month after ceasing the intervention product. Skin prick testing (SPT) to egg allergens, egg specific IgE and IgG4, Th1/Th2 cytokines and T cell phenotype were assessed at baseline and 7 months.
Results: After the intervention there was no difference in egg tolerance between groups, (23.5% (4/17) intervention group and 33.3% (6/18) control group) which was independent of age and amount of BE consumed (aOR 0.50 IQR 0.11-2.40 p=0.39). Both groups demonstrated decreased SPT wheal sizes and egg specific serum IgE titres and increased whole egg IgG4. No significant difference was observed between the groups in Th1/Th2 cytokines and T cell phenotype.
Conclusion: The results suggest that young BE tolerant, egg allergic children are evolving tolerance to raw egg which may not be influenced by regular inclusion of BE. Larger trials are required.

Clinical Implications

The results of this RCT suggest that young baked egg tolerant, egg allergic children are evolving tolerance to raw egg, which may not be influenced by short-term regular inclusion of baked egg.

Capsule Summary

Young baked egg tolerant and egg allergic children may be evolving tolerance to raw egg independent of regular inclusion of baked egg in their diets.

Key Words: food allergy, egg, egg allergy, baked egg, oral tolerance, randomized controlled trial

Abbreviations: Ig – Immunoglobulin; BE – Baked egg; SOTI – Specific oral tolerance induction; WE -whole egg; EW - egg white; EY - egg yolk; OVA – ovalbumin; OVM - ovomucoid; SPT – skin prick test; OFC - oral food challenge; IL – interleukin; TGF – transforming growth factor;

Material for the electronic repository:

Appendix 1 - Methods

Appendix 2 – Intervention Products

Appendix 3 – Immunological Methodology

Appendix 4 - Results

INTRODUCTION

Many egg allergic children tolerate baked egg (BE) before less well-cooked forms of egg as heating causes structural changes in some egg epitopes (1-4).

Inclusion of BE in the diet of egg allergic children, when tolerated, is accepted clinical practice (5-8). This is supported by the landmark BE studies which reported that regular consumption of BE is associated with increases in protective IgG (1 and 4), and decreases in IgE (1, 9), immunological changes similar to those observed during specific oral tolerance induction (SOTI) (10). In these studies individuals consuming BE tolerated lightly cooked egg earlier than those not consuming BE (11, 12), an observation also reported in a population based study (13). Similar changes have been observed in baked milk tolerant, cow's milk allergic children (14). As we reviewed (15) these observations led to supposition that consumption of heat-denatured proteins could promote tolerance to uncooked proteins.

For various reasons the BE studies were not intervention trials comparing children phenotyped for tolerance to BE, nor was tolerance to raw egg assessed at the end of the interventions. As the clinical effects of consumption of BE have not been compared using a randomized controlled trial methodology it is unclear if ingestion of BE affects the natural history of egg allergy when compared with strict avoidance, or if the observations are related to selection of a group of children moving towards natural resolution of their allergy, changes with time or other unidentified confounders.

Our study was designed to test the hypothesis that regular consumption of BE by children with raw egg allergy will hasten the resolution of allergy to raw egg. The primary aim of this study was to determine whether allergy to raw egg is better resolved by regular consumption of BE (intervention group) compared with the standard practice of an egg free diet (control group). The secondary aim was to examine the effect of regular BE exposure on immunity, particularly on patterns of evolving allergen-specific responses.

METHODS

STUDY DESIGN

Approval was granted by the local institutional review board (Human Research Ethics Committee; REC2400/9/14) of the Women's and Children's Health Network Adelaide, Australia, and the trial registered with the Australian New Zealand Clinical Trials Registry (ACTRN 12612000173897). Six month to 5 year old children with IgE mediated egg allergy were recruited from the Women's and Children's Hospital Allergy Clinic. Written informed consent was obtained prior to trial participation. Children were excluded if they were consuming BE; had parents or caregivers unable to provide informed consent; had non IgE mediated egg 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 oral egg challenge (OFC).

All children had SPT to egg allergens (whole egg (WE), egg white (EW), egg yolk (EY), ovalbumin (OVA) and ovomucoid (OVM)) (see Appendix 1 in this article's Online Repository) and a peripheral blood sample was collected to measure WE, EW, OVA and OVM specific IgE (sIgE) and WE specific IgG4 (sIgG4) and functional cell response profiles. Baseline characteristics including demographics, allergy history and anthropometrics (weight and length or height) were gathered at this appointment. For children with eczema the clinical severity of the child's eczema was scored using a SCORAD assessment (16).

Egg allergy was defined as children with a previous convincing clinical reaction to egg within the past 12 months (and evidence of current sensitisation on the basis of positive SPT to egg white OR evidence of current sensitisation consistent with a >95% likelihood of clinical reactivity (SPT to egg white >5mm if aged under 2yo, or >8mm in children aged 2 to 5yo)(17, 18). To assess BE tolerance, all children had an open, medically supervised BE OFC (muffin, containing 10 grams egg) (19). BE tolerant children with EW SPT

<5mm (6 months to 2yo) or <8mm (2 to 5yo), with no clinical reactions to raw egg in the previous 12 months had an open raw egg OFC (20) to confirm their egg allergy.

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 and 2 years 1 month to 5 years 11 months). A research assistant (with no contact with the study participants and with no involvement in the outcome assessments) was responsible for the baking and coding of dietary products for the trial.

DIETARY INTERVENTION

The study compared the effects of inclusion of egg or egg free baked products in the diet of egg allergic children for 6 months after randomisation. Both randomisation groups maintained an egg free diet and were provided with muffins, biscuits (cookies) or cake to be offered to the child for consumption two to three times per week for six months. The intervention group consumed the equivalent of 10g BE (1.3g egg protein) per serve. The control group consumed egg free products similar in terms of appearance, taste, and texture to the intervention group. The baked egg products were offered two to three times a week so as to be consistent with the NHMRC Australian Dietary Guidelines for inclusion of 'discretionary foods' in a child's diet (21). Recipes developed and tested for blinding by MN are in Appendix 2 in this article's Online Repository.

A review appointment was scheduled at one month after randomisation and participants were telephoned monthly for the duration of the study to assess compliance with the study protocol, tolerance to the study product, accidental exposure to egg and compliance with the egg free diet. Six months after randomization 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 (22).

OUTCOME ASSESSMENTS

The primary outcome of this study was to observe tolerance to raw egg one month after the end of the intervention. To assess raw egg tolerance, the children had an open medically supervised, graded pasteurised whole egg OFC (20). 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. Consistent with PRACTALL Guidelines (23), a positive reaction to an OFC was defined by the development of symptoms within 2 hours of the egg challenge and include 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) (24). A serious adverse event was defined as any death, admission to the intensive care unit, or anaphylactic reaction. Serious adverse events were reviewed and reported to the institutional review board. The children had repeat SPT, blood sampling and anthropometric measurements. Information was also collected regarding any illnesses requiring hospitalisation during the course of the study.

At the end of the study, after the final assessment all children who failed the raw egg OFC were offered another BE OFC, performed while the group allocation was still blinded, to ensure maintenance of BE tolerance during the intervention.

ANALYSIS OF IMMUNE OUTCOMES

To assess sensitisation SPT wheal size and egg allergen sIgE levels were measured. WE $sIgG_4$ 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 (25). To further assess immune activation peripheral blood mononuclear cells (PBMCs) sampled at baseline and the end of

the intervention were incubated with OVA or OVM and assessed for T cell CD69 expression (26) and cytokine excretion from the PBMCs was also measured to assess changes in the Th1/Th2 balance. The sIgE and sIgG4 were analysed at the completion of the trial, and cell culture experiments (maintaining blinding of the sample ID) were performed in batches as the trial progressed. See Appendix 3 in this article's Online Repository for full methods for the cell culture experiments. Representative flow plots are shown in E-Figure 1.

STATISTICAL ANALYSIS

A sample size estimate was calculated based on the known natural history of egg allergy expecting that after six months of treatment with an egg free diet 90% of children would still be egg allergic (2). We hypothesized that regular exposure to BE would result in 30% absolute reduction (ie from 90% to 60%) of egg allergy. To detect such a difference with 90% power and p=0.05, we estimated that 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 randomized group using STATA 13.1 (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 tolerant to egg at the end of the study was compared between groups. Secondary comparisons between groups included changes in SPT wheal size, sIgE and sIgG4 results and other immune outcomes. For SPT, sIgE and sIgG4 results, standard linear regression was performed including the 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 did not affect the conclusions. Change between groups was assessed using the Wilcoxon Rank-Sum Test.

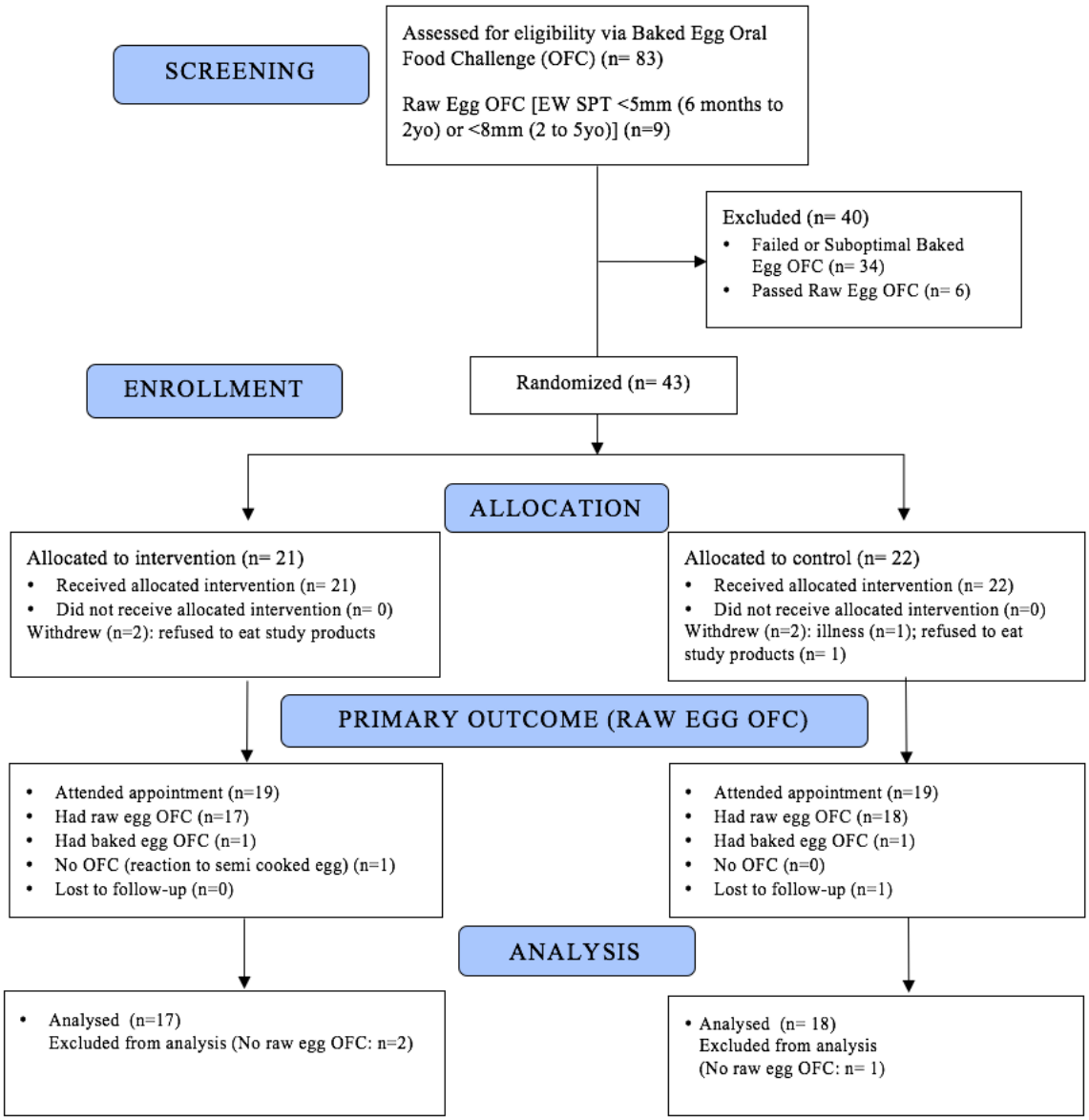
For the other immune outcomes a non-parametric approach was used for the analysis because of the highly skewed distributions of all variables and small sample sizes.

RESULTS

Randomization commenced on 22nd May 2012 and ceased 20th of January 2014. The final follow up appointment was completed on the 8th October 2014.

Forty-three children were randomized (n=21 intervention group; n=22 control group) (Figure 1). Both groups were similar in terms of family and clinical background (Table 1). Four parents withdrew consent for the following reasons: refusal to consume the study product (n=2 intervention group; n=1 control group) and illness (n=1, control group). One child (control group) was unable to be contacted. Thirty-eight children (19 from each group) attended the primary outcome assessment.

FIGURE 1: STUDY PARTICIPANT FLOW DIAGRAM



Legend to Figure 1: OFC= Oral food challenge

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%)
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%)

Legend to Table 1: Values are presented as follows: *mean (SD), †number (percentages) or ‡median (IQRs)

CLINICAL OUTCOMES

TOLERANCE TO RAW EGG AFTER THE INTERVENTION

Thirty-five children had raw egg OFCs (n=17 intervention group; n=18 control group). The 3 children who did not have raw egg OFC were excluded from analysis; one child (intervention group) had a recent reaction to semi-cooked egg and two had BE OFCs (n=1 intervention group; n=1 control group), because they did not consume the study products. 23.5% (4/17) children from the intervention group and 33.3% (6/18) control group passed

the raw egg OFC and were therefore egg tolerant. There was no difference between groups in the likelihood of tolerating raw egg (Odds Ratio (OR) 0.62 IQR 0.14-2.73 p=0.523), even when adjusted for age (aOR 0.50 IQR 0.11-2.40 p=0.390). Fewer children from the intervention group were egg tolerant, but this difference was not significant.

Per protocol analysis comparing children consuming 2-3 serves per week of study product 42.11% (4/19, intervention group), 68.42% (13/19, control group) demonstrated no difference between groups in the proportion passing the raw egg OFC (OR 1.200 IQR 0.185-7.770; p=0.848). Adjusted analysis was not performed because of small numbers.

COMPLIANCE WITH THE INTERVENTION

2 324 individual serves of intervention product were offered to the participants during the study. Children in the intervention group were offered fewer (1065) serves, consuming a median of 1.6 (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 serves per week (p=0.14) and the total intake of study product (p=0.10) were not significant.

ADVERSE EVENTS

Adverse events were reported by 9 children in the intervention group including; urticaria (unrelated to the study product n=2, related to study product n=1), flares in eczema (unrelated to study product n=2, unclear n=1), abdominal pain (possibly related to study product, n=1), vomiting (unrelated to study product n=1; vomiting after reintroduction of the study product post gastroenteritis n=2). Adverse events were reported by 8 children in the control group including flares in eczema (unrelated to study product n=5), vomiting (unrelated to study product n=3). One child (control group) was diagnosed with Eosinophilic Oesophagitis during the study. During the study period three children (n=1 intervention group; n=2 control group) had hospital admissions greater than 24 hours not

related to the trial. Three children (n=2 intervention group; n=1 control group) had anaphylaxis treated with adrenaline during the raw egg OFC.

TOLERANCE TO BAKED EGG WAS MAINTAINED DURING THE STUDY PERIOD

Twenty-two of 23 children (n=11 intervention group; n=12 control group) passed BE OFCs after the intervention. Two children (n=1 intervention group; n=1 control group) successfully reintroduced BE at home. One child (intervention group) failed the BE OFC. This participant refused to consume the study product, and although passed the BE screening at study entry, reportedly had a loose bowel action at home, and was possibly incorrectly classified as BE tolerant.

IMMUNOLOGICAL OUTCOMES

EGG ALLERGEN SKIN PRICK TEST AND SERUM SPECIFIC IgE RESULTS

Changes in egg allergen SPT wheal sizes and egg allergen sIgE levels did not differ between groups from baseline to the end of the intervention (Table 2). For both groups we observed significant reductions with time for EW and OVA SPT wheal size (BE group p=0.03, p=0.02; control group p=0.01, p=0.03) (E Figure 1) and WE, EW and OVA sIgE (E Figure 2).

WHOLE EGG SERUM SPECIFIC IgG4 AND IgE TO IgG4 RATIO RESULTS

There was no significant difference in the WE sIgG4 levels between groups from baseline to the end of the intervention. No difference in mean egg allergen specific IgE/WE IgG4 ratios was observed between groups over the duration of the intervention. However a decrease in the mean IgE/IgG4 ratio was observed with time in both BE intervention and control groups indicating evolving tolerance to egg.

TABLE 2 SKIN PRICK TEST, SPECIFIC IgE, IgG4 RESULTS AND IgE: IgG4 RATIOS IN BAKED EGG AND CONTROL GROUPS AT BASELINE AND THE END OF THE INTERVENTION

	BAKED EGG GROUP			**CONTROL GROUP**			**ESTIMATED DIFFERENCE**	
ALLERGEN	Baseline	7 Months	*Difference**	Baseline	7 Months	*Difference**	*Adjusted ***	*P* value
SKIN PRICK TEST RESULTS (mm)								
WE	5.00 (3.00-6.50)	5.00 (2.00-7.00)	-0.47 (4.06)	4.76 (3.00-6.89)	4.00 (1.00-8.50)	0.03 (4.51)	-0.29 (-3.06 to 2.48)	0.83
EW	8.50 (6.63-11.88	8.00 (5.00-11.50)	- 2.74 (4.83)	8.25 (4.25-10.75)	9.00 (4.50-11.50)	-1.55(4.82)	-0.47 (-3.48 to 2.55)	0.76
EY	6.00 (4.63-7.88)	6.00 (3.50-7.50)	-2.08 (3.62)	5.00 (3.00-8.75)	7.00 (4.00-8.50)	-0.63(4.14)	-0.89 (-3.32 to 1.54)	0.46
OVA	7.00 (4.00-8.00)	5.50 (3.00-7.50)	-1.55(2.82)	6.00 (5.00-9.00)	5.00 (3.00-8.00)	-1.63(3.39)	0.02 (-1.80 to 1.84)	0.98
OVM	0.0 (0.0-8.50)	7.00 (0.0-9.00)	-0.39(2.05)	2.50 (0.0-5.75)	6.50 (0.0-10.50)	0.34 (3.06)	-0.69 (-2.42 to 1.03)	0.42
SERUM SPECIFIC IgE RESULTS (kUA/L)								
WE	3.33 (0.73-11.62)	2.20 (0.47-8.01)	-1.35 (2.86)	1.71(0.19-6.94	1.26 (0.19-6.01)	-2.78 (9.68)	1.25 (-2.13 to 4.63)	0.456
EW	3.72 (0.82-11.62)	1.61 (0.42-8.17)	-1.40 (3.00)	1.95 (0.28-11.60)	1.66 (0.14-8.55)	-2.13 (5.98)	0.34 (-2.33 to 3.01)	0.797
OVA	2.57 (0.64-7.40)	1.23 (0.29-5.23)	-1.08 (1.78)	1.48 (0.33-5.29)	0.75 (0.15-4.05)	-1.28 (3.31)	-0.02 (-1.56 to 1.53)	0.984
OVM	1.87 (0.08-6.59)	0.66 (0.16-5.06)	-0.98 (2.02)	0.96 (0.23-7.27)	0.46 (0.11-6.67)	0.16 (3.39)	-1.08 (-3.05 to 0.88)	0.269
SERUM SPECIFIC IgG4 RESULTS (mgA/L)								
WE IgG4	0.16 (0.02-1.50)	0.64 (0.10-1.88)	0.297 (1.115)	0.16 (0.01-0.79)	0.27 (0.09-0.99)	0.288 (0.573)	0.00 (-0.645 to 0.645)	>0.999
IgE TO IgG4 RATIOS								
WE : WE	15.63 (3.51-33.00	2.91 (0.88-9.21)	-0.043 (0.107)	17.50 (3.86-50.18)	8.28 (0.74-14.13)	-0.054 (0.094)	0.029 (-0.014 to 0.073)	0.179
EW : WE	15.15 (3.58-21.67	2.97 (0.78-9.44)	-0.058 (0.166)	18.03 (4.72-58.82	7.22 (0.47-15.56)	-0.035 (0.047)	0.013 (-0.024 to 0.050)	0.489
OVA : WE	8.26 (2.13-18.32)	1.25 (0.39-4.11)	-0.043 (0.126)	8.07 (3.44-38.50)	4.20 (0.53-13.12)	-0.044 (0.091)	0.024 (-0.018 to 0.066)	0.256
OVM : WE	2.75 (0.92-10.90)	0.85 (0.21-3.29)	-0.027 (0.073)	7.71 (0.94-37.38)	3.00 (0.37-21.48)	-0.028 (0.056)	-0.003 (-0.016 to 0.010)	0.668

Legend to Table 2: Median result and interquartile ranges are shown. * Mean difference (SD) adjusted for baseline and age stratum **Linear Regression analysis for effect of group allocation on SPT outcome, adjusted for age stratum. WE (Whole Egg); EW (Egg White); EY (Egg Yolk); OVA (Ovalbumin); OVM (Ovomucoid); kUA/L (kilounits of Antibody/Litre); mgA/L (milligrams of Antibody/Litre)

CELLULAR IMMUNE ANALYSIS

1. CD4/CD8 RATIO

No difference in the CD4/CD8 Ratio was observed between the BE and control group (see E Table 1).

2. T CELL PHENOTYPES IN THE BAKED EGG AND CONTROL GROUPS

There was no significant difference in the percentage of naive ($CD4^+CD45RA^+$) or memory ($CD4^+CD45RO^+$) T cells between the intervention and control groups from baseline to the end of the intervention and no difference with time (see Figure 2 A and B).

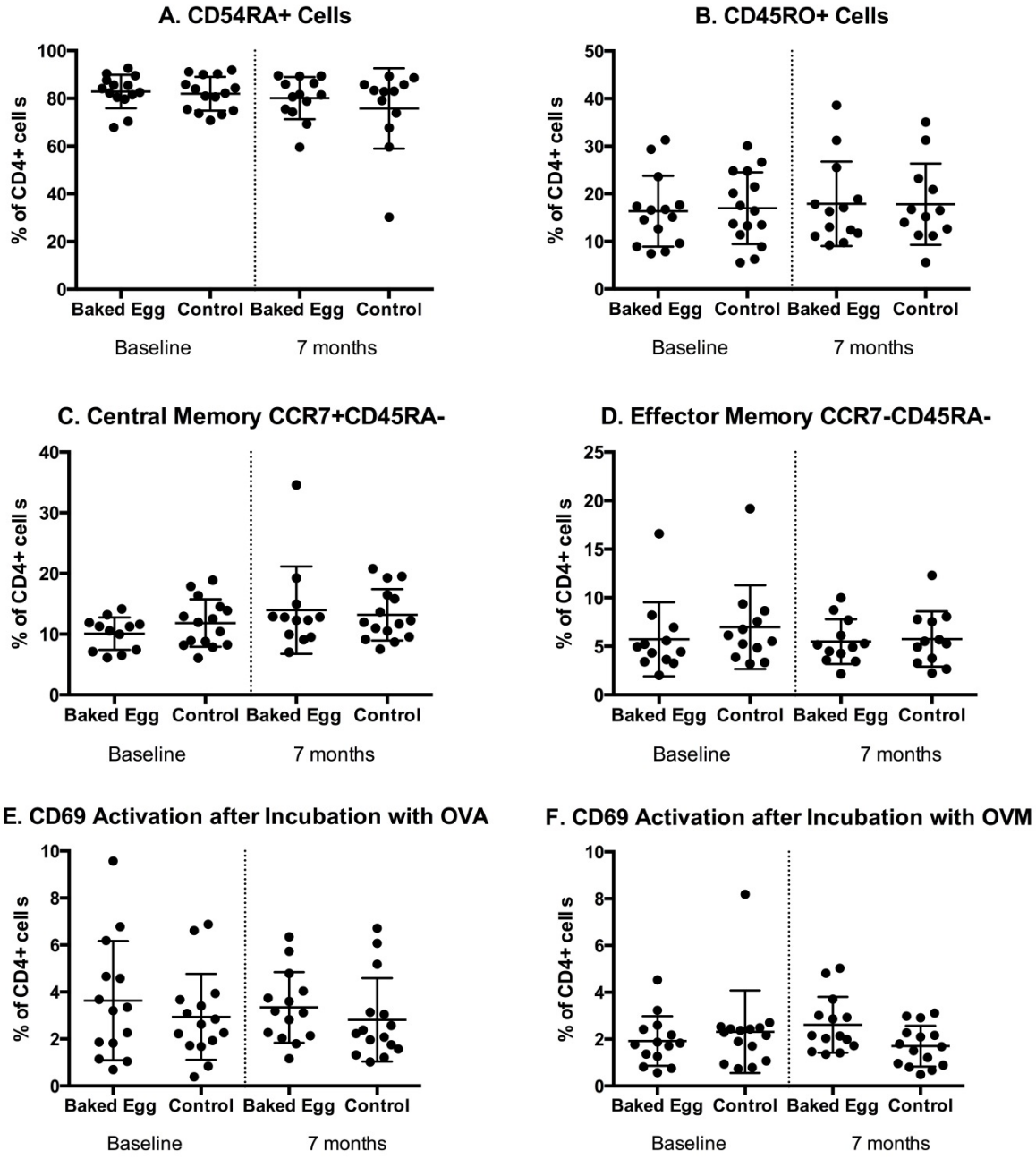
No difference in the mean percentage of central ($CD4^+CCR7^-CD45RA^-$) or effector memory ($CD4^+CCR7^+CD45RA^-$) T cells was observed between the intervention and control groups and no difference within groups from baseline to the end of the intervention, indicating that there was no overall effect on immunological memory (OVA stimulated cells; Figure 2 C and D). The result for the OVM stimulated cells is presented in E Figure 3.

After culture with ovalbumin OVA and ovomucoid OVM, $CD4^+$ cells were gated for CD69 as a marker of T cell activation (see Figure 2 E and F). No significant difference in activation was observed between the intervention and control groups at baseline and at the end of the intervention, and no difference observed with time.

3. CYTOKINE EXCRETION BY PBMCS AFTER INCUBATION WITH EGG ALLERGENS OVA AND OVM

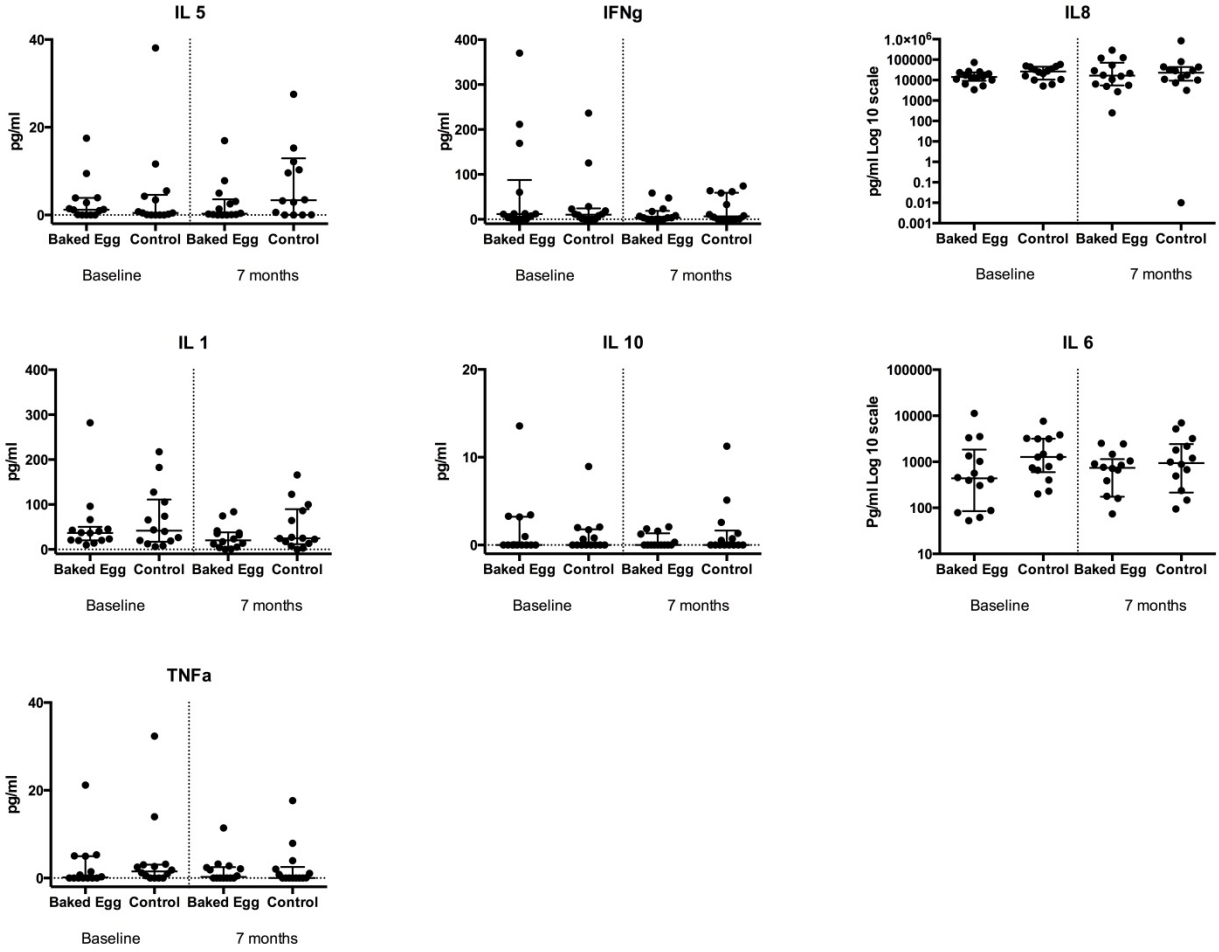
There were no significant differences in cytokine excretion between the BE and control groups at baseline and at the end of the intervention. Figure 3 shows the concentration of IL 5, IFN , IL 8, IL 1, IL 6, IL 10 and TNFα detected after incubation with OVA. No IL 4 or IL 12 was detected. No difference was observed in cytokine excretion by OVM-stimulated cells (E Fig 4 Online Repository).

Figure 2 T Cell Phenotypes in the baked egg and control groups at baseline and 7 months



Legend to Figure 2: A and B: Percentage of $CD4^+CD45RA^+$ and $CD4^+CD45RO^+$T cells. **C and D:** Central Memory ($CD4^+CCR7^+CD45RA^-$) and Effector Memory ($CD4^+CCR7^-CD45RA^-$) T cells after incubation with OVA. **E and F:** Percentage of $CD4^+CD69^+$ T cells after incubation with OVA and OVM. Baked egg and control groups at baseline and 7 months, bars indicate mean and standard deviation.

Figure 3 Cytokine Excretion after incubation of PBMCs with OVA



Legend to Figure 3: Cytokine excretion by PBMCs from the intervention and control group at baseline and 7 months after incubation with OVA less the amount secreted by untreated cells incubated with media alone. Bars denote median and interquartile range. Note: scales for y-axis vary between cytokines and results are presented in log10 scale for IL 8 and IL 6.

DISCUSSION

We report results of a double blind, randomized controlled trial comparing the effect of consumption of BE with avoidance of all egg in 1 to 5 year old BE tolerant, egg allergic children on the development of tolerance to raw egg. After the intervention, 23.5% (4/17) of the intervention group and 33.3% (6/18) of the control group tolerated raw egg implying that development of tolerance to egg may be independent of consumption of BE (OR 0.62 IQR 0.14-2.73). This was independent of the child's age, the amount of BE consumed and the time between last dose of intervention product and the raw egg OFC.

Clinical tolerance to egg in children consuming BE has been previously investigated (1, 9, 13, 27). In a population based study of two year-old BE tolerant, egg allergic children frequent ingestion of BE (≥ 5 times per month) compared with less frequent ingestion (0-4 times per month) was associated with earlier resolution of egg allergy (aOR, 3.52; 95%CI, 1.38-8.98; p=0.009)(13). This may reflect a phenotype that outgrows their egg allergy more quickly compared to 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 (13).

Immune changes occurring after ingestion of BE have not been fully investigated. Decreased SPT wheal sizes and egg sIgE, increased sIgG4 have been reported after 3 to 6 months exposure to BE (1, 9), results similar to those observed during egg allergy SOTI (28-32), leading to conjecture that inclusion of BE, when tolerated, in the diets of egg allergic children may modulate the immune system (8, 15, 33, 34). In our group of BE tolerant children we observed reductions in egg allergen SPT wheal size, and egg sIgE levels along with increases in WE sIgG4 levels in both the BE consuming and egg free groups with no difference between groups. Our results are consistent with Tey et al (35), who reported no difference in the rate of decline in EW SPT wheal size in 3 to 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. Little is known about the effect on cell phenotypes when egg allergic children consume BE so to investigate this in more detail, and to support our clinical findings, we investigated more extensive immune changes. These have not been reported in other BE studies and of the egg SOTI trials, four reported immune outcomes beyond SPT, sIgE and sIgG4 (28, 29, 31, 36), however the immune parameters measured and methodologies used varied.

Strengths of our study include the design of the blinded intervention and the consistent dosing protocol. The randomization groups were of similar age, allergy background and egg allergy phenotype, and the timing of assessments for clinical and immunological outcomes add strength to the outcomes we report. The study was limited by the small sample size. Fifty percent of the children screened for inclusion in this study tolerated BE, less than expected (1, 13), and many potential candidates were already consuming BE. There were three protocol deviations due to clinical decisions to not proceed with the end of study raw egg OFC. Two children (intervention group n=1; control group n=1) had BE OFCs instead of raw egg OFCs and it is not known if these children would have passed the raw egg OFC if given. One child (intervention group) reacted to semi-cooked egg the week prior to the appointment and was not given a raw egg OFC. Even if this child were included in the analysis, this would not have affected the final outcome of the trial. While a more extensive examination of immune outcomes would have been ideal we were limited by the volume and number of samples we could request from the children in the study.

Withdrawal rates from 15 to 42% due to refusal to consume BE products in SOTI trials have been reported (37), and poor compliance has been reported in other immunotherapy trials (38, 39). 6.9% (three children n=2 intervention group; n=1 control group) withdrew from our study due to refusal to consume the intervention products. This may have been due to finicky eating, common in young children, or related to the texture of the study products. A group of children in our study group may have lost tolerance to BE reflected by refusal to consume the

study product (40). Development of symptoms upon consumption of BE has been reported in one third of 3 year old children previously passing a BE OFC and refusal to consume BE products was also observed (19).

In attempts to comply with healthy eating guidelines related to consumption of 'discretionary foods' (21) we limited the fat and sugar content of the study foods and asked the children to consume the study foods two or three times per week. This dose rate is consistent with several egg SOTI studies (28, 31, 41), but was less frequent than Lemon-Mule et al (1) who dosed one to three times daily. Adjusting for total consumption of BE in our final analysis made no difference to the outcome.

We studied the effect of BE in 1 to 5 year olds as these children have a high incidence of egg allergy. Whilst this study was underpowered, there were no trends suggestive of an effect in either the clinical or immunological outcomes.

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 (1, 9). Further studies investigating inclusion of BE in older children with egg allergy are also warranted as BE may modulate the immune system in children with more resistant phenotypes of egg allergy.

CONCLUSION

The results of this double blind RCT suggest that BE tolerant 1 to 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 sizes are required to further test this hypothesis.

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APPENDICES FOR ON LINE REPOSITORY

APPENDIX 1: METHODS

SKIN PRICK TESTING

To assess sensitisation to egg allergens, the children were skin prick tested to the common egg allergens, according to standard methods (42). The allergen extracts used were egg white (Alyostal # 143, AUST R32582)(1:20w/v), egg yolk (Alyostal # 144, AUST R32582)(1:20w/v), (The Link Group / Stallergenes, Suburb, NSW), whole egg (ALK-Abello USA) (1:100w/v), ovalbumin (ALK–Abello Spain; Allergen # 6.22)(1:20w/v), and ovomucoid (ALK-Abello Spain; Allergen # 6.23)(1:20w/v), (Australasian Medical and Scientific Ltd, Suburb, NSW). The negative control used was 50% (w/v) glycerin / saline (Holister-Steir Laboratories, Spokane, WA, USA) and the positive control was histamine phosphate (10mg/ml, B 0911308) supplied by The Department of Pharmacy, Royal Adelaide Hospital, Adelaide, South Australia. A positive skin prick test to an allergen was defined as a mean of two perpendicular wheal diameters of 3mm or greater in size than the mean wheal of the negative control site at 15 minutes. If the egg white skin prick test result was <5mm (6 months to 2yo) or <8mm (2-5yo), and the child had not had a clinical reaction to raw egg in the previous 12 months, the egg allergy was confirmed with a pasteurised raw egg oral food challenge according to standard protocol ((20). If the child passed the BE but failed the raw egg challenge they were randomized into the study.

Baseline characteristics including demographics and history of allergy were gathered at the initial screening appointment, weight and height or length were measured, and a peripheral blood sample was collected to measure egg allergen serum specific IgE and IgG4 and functional cell response profiles. For children with eczema, a SCORAD assessment (16) was performed.

MEASUREMENT OF ANTIGEN SPECIFIC IGE

Serum whole egg, egg white, ovalbumin and ovomucoid specific IgE and whole egg specific IgG4 concentrations in plasma were measured using the Phadia CAP system by the Department of Immunopathology, SA Pathology at the Women's and Children's Hospital, using NATA accredited methodologies as per SA Pathology protocols.

APPENDIX 2 INTERVENTION PRODUCTS

Chocolate Biscuit	
Final Study Recipe - Egg Containing	**Final Study Recipe- Egg Free Placebo**
Ingredients 100g Nuttelex™ milk free margarine ¾ cup caster sugar 1 x 60g egg 1 Tbs Farm Pride Pasteurised egg powder 1 cup plain flour + extra if dough looks too sticky. ¼ cup SR flour 1 tsp vanilla extract 1/3 cup cocoa powder 60ml water – approximately **Method** Cream Nuttelex™ margarine and sugar. Add vanilla extract, egg and egg powder mix until combined. Add dry ingredients, and mix. Mix until mixture comes together. Refrigerate for 10-15 mins. Put through biscuit forcer. Bake 15 mins at 180C. Should make about 48 biscuits. 12 biscuits = 1 egg. 2 biscuits = 1 serve.	**Ingredients** 100g Nuttelex™ milk free margarine ¾ cup caster sugar 1 cup plain flour ¼ cup SR flour 1 tsp vanilla extract 1/3 cup cocoa powder 1 tsp 'No Egg' egg replacer 60ml water – approximately **Method** Cream Nuttelex™ margarine and sugar. Add vanilla extract, and mix until combined. Add dry ingredients, and mix. Gradually add water until mixture comes together. Refrigerate for 10-15 mins. Put through biscuit forcer. Bake 15 mins at 180C. Should make about 48 biscuits.
Apricot and Coconut Muffins	
Final Study Recipe- Egg Containing	**Final Study Recipe- Egg Free Placebo**
Ingredients 1/2 cup chopped dried apricots 1/2 cup apricot nectar 50g Nuttelex™ milk free margarine 2 Tbs caster sugar 1 egg 2/3 cup coconut 2/3 cup SR Flour **Method** Preheat oven to 155°C. Combine apricots and nectar in a bowl, stand 1 hour Cream margarine and sugar in a bowl. Beat in eggs one at a time. Stir in coconut, then half of the sifted flour and half the apricot mixture. Stir in remaining flour and apricots. Spread into prepared tin or place in muffin tins. Bake in moderate oven for 1 hour. Dust with icing sugar. Makes 6 muffins. 1 muffin is 1 serve.	**Ingredients** 1/2 cup chopped dried apricots 1/2 cup apricot nectar + 2 Tbs water 50g Nuttelex™ milk free margarine 1 Tbs caster sugar + 1 Tbs raw caster sugar 1 tsp egg replacer 2/3 cup coconut 2/3 cup SR Flour **Method** Preheat oven to 155°C. Combine apricots and nectar in a bowl, stand 1 hour Cream margarine and sugar in a bowl. Beat in eggs one at a time. Stir in coconut, then half of the sifted flour and half the apricot mixture. Stir in remaining flour and apricots. Spread into prepared tin or place in muffin tins. Bake in moderate oven for 1 hour. Dust with icing sugar Makes 6 muffins. 1 muffin is 1 serve.

Date and Apple Loaf	
Final Study Recipe- Egg Containing	**Final Study Recipe- Egg Free Placebo**
Ingredients 1 medium sized eating apple 150g dates ¾ cup water ¾ tsp bicarbonate of soda 125g Nuttelex™ milk free margarine 1 cup sugar 2 eggs 1 cup plain flour ½ cup SR flour **Method** Chop dates, peel and chop apple. Soak in ¾ cup boiling water and ¾ tsp bicarbonate of soda for ½ hour. Cream Nuttelex™ margarine and sugar. Add eggs. Stir in flour alternately with apple and date mixture. Divide mixture evenly between two prepared loaf tins. Bake at 180°C for 1 hour or until well cooked. Makes two loaves, slice each into 6 slices. Each slice is one serve.	**Ingredients** 1 medium sized eating apple 150g dates 1 cup water ¾ tsp bicarbonate of soda 125g Nuttelex™ milk free margarine 1 cup sugar 2 tsp egg replacer 1 cup plain flour ½ cup SR flour **Method** Chop dates, peel and chop apple. Soak in ¾ cup boiling water and ¾ tsp bicarbonate of soda for ½ hour. Cream Nuttelex™ margarine and sugar. Stir in flour and egg replacer alternately with apple and date mixture. Divide mixture evenly between two prepared loaf tins. Bake at 180°C for 1 hour or until well cooked. Makes two loaves, slice each into 6 slices. Each slice is one serve.
Banana Bread	
Final Study Recipe- Egg Containing	**Final Study Recipe- Egg Free Placebo**
Ingredient 1 ½ cup SR Flour ½ tsp mixed spice 2/3 cup brown sugar 2 large over ripe bananas 2 eggs 1/3 cup canola oil ¼ cup soy milk **Method** Heat oven to 180C. Sift dry ingredients. Mix wet ingredients together. Gradually combine. Divide mixture evenly between two greased and lined two loaf tins. Bake for 1 hour. Makes two loaves, slice each into 6 slices. Each slice is one serve.	**Ingredients** 1 ½ cup SR Flour 1 tsp baking powder ½ tsp mixed spice 2/3 cup brown sugar 2 large over ripe bananas 2 tsp egg replacer 4 Tbs water 1/3 cup canola oil ¼ cup soy milk **Method** Heat oven to 180C. Sift dry ingredients. Mix wet ingredients together. Gradually combine. Divide mixture evenly between two greased and lined two loaf tins. Bake for 1 hour. Makes two loaves, slice each into 6 slices. Each slice is one serve.

APPENDIX 3 IMMUNOLOGICAL METHODOLOGY

BLOOD SAMPLING AND PROCESSING

Peripheral blood samples were collected at the screening and 7 month visits and processed immediately. Plasma was collected after centrifugation etc g and stored at -80C for determination of serum specific IgE / IgG4 levels. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque (Alexis-Shield, Oslo, Norway) gradient centrifugation and isolated cells cryopreserved in 80% heat inactivated FCS (Sigma-Aldrich, Sydney, Australia) and 20% dimethyl sulfoxide (Ajax Finechem, Taren Point, NSW, Australia). Cell number and viability was assessed by Trypan Blue.

CELL CULTURE EXPERIMENTS, IMMUNOPHENOTYPING AND OVA AND OVM – SPECIFIC T-CELL ACTIVATION AND MEMORY DEVELOMENT

For the cell culture experiments, PBMCs (10^6 cells/ ml) were cultured with 100μg/ml egg allergens OVA and OVM (Sigma-Aldrich, Sydney, Australia) for five days at 37°C and 5% CO2. Cells cultured with AIM-V medium (Invitrogen, Life Technologies Sydney, Australia) alone served as a no-antigen control, and phytohemagglutinin-L (PHA-L) (1 ug/ml) (Roche Diagnostics, Australia or Remel, KS, USA) was used as the positive control. At day 3 (for PHA-L stimulated cells) and day 5 (for OVA, OVM and no antigen control) all available cells were harvested and analysed. Cells were centrifuged at 300xg for 5 min and the resulting supernatants collected and stored at -80°C for cytokine analysis. The cell pellet was then equally divided into FACS tubes for labelling with mouse anti human conjugated monoclonal antibodies specific for cell surface antigens. Combinations of fluorophores PE, PE-Cy7, FITC, APC, APC-Cy7 or PerCP were used to assess the phenotype of the cells. All antibodies (except CCR7) were purchased from BD Biosciences (San Jose, CA, USA). CCR7 was purchased from (Miltenyi Biotec, Auburn, SD, USA). Cells were acquired on a BD Biosciences FACS Canto flow cytometer (Becton Dickinson, CA, USA). Isotype controls were used to set up the instrument and the positive gating, and these settings were maintained throughout. After selecting a lymphocyte gate based on forward and side-scatter

characteristics, events within the lymphocyte gate were analysed using BD FacsDiva™ software version 6.1.3 (BD Biosciences, San Jose, CA, USA).

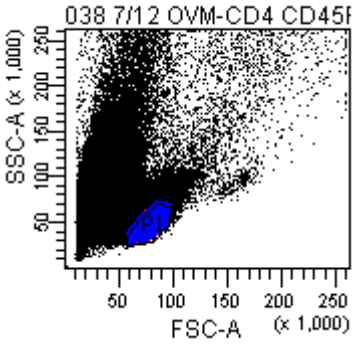
For the immunophenotyping cells were stained at baseline for CD4, CD8, CD14, CD19 and HLA DR. To assess activation, cells were stained with CD69 at baseline and after incubation with OVA and OVM and to assess memory markers cells were stained with CD45RO, CCR7, CD27 and CD28.

IN VITRO CYTOKINE RESPONSES

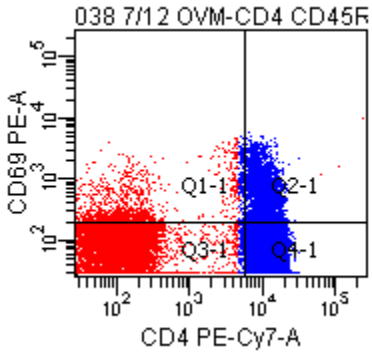
The cytokine concentration in supernatants collected on days 3 and 5 after incubation with OVA or OVM was assessed using a BD Cytometric Bead Array Human Inflammatory Cytokine Kit (IL-8, Il-1, IL-6, TNF, IL-12 and IL-10) and individual cytokines IL-4, IL-5 and IFNγ were measured using the BD Biosciences enhanced sensitivity flex sets for Human IL-4, Human IL-5 and Human IFNγ and a BD Cytometric Bead Array (CBA) Human enhanced Sensitivity Master Buffer Kit (BD Biosciences, San Jose, CA, USA). (Minimum levels of detection: IL-8, 2.6 pg/ml; IL-1, 7.2 pg/ml; IL-6 2.5 pg/ml; IL-10 3.3 pg/ml; TNF, 3.7pg/ml; IL-12, 1.9 pg/ml). Beads were acquired on a BD Biosciences FACS Canto flow cytometer (Becton Dickinson, San Jose, CA, USA) and analysed using BD FacsDiva™ software version 6.1.3 (BD Biosciences, San Jose, CA, USA).

E FIGURE 1 EXAMPLE GATING PROTOCOLS

Example 1 Lymphocyte population

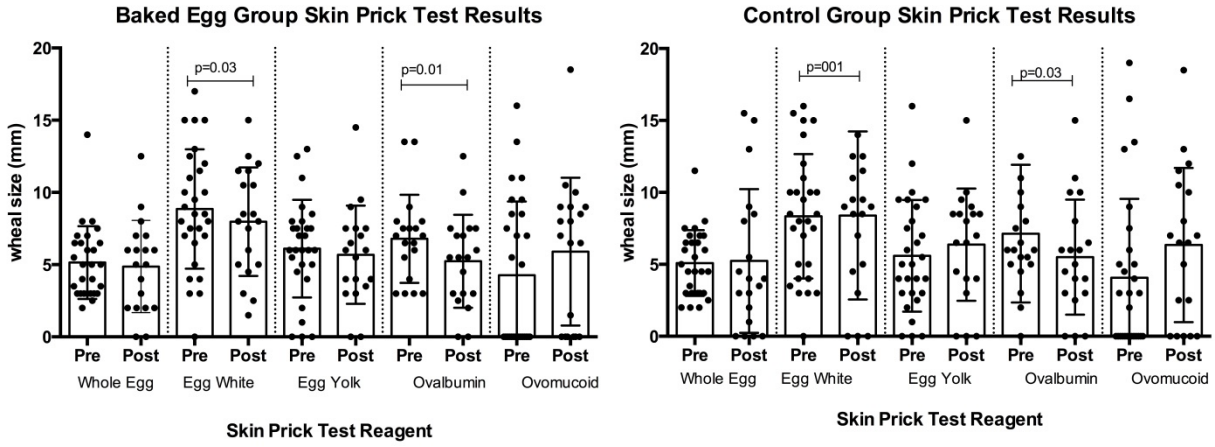


Example 2. Gating for CD4+CD69+

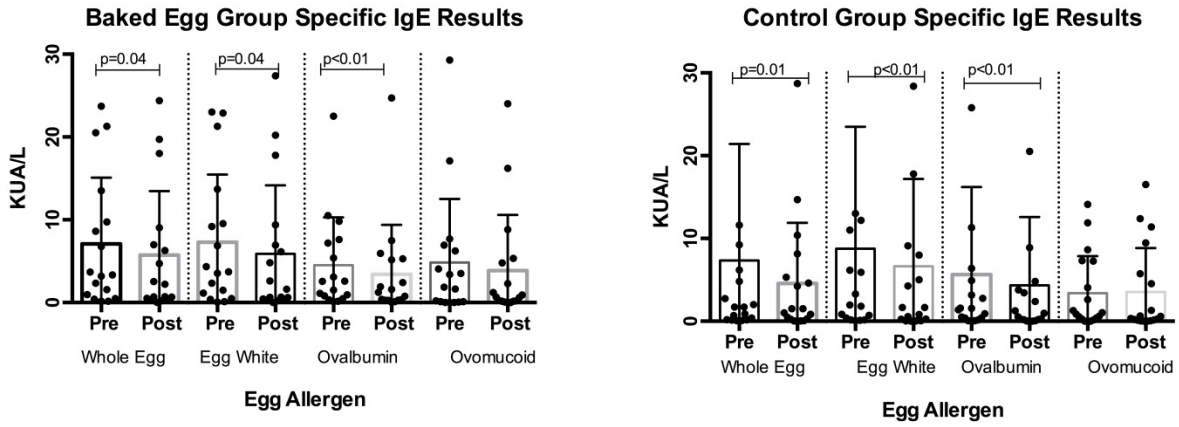


APPENDIX 4 RESULTS

E-FIGURE 1 CHANGES IN SKIN PRICK TEST WEAL SIZES FROM BASELINE TO END OF INTERVENTION



E - FIGURE 2 CHANGES IN EGG SPECIFIC IGE LEVELS FROM BASELINE TO END OF INTERVENTION

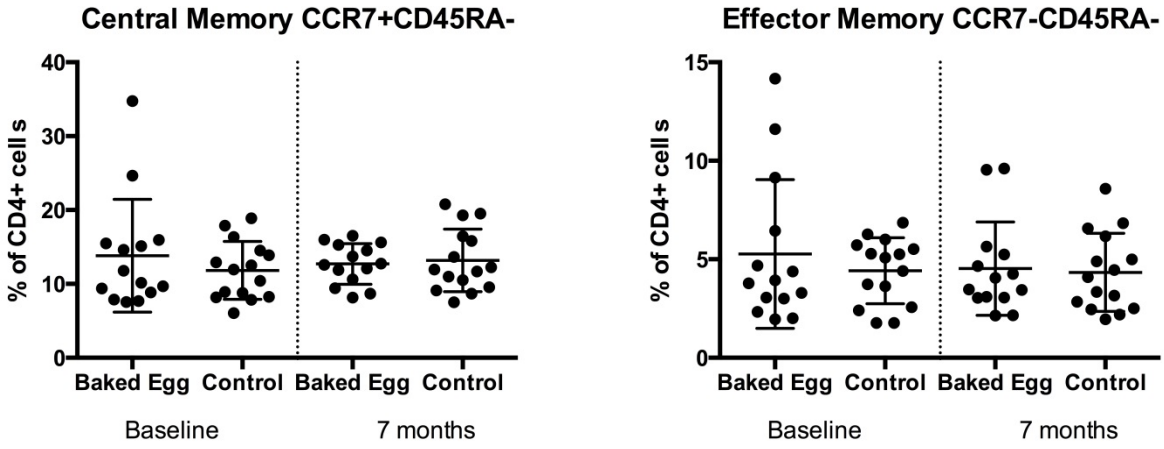


E-TABLE 1. CD4/CD8 PROFILES

	% $CD4^+$ T Cells		% $CD8^+$ T Cells		CD4/CD8 Ratio	
	Baseline	**7 months**	**Baseline**	**7 months**	**Baseline**	**7 months**
Placebo Group	51.07 +/- 6.90	48.89 +/- 10.89	22.12 +/- 6.17	22.56+/- 5.29	2.57 +/- 1.13	2.39+/- 1.04
Baked Egg group	51.86+/- 0.01	45.27 +/- 13.13	20.94+/- 7.14	22.87 +/- 6.59	2.84 +/- 1.34	2.24 +/- 1.11

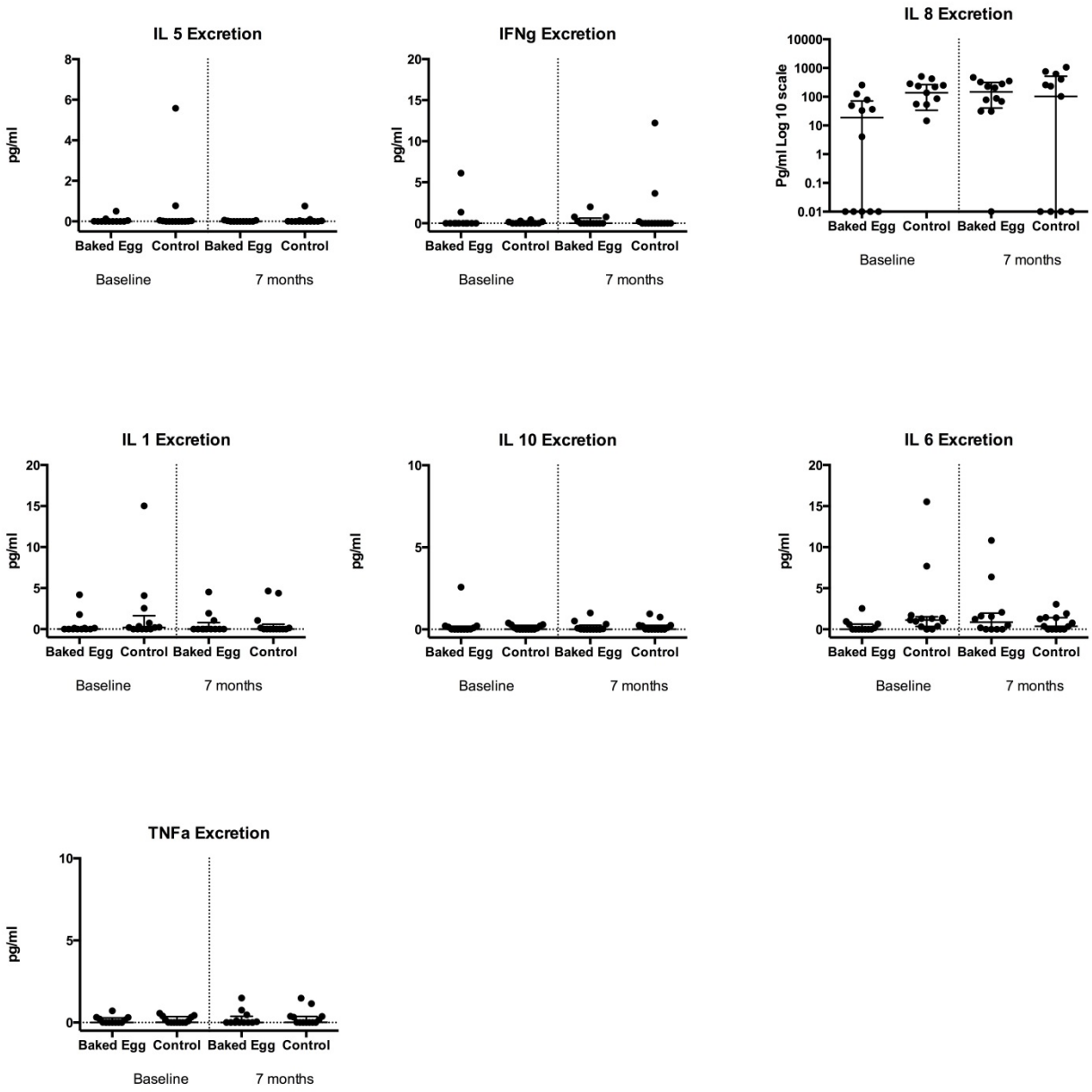
Legend to E-Table 1: Mean +/- SD

E-FIGURE 3 PERCENTAGE OF CENTRAL MEMORY ($CD4^+$ $CCR7^+CD45RA^-$) AND EFFECTOR MEMORY ($CD4^+$ $CCR7^-CD45RA^-$) T CELLS AFTER STIMULATION WITH OVM



Legend to E-Figure 3 Percentage of Central Memory (CD4+ CCR7+CD45RA-) and Effector Memory (CD4+ CCR7-CD45RA-) T cells in the baked egg and control groups at baseline and 7 months after stimulation with OVM showing mean and standard deviation.

E-FIGURE 4 CYTOKINE EXCRETION AFTER INCUBATION OF PBMCS WITH OVM



Legend to E-Figure 4: Cytokine excretion by PBMCs from the baked egg and control group at baseline and 7 months after incubation with OVM. Bars denote median and interquartile range. Note that scales for y-axis vary between cytokines and that results are presented in log10 scale for IL 8.

CHAPTER 7

DISCUSSION, CONCLUSION AND DIRECTIONS FOR FUTURE RESEARCH

7.1 MAJOR FINDINGS REPORTED IN THIS THESIS

- A systematic review of maternal diet during pregnancy and lactation and atopic outcomes in the offspring supports current policy documents recommending no specific restrictions in the maternal diet during pregnancy to prevent the development of atopic disorders in the newborn. However, dietary patterns with high Mediterranean diet scores, diets rich in fruits and vegetables, fish, and vitamin D containing foods were suggestive of benefit, requiring further evaluation.
- Pasteurised egg is safe, and is as effective as fresh raw egg in raw egg oral food challenges.
- Baked egg is tolerated by 50% of 1 to 5 year children with IgE mediated egg allergy.
- Common egg allergens used for SPT and sIgE perform poorly when used to predict tolerance to baked egg. When predicting tolerance to baked egg, component allergen testing with ovalbumin is as effective as egg white however ovomucoid performs poorly when compared to other egg allergens. The values of egg allergen sIgE to IgG4 ratios in prediction of baked egg tolerance require further evaluation. A baked egg OFC remains the definitive test for tolerance to baked egg.
- The results of an RCT (the CAKE Study) comparing clinical and immunological changes after consumption of baked egg for 6 months with an egg free diet by 1 to 5 year-old baked egg tolerant, raw egg allergic children suggest that tolerance to baked egg is indicative of a phenotype of egg allergy that is outgrown, and may not be influenced by consumption of baked egg for six months. Larger studies are required to confirm these results.

7.2 DISCUSSION

This thesis strengthens the evidence base for standard protocols for management of IgE mediated egg allergy in children, and provides important information regarding the influence of the perinatal maternal diet on atopy development. Food allergy is an early manifestation of atopic disease, and a diagnosis of food allergy carries significant medical and psychosocial costs. Many food allergic children develop longer-term health issues such as asthma, further increasing the burden of disease. The incidence of food allergy is predicted to increase worldwide as developing countries adopt western life style habits[1, 144], with warnings of a potential 'allergy tsunami'[20]. For these reasons it is important to investigate questions that address both the prevention and management of food allergies.

7.2.1 HETEROGENEITY AND HARMONISATION

Throughout this thesis the themes of immune modulation and tolerance development, heterogeneity and harmonisation recurred during the discussions of prevention, diagnosis and management of allergy.

Chapters 1, 5 and 6 addressed dietary aspects of prevention and management of food allergy, focusing on the potential of whole foods, components of foods or dietary patterns to influence programming of the immune system or, for those with allergies, to induce tolerance to allergens. Dietary modulation of the immune system may be important in the maternal diet during the neonatal period for the prevention of allergy, and also has potential as treatment for children with IgE mediated allergies.

The influence of the maternal diet during pregnancy and lactation on development of childhood atopy was the focus of several RCTs and cohort studies, but prior to our publication the results had not been systematically reviewed. It is unlikely that RCTs investigating avoidance of allergens in the maternal diet will be repeated for ethical reasons given the potential for restricted diets to adversely affect fetal growth and adversely affect tolerance development, which may begin in utero[144, 145]. As such it was timely to consider

these studies as a whole, particularly as the emphasis of current research addressing questions related to the prevention of food allergy has shifted from maternal allergen avoidance to early infant feeding studies considering promotion of tolerance by early introduction of allergens into the child's diet. Management of children with IgE mediated food allergies has also shifted from complete avoidance of allergens to inclusion of the allergen if tolerated[138, 141], and forms of SOTI are being investigated as potential treatment strategies. SOTI induces tolerance development by modification of the Th1/Th2 balance of the immune system, however SOTI with raw proteins is not free of risk and there is interest in modified allergens as potentially safer, more effective vehicles for SOTI.

The systematic reviews in Chapters 1 and 5 highlighted heterogeneity in study protocols and outcome assessments, which made it more complex to synthesise the overall results. In the perinatal maternal diet systematic review[58] the major differences between studies included, but were not limited to, the tools used for dietary assessment of maternal dietary intake, and the methods used to assess primary allergic outcomes in the children (also an issue highlighted in the SOTI review). Many of the cohort studies relied on retrospective dietary recall questionnaires that are less accurate than dietary data collected prospectively. Some studies used internationally recognised, standardised tools to report medically diagnosed atopic outcomes, whereas others reported results of parental questionnaires. Where sensitisation was reported as an outcome, the methods of diagnosis varied (from SPT or sIgE to common allergens), and for food allergy, OFC to confirm the diagnosis were not performed. In the review of specific oral tolerance induction[146], there were differences in criteria for recruitment, types of intervention products, the length of intervention periods, dosing rates along with the timing and type of outcome assessments. Few studies reported extensive analysis of immune outcome, and where more extensive analysis occurred there were differences in the outcomes measured and analytical methodology. SOTI is an area of active research, and to facilitate comparison of results of these types of studies there is a need for harmonised study protocols and harmonisation of

reporting studies. Large international collaborations such as EuroPrevall foster harmonisation in allergy research by development of standard protocols and this is reviewed in the EAACI European Declaration on Immunotherapy[147], and the standardisation of cellular immune phenotype analysis was also recently reviewed[148].

Egg protein is a complex glycoprotein and its structure and allergenicity are affected by food processing including heating. To inform the screening process for entry into the CAKE Study, the use of egg allergen SPT, specific IgE and OFC protocols to diagnose and manage egg allergy were systematically reviewed, which highlighted further sources of heterogeneity. Egg OFC protocols varied in relation to the form of egg (raw vs cooked), dosing rates, total dose, and the extent of cooking for cooked egg challenges. Use of standardised egg OFC protocols will facilitate consistent clinical care and comparison between studies reporting outcomes of OFCs. This is of particular relevance for studies reporting outcomes of SOTI protocols where an OFC may be used to define entry criteria and to evaluate the clinical outcome of the SOTI protocol. Clinicians should be mindful of the study methodology used to report predictive values for SPT and specific IgE levels as these may be influenced by the form of egg used for the OFC, the clinical history and age of the children investigated, and the statistical methods used to calculate the 'cut off' values.

7.2.2 BAKED EGG IN THE DIETS OF EGG ALLERGIC CHILDREN

One of the major changes in the approach to dietary management of egg allergy in the last decade has been the active inclusion of baked egg in the diets of egg allergic children, if tolerated[138, 139]. Although inclusion of BE improves the quality of life and does not adversely affect growth or measures of gut integrity[13], the change to clinical practice was made in the absence of evaluation using RCT methodology and consumption of BE may not alter the natural history of egg allergy. Consumption of BE by BE tolerant, raw egg allergic children is associated with immunological changes suggestive of evolving tolerance to all forms of egg however the results of the RCT (the CAKE Study) reported in Chapter 6 suggest that these

changes may be independent of consumption of BE, and tolerance to BE is likely to reflect a phenotype of egg allergy that gains tolerance to raw egg earlier than children who do not tolerate BE.

There were several difficulties in the management of the RCT including recruitment rates, consumption of the intervention product and protocol violations. Recruitment was slow, possibly because the incidence of egg allergy in the community may have been overestimated, and partly because many children (particularly from the older age strata) were already consuming BE. Also, independent of our allergy unit, many potential recruits were already undergoing egg desensitisation in the community. Consumption of the intervention product by study participants was variable. This was identified as a potential issue when planning the RCT as the children in the study group were of an age where finicky eating is often a problem, additionally many children with food allergies have associated feeding disorders, and food refusal may be a child's way of limiting exposure to an allergen[149]. We attempted to address potential taste fatigue and textural issues by provision of more than one intervention product and providing one product of different texture (a biscuit). To limit the potential impact of the study on the participant's innate feeding regulation, parents were instructed to allow the children to maintain control of their own intake of study product, which was offered 2 to 3 times a week, to limit the impact on their intake of other foods. These issues are not commonly identified by researchers as potential barriers to effective dosing for SOTI trials, and should be both practical and ethical considerations when designing similar SOTI protocols.

A strength of the CAKE Study was the extensive immunological evaluation. Few studies report immune changes after consumption of BE, and consistent with these we measured SPT, sIgE and IgG4 before and at the end of the intervention period. Little is known about the effect on cell phenotypes when egg allergic children consume BE so to investigate this in more detail, and to support our clinical findings, we investigated more extensive immune

changes. These have not been reported in other BE studies and of the egg SOTI trials, four reported immune outcomes beyond SPT, sIgE and sIgG4[150-153], however the immune parameters measured and methodologies used varied. In an RCT of egg SOTI Meglio et al[150] measured serum cytokines (IL 4, IL5, IL6, IL 10, IL12, IL13, IFN , TNFα) and TGFß, and reported an increase in IL5 excretion in the desensitisation group compared with the control group. Fuentes et al[152] reported no change in concentration and percentage of B cells, NK cells, and CD8+ T cells, a decrease in effector memory CD4+CD45RA+CD27- T cells (T_{EM}), and decreases in both Th1 and Th2 cytokines in 16 children successfully completing a 9-week egg desensitisation protocol. Compared with non-allergic controls, allergic children had more T_{EM} at the start and by the end of the intervention the concentration had decreased to similar numbers[152]. Ito et al[151] reported a decreased Th1/Th2 ratio at 6, but not 12 months, a decrease in IL 10, an increase in TGF ß and no change in IL4, IFN in samples from 6 children after egg SOTI. For the more extensive immune analysis a range of immune cell markers reflecting changes in immune memory and regulation of tolerance were selected. However, the low sample volume, and the children's naturally low cell counts limited the number of immune parameters that could be measured. This was further limited by the capacity of the cytometer to detect only a limited number of flourophores during each experiment. The results of our study indicate that both the BE intervention group and control group were developing immune memory at the same rate. We observed no change in percentage of CD45RA and CD45RO positive cells and no difference between groups in the percentage of T_{CM} and T_{EM} cells. There was no difference between groups in cytokine excretion, particularly in IL 10, which plays a role in regulation of the immune response. After incubation of cells with egg allergens OVA and OVM no difference in expression of the T cell activation marker, CD69, was observed between cells obtained from the intervention and control groups from baseline to the end of the intervention. A more comprehensive immune evaluation would have included more markers of immune regulation such as Fox P3 and TGFß. It would also have been interesting to measure the effect of the

intervention on basophil activation. As more is learned about the basic mechanisms of SOTI, standardised markers to evaluate the effectiveness of the intervention will be identified[148].

Based on the results reported in Chapter 6, directions for future studies include investigation of the clinical and immunological effects of inclusion of BE in the diets of children of different age strata, at different doses and dosing rates. High frequency (eg several times a day) and low frequency immunotherapy induce different immune tolerance pathways, and it would be interesting to compare the effectiveness of these two different strategies. However, power calculations indicate that the numbers of recruits required for these studies would be large. For example, for the 1 to 5 year old age group enrolled in the CAKE study the observed proportions of IgE-mediated egg allergy at 7 months were 0.8 (Intervention Group) vs 0.7 (Placebo Group), or an absolute difference of 10%. If we wish to have 80% power to detect a difference of this magnitude when the underlying proportion of egg allergy at 7 months is 80%, with two-sided alpha=0.05, 313 children will be needed in each group (total sample size=626).

7.3 IMPLICATIONS FOR CLINICAL PRACTICE

The work reported in this thesis has the following implications for clinical practice:

2 For pregnant and breast feeding women, the current Australian dietary guidelines encouraging a varied diet, rich in fruits and vegetables with no specific dietary restrictions for allergy prevention do not need to be modified.

3 Skin prick and specific IgE testing to common egg allergens does not reliably predict tolerance to baked egg. A baked egg oral food challenge remains the gold standard for determining if a child tolerates baked egg.

4 Tolerance to baked egg in egg allergic children may indicate that a child is moving towards tolerance to raw egg. Although inclusion of baked egg in the diet may not hasten development of tolerance to raw egg it improves the quality of life.

5 The use of standard oral food challenge protocols in both clinic and research settings is essential to allow comparison of outcomes.

7.4 OTHER RESEARCH QUESTIONS RAISED BY THIS THESIS

7.4.1 MATERNAL AND INFANTILE DIET DURING EARLY LIFE AND ATOPY DEVELOPMENT

There are many potential research questions related to the influence of maternal diet on childhood allergy. Specific areas that are worthy of research include the effect of atopic parents compared with non-atopic parents, high compared with low or sporadic consumption of allergens, effects of timing of exposures during gestation. Questions should also consider other immune modulating components in the diet, the mode of birth and environmental considerations such as microbial exposure, and intake of pre and probiotics. Potential maternal dietary interventions could include a fruit and vegetable rich diet with supplementation of Vitamin D, probiotics and omega 3 long chain fatty acids, along with regular consumption of common allergens. The intervention could extend to the first six months of life, and timing of introduction of foods into the infant's diet and early identification and management of eczema could also be considered. Such a trial with so many interventions would be difficult to conduct.

7.4.2 MANAGEMENT OF INFANTILE FOOD ALLERGIES BY MANIPULATION OF THE MATERNAL DIET DURING LACTATION

Although it is common clinical practice there is a lack of scientific literature validating protocols for management of IgE and non-IgE mediated allergy in breast fed infants by manipulation of the maternal diet during lactation. This area is deserving of further research as restrictive maternal diets during the vulnerable postnatal period add to the burden of care for a child with a food allergy.

7.4.3 CLINICAL RELEVANCE OF EGG YOLK ALLERGENS AND POTENTIAL FOR BAKED EGG YOLK SPECIFIC ORAL TOLERANCE INDUCTION

The experiment reported in Chapter 3 compared the binding of serum IgE from egg allergic children to pasteurised whole egg powder with fresh whole raw egg. Serum IgE from children allergic to both raw and baked egg bound to both egg white and egg yolk allergens,

and although egg yolk allergens are major egg allergens this was an interesting finding as the clinical impact of allergy to egg yolk allergens, and the potential use of egg yolk allergens in SOTI has not been fully investigated.

7.4.4 CLINICAL ALGORITHMS TO PREDICT TOLERANCE TO BAKED EGG

As reported in Chapter 4, tolerance to BE is poorly predicted by egg allergen SPT and sIgE levels, and a baked egg OFC is recommended to assess tolerance to baked egg. The use of several parameters (eg age, clinical history, along with total IgE, egg allergen specific IgE and IgG4) may have greater predictive value[99, 102], and further statistical modeling may help to identify an algorithm that accurately predicts tolerance to BE in young egg allergic children.

7.5 CONCLUDING REMARKS

This thesis provides an overview of the current understanding of nutritional strategies for the prevention, diagnosis and management of food allergy, with a specific focus on egg allergy. More research is required into the basic prevention of food allergy, including a comparison of multiple interventions in both the maternal and infantile diets. It may be possible that differing atopy phenotypes require differing interventions.

For children with egg allergy early phenotyping for BE tolerance and subsequent inclusion of BE in the diet improves quality of life, but may not hasten the time to develop overall tolerance to egg. Larger trials including children of different ages are required to confirm the findings described in this thesis.

The most useful studies will be those that use harmonised strategies and standardised reporting of outcomes. This will allow comparison and pooling of results from small studies and those with different cohorts, ultimately providing better quality results able to be transferred to the clinic setting.

CHAPTER 8

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APPENDIX 1

CONFERENCE PRESENTATIONS AND ACCEPTED ABSTRACTS SUPPORTING THIS THESIS

INVITED PRESENTATIONS:

Lactation Consultants Australia and New Zealand Annual Conference, 2011. "Dietary Management of Breast-fed Babies with Allergies."

16th International Congress of Nutrition and Dietetics. Adverse Reactions to Foods Symposium, 2012. "Food Allergy Research in Australia."

Centre of Food Allergy Research NHMRC CRE Symposium, 2014 "Does Maternal Diet during Pregnancy and Lactation Affect Allergy Outcomes in Their Offspring? A Systematic Review of Food Based Approaches."

26th Annual Conference of the Australasian Society of Clinical Immunology and Allergy (ASCIA), 2014 "Introduction of Heat Treated Egg and Cow's Milk. Does this have a place in allergy management?"

27th Annual Conference of the Australasian Society of Clinical Immunology and Allergy (ASCIA), 2015 "Results of a Randomised Controlled Trial of Consumption of Baked Egg by 1 to 5 year old Egg Allergic Children."

ACCEPTED ABSTRACTS:

EAACI Food Allergy and Anaphylaxis Meeting (FAAM 2013), Nice, France February 2013, "Does Maternal Diet during Pregnancy and Lactation Affect Allergy Outcomes in Their Offspring? A Systematic Review of Food Based Approaches." (Oral presentation and abstract)

European Academy of Allergy & Clinical Immunology (EAACI) Congress, Copenhagen, Denmark 2014. "Allergenicity of Pasteurised Whole Raw Egg Powder compared with Fresh Whole Raw Egg." (Poster and abstract)

EAACI 4th Paediatric Allergy and Asthma Meeting (PAAM 2015), Berlin, Germany October 2015, "The Clinical and Immunological Outcomes After Consumption Of Baked Egg By 1-5 Year Old Egg Allergic Children. Results Of A Randomised Controlled Trial." (Oral presentation and abstract)

27th Annual Conference Of The Australasian Society Of Clinical Immunology And Allergy (ASCIA), 2015 "Skin Prick Testing To Common Egg Allergens Does Not Predict Baked Egg Tolerance In Young Egg Allergic Children." (Poster and abstract)

27th Annual Conference Of The Australasian Society Of Clinical Immunology And Allergy (ASCIA), 2015 "Whole Egg And Egg White, But Not Ovalbumin Or Ovomucoid Specific Ige May Help Predict Baked Egg Tolerance In Young Egg Allergic Children." (Poster and abstract)

27th Annual Conference Of The Australasian Society Of Clinical Immunology And Allergy (ASCIA), 2015 "Egg Allergen Specific Ige To Whole Egg Igg4 Ratios May Help Predict Baked Egg Tolerance In Young Egg Allergic Children." (Poster and abstract)

APPENDIX 2

E-TABLES FROM CHAPTER 1

Appendix 2 contains copies of the electronic tables published in the online repository for the paper "Does Maternal Diet during Pregnancy and Lactation Affect Outcomes in Offspring? A Systematic Review of Food-Based Approaches" by Merryn J Netting, Philippa F Middleton and Maria Makrides *Nutrition* 30 (2014) 1225–1241. DOI: 10.1016/j.nut.2014.02.015

DOES MATERNAL DIET DURING PREGNANCY AND LACTATION AFFECT ALLERGY OUTCOMES IN THEIR OFFSPRING? A SYSTEMATIC REVIEW OF FOOD BASED APPROACHES.

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TABLE OF CONTENTS: ELECTRONIC TABLES

PROSPECTIVE AND RETROSPECTIVE COHORTS

TABLE E 1 MATERNAL DIET AND ECZEMA IN CHILDREN

Study details	Maternal dietary pattern/component	Effect size: aOR and 95% CI (except where stated otherwise)	P-value
Prospective and Retrospective cohort studies			
- 3-4 months age			
Saito 2010 (1) (n = 771) Diet during pregnancy OMCHS, Japan *ECZEMA defined as suspected atopic eczema (diagnosed by a doctor as having atopic eczema or possibly having atopic eczema)* *Low risk of bias*	Total dairy products	Q1 (52.7 g/day) 13/192 1.00 Q2 (126.0 g/day) 16/193 1.39 (0.62 to 3.20) Q3 (191.0 g/day) 18/193 1.63 (0.73 to 3.72) Q4 (288.3 g/day) 18/193 1.84 (0.82 to 4.27)	Ptrend = 0.13
	Eggs	Q1 (9.7 g/day) 17/192 1.00 Q2 (22.9 g/day) 15/193 0.87 (0.40 to 1.89) Q3 (40.7 g/day) 19/193 1.37 (0.66 to 2.86) Q4 (61.3 g/day) 14/193 0.73 (0.33 to 1.61)	Ptrend = 0.74
	Fish	Q1 (23.0 g/day) 14/192 1.00 Q2 (37.8 g/day) 15/193 0.93 (0.41 to 2.13) Q3 (51.4 g/day) 21/193 1.60 (0.75 to 3.51) Q4 (73.1 g/day) 15/193 1.15 (0.51 to 2.62)	Ptrend = 0.44
	Meat	Q1 (33.4 g/day) 10/192 1.00 Q2 (49.1 g/day) 14/193 1.46 (0.61 to 3.62) **Q3 (63.6 g/day) 19/193 2.41 (1.06 to 5.75)** **Q4 (89.8 g/day) 22/193 2.59 (1.15 to 6.17)**	Ptrend = 0.01
	Adjusted for maternal age, gestation at baseline, family income, maternal and paternal education, maternal and paternal history of asthma, atopic eczema and allergic rhinitis, mite allergen level from maternal bedclothes, vacuuming living room, mould in kitchen, changes in maternal diet in the previous 1 month, season when data at baseline were collected, baby's older siblings, baby's sex, baby's birth weight, breastfeeding and bathing or showering infant		
- First year of life/at 1 year age			
Hoppu 2005 (2) (n = 34 mothers with atopic disease) Diet during lactation Turku, FINLAND ECZEMA defined as the presence of atopic eczema during the first year of life and a positive SPT at 12 months of age Moderate risk of bias	Fruit and vegetables (specifically recommended an abundant intake of fresh fruit, berries and vegetables during breastfeeding)	**OR 0.30 (0.09 to 0.94)** (with increased vitamin C concentration in breast milk – attributed to dietary intake of vitamin C)	
Romieu 2007 (3) (n = 458) Menorca, SPAIN Diet during pregnancy ECZEMA defined as a medical diagnosis of eczema as reported by the parents at 1 year of age Low risk of bias	Fish – never, once per year, once per month, once per week, once per day)	**0.73 (0.55 to 0.98)** (per unit increase of log transformed weekly fish consumption)	0.036
	Adjusted for maternal asthma, type of fish, smoking during pregnancy		
Jedrychowski 2011 (4) (n = 469) Diet during pregnancy New York, USA and Krakow, POLAND ECZEMA defined as dry skin with itchy rash and typical	Fish	≤ 90 g/week (76/176): 1.00 91 – 205 g/week (69/168) 0.91 (0.59 to 1.41) **> 205 g/week (38/125) 0.57 (0.35 to 0.93)**	
	Fine particulate matter and environmental tobacco smoke exposure; modified by fish intake	$PM_{2.5}$ > 53 µg/m^3 & smoking in house*: aRR 1.89 (0.99 to 2.44)	
		> 205 g/week: aIRR 0.72 (0.52 to 0.99)	
	Adjusted for maternal characteristics (age, education, atopy), duration of exclusive breastfeeding,		

TABLE E 1 MATERNAL DIET AND ECZEMA IN CHILDREN (cont)

localisation, confirmed by a doctor Low-moderate risk of bias	*presence of older siblings, and damp/mouldy house, gender of child* *$PM_{2.5}$ = Personal Measurement of fine particulate matter IRR = incidence rate ratio		
- 2 years age			
Sausenthaler 2007 (5) (n = 2518; 446 with eczema) Diet during pregnancy LISA, Germany Eczema defined as doctor's diagnosis of allergic or atopic eczema in the past 6 months Low risk of bias	Milk (high versus low intake) High intake = more than sometimes	High: 304/1710 (17.8%) Low: 142/803 (17.7%) aOR 1.04 (0.80 to 1.34)	
	Yoghurt (high versus low intake) High intake = more than sometimes	High: 249/1370 (18.2%) Low: 197/1146 (17.2%) aOR 0.99 (0.78 to 1.27)	
	Cheese (high versus low intake) High intake = ≥ 4 times/week	High: 174/964 (18.0%) Low: 271/1551 (17.5%) aOR 0.87 (0.68 to 1.13)	
	Cream (high versus low intake) High intake = 3-4 times a week	High: 122/701 (17.4%) Low: 322/1786 (18.0%) aOR 1.02 (0.78 to 1.34)	
	Eggs (high versus low intake) High intake = 1-2 times a week	High: 318/1838 (17.3%) Low: 127/670 (19.0%) aOR 0.81 (0.62 to 1.06)	
	Butter (high versus low intake) High intake = 3-4 times a week	High: 274/1616 (17.0%) Low: 162/857 (18.9%) aOR 1.08 (0.79 to 1.46)	
	Margarine (high versus low intake) High intake = ≥ 4 times/week	High: 163/801 (20.3 %) Low: 267/1626 (16.4 %) **aOR 1.49 (1.08 to 2.04)**	
	Vegetable oils (high versus low intake) High intake = 3-4 times a week	High: 160/789 (20.3%) Low: 281/1703 (16.5%) **aOR 1.48 (1.14 to 1.91)**	
	Seeds (high versus low intake) High intake = 1-2 times a week	High: 130/622 (20.9%) Low: 311/1854 (16.8%) aOR 1.24 (0.94 to 1.64)	
	Deep-frying vegetable fat (high versus low intake) High intake = ≥ 2-3 times a month	High: 157/860 (18.3%) Low: 281/1605 (17.5%) aOR 1.10 (0.87 to 1.41)	
	Nuts (high versus low intake) High intake = 1-2 times a week	High: 92/548 (16.8%) Low: 351/1944 (18.1%) aOR 0.85 (0.63 to 1.14)	
	Raw carrots (high versus low intake) High intake = 1-2 times a week	High: 323/1773 (18.2%) Low: 120/727 (16.5%) aOR 1.12 (0.85 to 1.46)	
	Spinach (high versus low intake) High intake = ≥ 2-3 times a month	High: 270/1464 (18.4%) Low: 172/1027 (16.7%) aOR 1.26 (0.99 to 1.61)	
	Cabbage (high versus low intake) High intake = 1-2 times a week	High: 178/955 (18.6 %) Low: 268/1548 (17.3%) aOR 1.24 (0.96 to 1.59)	
	Celery (high versus low intake) High intake = ≥ 2-3 times a month	High: 68/398 (17.1%) Low: 367/2047 (17.9%) aOR 0.94 (0.67 to 1.31)	
	Raw tomatoes = 3-4 times a week	High: 133/774 (17.2%) Low: 310/1726 (18.0%) aOR 0.83 (0.63 to 1.10)	
	Raw sweet pepper (high versus low intake) High intake = ≥ 2-3 times a month	High: 309/1750 (17.7%) Low: 133/744 (17.9%) aOR 0.97 (0.75 to 1.27)	
	Salad (high versus low intake) High intake = 3-4 times a week	High: 139/797 (17.4%) Low: 305/ 1707 (17.9%) aOR 0.92 (0.69 to 1.22)	
	Vegetable juice (high versus low intake)	High: 96/550 (17.5%) Low: 345/1927 (17.9%)	

TABLE E 1 MATERNAL DIET AND ECZEMA IN CHILDREN (cont)

	High intake = ≥ 2-3 times a month	aOR 0.91 (0.68 to 1.22)	
	Citrus fruit (high versus low intake) High intake = 3-4 times a week	High: 157/909 (17.3%) Low: 284/1570 (18.1%) aOR 1.03 (0.78 to 1.35)	
	Apples (high versus low intake) High intake = ≥ 4 times/week	High: 150/857 (17.5%) Low: 296/1651 (17.9%) aOR 0.92 (0.72 to 1.21)	
	Exotic fruit (high versus low intake) High intake = 3-4 times a week	High: 282/1623 (17.4%) Low: 162/860 (18.8) aOR 0.85 (0.66 to 1.11)	
	Bananas (high versus low intake) High intake = ≥ 4 times/week	High: 346/1946 (17.8%) Low: 98/561 (17.5%) aOR 1.03 (0.77 to 1.38)	
	Strawberries (high versus low intake) High intake = 1-2 times a week	High: 148/857 (17.3%) Low: 295/1640(18.0%) aOR 1.02 (0.77 to 1.35)	
	Fruit juice (high versus low intake) High intake = 3-4 times a week	High: 325/1782 (18.2%) Low: 120/722 (16.6%) aOR 1.18 (0.90 to 1.54)	
	Fish (high versus low intake) High intake = 1-2 times a week	**High: 122/771 (15.8%)** **Low: 322/1727 (18.6%)** **aOR 0.75 (0.57 to 0.98)**	
Adjusted for study area, sex, maternal age at delivery, smoking during second or third trimester of pregnancy, parental education, exclusive breastfeeding for ≥ 4 months, family history of atopy, season of birth, and all dietary variables			
Miyake 2009 (6) Prospective (n = 763 with eczema at 16-24 months) Diet during pregnancy ISAAC definition for eczema Moderate risk of bias	Fish	Q1* (23.4 g/day) 39/763 (5%) 1.00 Q2 (38.7 g/day) 35/763 (5%) 1.10 (0.59 to 2.05) Q3 (51.7 g/day) 40/763 (5%) 1.33 (0.65 to 2.72) Q4 (73.2 g/day) 28/763 (4%) 0.73 (0.30 to 1.75)	Ptrend = 0.68
	Meat	Q1 (33.8 g/day) 40/763 (5%) 1.00 Q2 (49.0 g/day) 26/763 (3%) 0.68 (0.38 to 1.21) Q3 (63.6 g/day) 32/763 (4%) 0.80 (0.46 to 1.39) Q4 (90.8 g/day) 44/763 (6%) 1.31 (0.78 to 2.22)	Ptrend = 0.28
*quartile medians in g/day energy adjusted intake *Adjusted for maternal age, gestation at baseline, residential municipality at baseline, family income, maternal and paternal education, maternal and paternal history of asthma, atopic eczema and allergic rhinitis, maternal intake of vitamins D and E during pregnancy, changes in maternal diet in previous month, season, maternal smoking during pregnancy, baby's older siblings, baby's sex, birthweight, household smoking, breastfeeding duration and time of delivery before third survey*			
Oien 2010 (7) Retrospective (n = 3086; 466 with eczema) Diet during pregnancy PACT, NORWAY Eczema defined as an itchy rash coming and going for at least 6 months. Moderate-high risk of bias	Fish (never or < once a week versus ≥ once per week)	Never or <once per week (n=961) OR 1.00 ≥ once per week (n = 2052) OR 1.02 (0.82 to 1.26)	p=0.88
	Vegetables (≤ once a week versus 1 to 5 times per week versus almost daily)	≤ once a week (n=275) OR 1.00 1 - 5 times / week (n= 1505) OR 0.76 (0.55 to 1.06) almost daily (n= 985) OR 0.72 (0.51 to 1.02)	 p=0.11 p=0.06
	The sum of n varies for the different dietary factors due to missing data.		
Miyake 2010a (8) (n = 763; 18.6% with eczema) Diet during pregnancy OMCHS, Japan Eczema defined as	Total dairy products	Q1: (43.6 g/day)* 43/763 (6%) 1.00 Q2: (120.8 g/day) 22/763 (3%) **0.45 (0.25 to 0.81)** Q3: (184.5 g/day) 38/763 (5%) 0.94 (0.55 to 1.59) Q4: (280.7 g/day) 39/763 (5%) 1.01 (0.59 to 1.73)	Ptrend = 0.50
	Milk	Q1: (16.1 g/day) 37/763 (5%) 1.00 Q2: (77.9 g/day) 33/763 (4%) 0.94 (0.54 to 1.63) Q3: (144.7 g/day) 35/763 (5%) 1.12 (0.65 to 1.94)	Ptrend = 0.44

TABLE E 1 MATERNAL DIET AND ECZEMA IN CHILDREN (cont)

maternal affirmation of child ever having an itchy rash which was coming and going for at least 6 months, this itchy rash at any time in the last 12 months and in any of folds of the elbow, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes (ISAAC) Moderate risk of bias		Q4: (194.0 g/day) 37/763 (5%) 1.19 (0.69 to 2.06)	
	Yoghurt	Q1: (4.4 g/day) 41/763 (6%) 1.00 Q2: (16.9 g/day) 33/763 (4%) 0.72 (0.42 to 1.23) Q3: (40.3 g/day) 33/763 (4%) 0.73 (0.42 to 1.27) Q4: (94.6 g/day) 35/763 (5%) 0.79 (0.46 to 1.37)	Ptrend = 0.41
	Cheese	Q1: (0.0 g/day) 41/763 (5%) 1.00 Q2: (2.2 g/day) 38/763 (5%) 0.79 (0.46 to 1.34) Q3: (4.5 g/day) 33/763 (4%) 0.74 (0.43 to 1.29) Q4: (12.5 g/day) 30/763 (4%) 0.67 (0.38 to 1.16)	Ptrend = 0.16
	*quartile medians in g/day energy adjusted intake *Adjusted for maternal age, gestation at baseline, residential municipality at baseline, family income, maternal and paternal education, maternal and paternal history of asthma, atopic eczema and allergic rhinitis, changes in maternal diet in the previous 1 month, season when data at baseline were collected, maternal smoking during pregnancy, older siblings, sex, birthweight, household smoking in same room, breastfeeding duration and age at third survey*		
Miyake 2010b (9) (n = 763; 18.6% with eczema) Diet during pregnancy OMCHS, Japan Eczema defined as maternal affirmation of child ever having an itchy rash which was coming and going for at least 6 months, this itchy rash at any time in the last 12 months and in any of folds of the elbow, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes (ISAAC) Moderate risk of bias	Total vegetables	Q1 (90.9 g/day)# 45/763 (6%) 1.00 Q2 (144.4 g/day) 29/763 (4%) 0.60 (0.34 to 1.02) Q3 (189.8 g/day) 32/763 (4%) 0.61 (0.35 to 1.05) Q4 (288.4 g/day) 36/763 (5%) 0.70 (0.41 to 1.19)	Ptrend = 0.22
	Green and yellow vegetables	Q1 (27.6 g/day) 54/763 (7%) 1.00 Q2 (54.9 g/day) 22/763 (3%) **0.30 (0.16 to 0.52)** Q3 (77.5 g/day) 34/763 (4%) **0.53 (0.31 to 0.89)** Q4 (125.8 g/day) 32/763 (4%) **0.41 (0.24 to 0.71)**	**Ptrend = 0.01**
	Other vegetables	Q1 (53.2 g/day) 39/763 (5%) 1.00 Q2 (79.3 g/day 35/763 (5%) 0.84 (0.49-1.44) Q3 (112.7 g/day) 33/763 (4%) 0.80 (0.46-1.37) Q4 (167.2 g/day) 35/763 (5%) 0.85 (0.50-1.46)	Ptrend = 0.54
	Total fruit	Q1 (49.6 g/day) 42/763 (6%) 1.00 Q2 (114.3 g/day) 36/763 (5%) 0.99 (0.58-1.68) Q3 (176.4 g/day) 34/763 (4%) 0.89 (0.52-1.52) Q4 (290.8 g/day) 30/763 (4%) 0.78 (0.45-1.35)	Ptrend = 0.34
	Apples	Q1 (0.3 g/day) 40/763 (5%) 1.00 Q2 (7.6 g/day) 44/763 (6%) 1.15 (0.69-1.93) Q3 (16.7 g/day) 23/763 (3%) **0.55 (0.30-0.98)** Q4 (45.0 g/day) 35/763 (5%) 0.87 (0.49-1.52)	Ptrend = 0.24
	Citrus fruit	Q1 (-0.5 g/day) 49/763 (6%) 1.00 Q2 (15.2 g/day) 34/763 (4%) 0.61 (0.36 -1.02) Q3 (34.8 g/day) 31/763 (4%) **0.57 (0.33-0.98)** Q4 (87.1 g/day) 28/763 (4%) **0.53 (0.30-0.93)**	**Ptrend = 0.03**
	#quartile medians in g/day energy adjusted intake		
	Adjusted for maternal age, gestation at baseline, residential municipality at baseline, family income, maternal and paternal history of asthma, atopic eczema, and allergic rhinitis, changes in maternal diet in previous 1 month, season when data at baseline were collected, maternal smoking during pregnancy, baby's older siblings, baby's sex, baby's birth weight, household smoking in same room as infant, breastfeeding duration and age of infant at survey.		
Miyake 2011b (10) (n = 763) Diet during pregnancy OMCHS, Japan ECZEMA defined as "maternal assessment of itchy rash in last 12 months" (ISAAC) Moderate risk of bias	Healthy (high intake of green and yellow vegetables, seaweed, mushrooms, white vegetables, pulses, potatoes, fish, sea products, fruit and shellfish; low intake of confectionery and soft drinks)	Q1 44/190 (23%) 1.00 Q2 33/191 (17%) 0.76 (0.44 to 1.28) Q3 30/191 (17%) 0.62 (0.35 to 1.07) Q4 35/191 (18%) 0.70 (0.41 to 1.20)	Ptrend = 0.14
	Western (high intake of vegetable oil, salt-containing seasonings, beef and pork, processed meat, eggs, chickens and white vegetables and low intake of	Q1 38/190 (20%) 1.00 Q2 35/191 (18%) 0.85 (0.49 to 1.46) Q3 27/191 (14%) 0.69 (0.39 to 1.21) Q4 42/191 (22%) 1.09 (0.64 to 1.85)	Ptrend = 0.92

TABLE E 1 MATERNAL DIET AND ECZEMA IN CHILDREN (cont)

	fruit, soft drinks and confectionery)		
	Japanese (high intake of rice, miso soup, sea products, and fish)	Q1: 32/190 (17%) 1.00 Q2: 37/191 (19%) 1.15 (0.66 to 1.99) Q3: 36/190 (19%) 1.13 (0.64 to 2.00) Q4: 37/190 (19%) 1.11 (0.64 to 1.94)	Ptrend = 0.76
	Adjusted for maternal age, gestation at baseline, residential municipality, family income, maternal and paternal education, maternal and paternal history of asthma, atopic eczema, allergic rhinitis, changes in maternal diet in the previous 1 month, season when data at baseline were collected, maternal smoking during pregnancy, baby's older siblings, baby's sex, baby's birthweight, household smoking in the same room as the infant, breastfeeding duration, and time of delivery before the third survey		
- 2.5 years age			
Shaheen 2009 (11) (n = 9516; 2509 with eczema at 2.5 years) Diet during pregnancy ALSPAC, UK Eczema defined as "itchy dry skin rash in joints and creases of his/her body (eg, behind the knees, under the arms) since he/she was 18 months old" Moderate risk of bias	Health conscious: salad, fruit, fruit juices, rice, pasta, oat/bran based breakfast cereals, fish pulses, cheese, non-white bread	1.06 (0.99 to 1.12) per standard deviation of dietary pattern score	
	Traditional: vegetables, red meat, poultry	1.00 (0.95 to 1.05) per standard deviation of dietary pattern score	
	Processed: meat pies, sausages, burgers, fried foods, pizza, chips, crisps, white bread, eggs, baked beans	0.97 (0.91 to 1.03) per standard deviation of dietary pattern score	
	Confectionery: chocolate, sweets, biscuits, cakes, pudding	1.03 (0.97 to 1.08) per standard deviation of dietary pattern score	
	Vegetarian: meat substitutes, pulses, nuts, herbal tea	0.99 (0.94 to 1.04) per standard deviation of dietary pattern score	
	Adjusted for energy intake, maximum smoked, infections, antibiotics and paracetamol use during pregnancy; maternal educational level, housing tenure, financial difficulties, pre-pregnancy body mass index, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine, sex of child, gestational age, breastfed in first 6 months, day care at 8 months, multiple pregnancy, pets in infancy, damp/condensation/mould, child exposed to environmental tobacco smoke at weekends, season of birth, season of food frequency questionnaire completion, birthweight, head circumference, birth length.		
- 3 years age			
Lange 2010 (12) (n = 1376; 35% with eczema) Diet during pregnancy PROJECT VIVA, USA *ECZEMA defined as a parental report of a doctor's diagnosis of eczema* *Low risk of bias*	Mediterranean diet score (0 to 9 points)	1.00 (0.94 to 1.06) per 1 point increase in score (one point if above median consumption for dairy, fish, fruit, legumes, nuts, unsaturated-to-saturated fat ratio, vegetables and whole grains; and one point if intake of red and processed meats was below the median value)	
	AHEI-P (90 point scale)	0.94 (0.82 to 1.08) per 10 points (each of the following nine components can have 10 possible points: vegetables, fruit; ratio of white to red meat; fibre; trans fat; ratio of polyunsaturated to saturated fatty acids; and folate, calcium and iron from foods)	
	Prudent pattern	0.95 (0.83 to 1.09) (fruits, tomatoes, cabbages, green leafy vegetables, poultry and fish)	
	Western pattern.	1.06 (0.93 to 1.22) (red meat, processed meat, refined grains, snacks, sweets, desserts, French fries, and pizza)	
	Adjusted for child's sex, maternal race, maternal education level, household income, maternal and paternal history of eczema, presence of children < 12 years of age at home, maternal prepregnancy BMI, breastfeeding duration, and passive smoke exposure		
Miyake 2011 (13) N=582 mother infant pairs	Maternal hair mercury levels at 29-39 months	**ECZEMA** **Risk (%)** Maternal hair mercury (μg/g)	

TABLE E 1 MATERNAL DIET AND ECZEMA IN CHILDREN (cont)

OMCHS, Japan Diet during pregnancy ISAAC definition for eczema Moderate risk of bias	postpartum were used as a surrogate for antenatal mercury exposure (fish)	≤ 1.09 28/145 (19.3) 1.00 1.10-1.51 22/139 (15.8) 0.86 (0.45-1.63) 1.52-2.10 22/150 (14.7) 0.77 (0.40-1.45) ≥ 2.11 28/148 (18.9) 0.93 (0.49-1.75)	p-trend 0.74
	Adjusted for maternal age, residential municipality at baseline, maternal and paternal education, maternal and paternal history of allergic disorders, maternal energy-adjusted fish intake during pregnancy, maternal smoking during pregnancy, number of child's older siblings, child's sex, household smoking in the same room as the child, breastfeeding and children's fish intake		
- 5 years of age			
Willers 2007 (14) (n = 1253) Diet during pregnancy Diet during pregnancy Aberdeen, SCOTLAND ECZEMA defined as parental response to ISAAC questionnaire Low risk of bias	Total fish (never (9%) versus < once/week (21%) versus ≥ once/week (70%))	<u>Eczema (doctor confirmed): 380/979 (30.4%)</u> Never (n = 107) 1.00 < once/week (n = 255) 0.79 (0.47 to 1.32) **≥ once/week (n = 831) 0.57 (0.35 to 0.92**)	**Ptrend = 0.008**
		<u>Current eczema medication: 191/982 (15.3%)</u> Never (n = 107) 1.00 < once/week (n = 255) 0.88 (0.46 to 1.67) ≥ once/week (n = 831) 0.58 (0.32 to 1.06)	**Ptrend = 0.028**
		<u>Ever had eczema: 406/983 (32.4%)</u> Never (n = 107) 1.00 < once/week (n = 255) 0.91 (0.54 to 1.53) ≥ once/week (n = 831) 0.68 (0.43 to 1.10)	**Ptrend = 0.05**
	"No consistent linear associations were found between maternal intake of total fruit, citrus/kiwi fruit, total vegetables, green leafy vegetables, fruit juice, whole grain products, fat from dairy products or butter versus margarine/low fat spread use and respiratory or atopic outcomes in the 5-year old children"		
	Adjusted for energy intake, maternal age, paternal social class, maternal age of leaving fulltime education, maternal smoking during pregnancy, maternal atopy, child's birth weight, child's sex, presence of older siblings, breastfeeding, smoking in the child's home		
Erkkola 2009 (15) (n = 1669; 37% eczema) Diet during pregnancy FINLAND ECZEMA defined from parental responses to ISAAC questionnaire Low risk of bias	Vitamin D from food	Q1: (< 0.31 µg/MJ) 1.07 (0.83 to 1.39) Q2/3: (0.31-0.54 µg/MJ) 1.00 Q4: (> 0.54 µg/MJ) 0.92 (0.71 to 1.20) <u>Absolute intake (log-transformed)</u>: 0.94 (0.84 to 1.04) 0.95 (0.82 to 1.09)*	
	Adjusted for sex, area of birth, duration of gestation, maternal age, maternal basic education, ,maternal smoking during pregnancy, number of siblings, parental asthma, parental allergic rhinitis, and pets inside the house before the age of 1 year **additionally adjusted for energy-adjusted maternal intake of fruits and vegetables, vitamin C, vitamin E, selenium and zinc*		
Grandjean 2010 (16) (n = 464) Faroe Islands, Denmark ECZEMA determined by paediatric assessment Moderate risk of bias	Fish (as a source of PCBs and mercury)	**No allergy* N = 378** **atopic eczema n = 60** PCB (µg/g serum lipid) geometric mean, IQR Antenatal 1.24 (0.83-2.0) 0.70 (0.55-1.58) 5 years 1.17 (0.73-1.96) 0.56 (0.58**-1.85) Methylmercury (µg/L blood) geometric mean, IQR Antenatal 12.8 (7.2-21.1) 13.2 (7.4-24.2) 5 years 2.5 (1.34-4.7) 3.1 (1.41-6.7) *Unadjusted analyses (adjusted analyses gave similar results) ** On paper value looked implausible	**p-value** **0.01** 0.07 0.80 0.13
- 7 to 7.5 years age			
Shaheen 2009 (11) (n = 7693) Diet during pregnancy ALSPAC, UK ECZEMA defined as "itchy dry skin rash in joints and creases of his/her body (eg, behind	Health conscious: salad, fruit, fruit juices, rice, pasta, oat/bran based breakfast cereals, fish pulses, cheese, non-white bread	1.04 (0.95 to 1.13)[xx]	p-value = 0.38
	Traditional: vegetables, red meat, poultry	0.99 (0.92 to 1.05)	p-value = 0.71

TABLE E 1 MATERNAL DIET AND ECZEMA IN CHILDREN (cont)

the knees, under the arms) since he/she was 18 months old" Moderate risk of bias	Processed: meat pies, sausages, burgers, fried foods, pizza, chips, crisps, white bread, eggs, baked beans	0.96 (0.88 to 1.05)	p-value = 0.36
	Confectionery: chocolate, sweets, biscuits, cakes, pudding	1.03 (0.95 to 1.11)	p-value = 0.51
	Vegetarian: meat substitutes, pulses, nuts, herbal tea	1.01 (0.95 to 1.08)	p-value = 0.64
Grandjean 2010 (16) (n = 464) Faroe Islands, Denmark ECZEMA determined by paediatric assessment Moderate risk of bias	Fish (as a source of PCBs and mercury)	No allergy* N = 378 atopic eczema n = 60 PCB (µg/g serum lipid) geometric mean, IQR Antenatal 1.24 (0.83-2.0) 0.70 (0.55-1.58) 7 years 0.77 (0.44-1.39) 0.60 (0.29-1.19) Methylmercury (µg/L blood) geometric mean, IQR Antenatal 12.8 (7.2-21.1) 13.2 (7.4-24.2) 7 years 2.0 (1.00-4.6) 2.2 (1.07-4.6) *Unadjusted analyses (adjusted analyses gave similar results)	**p-value** **0.01** **0.04** 0.80 0.65

ECZEMA MANAGEMENT ARTICLES			
< 1 Year old			
Cant 1986 (17) UK N = 37 Breast fed infants with eczema aged 6 weeks to 6 months RCT (crossover trial) [plus non-randomized crossover trial designed to see if the soy substitute might have provoked symptoms in the RCT] Moderate risk of bias	Dairy foods (cow's milk) and egg **Trial 1:** Exclusion of cow milk, egg, chocolate, wheat, nuts, fish, beef, chicken, citrus fruits, colourings, and preservatives, with use of soya-based milk substitute for 4 weeks, *versus* same dietary exclusions for same duration (4 weeks) but substitute contained cow milk and egg, *versus* normal maternal diet (4 weeks – control period)	**Trial 1: (n= 17)** Non-significant reduction in eczema activity score – mean 10.8 in exclusion period; 12.2 in control period Non-significant reduction in eczema area score – mean 8.6 in exclusion period; 9.4 in control period	
	Trial 2: Phase 1. Normal diet (milk and egg containing) (2 weeks) vs exclusion diet as above (2 weeks) v normal diet (2 weeks). Phase 2. Infants with eczema scores >20% difference between groups. Exclusion	**Trial 2: non RCT (n = 18):** In 2 children the eczema activity score decreased by > 20% when their mothers took the exclusion diet and then increased but > 20% when their mothers returned to a normal diet; In 2 children the eczema activity scores remained unchanged when their mothers took the exclusion diet but then deteriorated when the mothers took a normal diet	

TABLE E 1 MATERNAL DIET AND ECZEMA IN CHILDREN (cont)

	diet as above v milk and egg containing diet (2 weeks) v potato starch placebo (2 weeks)		
Palmer 2008 (18) 32 Mother – Infant pairs. Breast fed babies with IgE mediated egg allergy and eczema. RCT. Maternal egg exclusion and rechallenge Australia Low risk of bias	Egg	***Egg Group (n=14)*** ***Control Group (n=16)*** Objective standardised scoring system for atopic dermatitis (SCORAD) Day 0 21.9 +/- 6.5 21.4 +/- 4.8 Day 24 11.1 +/- 7.3 9.5 +/- 5.5 Total SCORAD Day 0 34.3 +/- 9.9 32.0 +/-6.4 Day 24 17.6 +/- 12.1 14.3 +/- 7.1 Infants' Dermatitis Quality of Life Index (IDQOL) Day 0 10.9 +/- 5.3 8.4 +/- 3.5 Day 12 7.6 +/- 3.8 6.4 +/- 4.2 Day 24 5.9 +/- 4.9 5.1 +/- 3.4 ***Improvement with time for both groups, but no statistical difference between groups.***	 p<0.001 p<0.001 p<0.001
Uenishi 2011 (19) N= 92 Japan Lactation Prospective cohort, then maternal food exclusion-challenge Eczema (as defined by Japanese Dermatology Association criteria for diagnosis of atopic dermatitis) High risk of bias	Tree-nut related foods (chocolate, coffee) and fermented foods (cheese, yoghurt, soy sauce, miso soup and fermented soy beans) Mothers excluded the 8 foods for 2 weeks; Then mothers took one of the excluded foods at breakfast for 2 successive days	Improvement was assessed on a five point scale: greatly improved (> 75% improvement); fairly improved (50-75% improvement); slightly improved (< 50% improvement); unchanged or worsened; Positive rating was regarded as greatly or fairly improved Exacerbation was assessed on a similar scale ***Eczema after 2 weeks maternal dietary exclusion*** Greatly improved 43 Fairly improved 24 Slightly improved 12 Unchanged 13 Worsened 0 i.e. 67/92 (73%) had a positive response ***Maternal challenges*** Of the 67 positive infants; skin lesions were moderately or severely exacerbated in all 107 maternal challenges – 43 severely and 64 moderately (mean 1.6 challenges per infant; range 1 to 4) "predominant offending foods were soy sauce, chocolate, yoghurt and miso sauce"	

TABLE E 2 MATERNAL DIET AND ASTHMA IN CHILDREN

Study details	Maternal dietary pattern/component	Effect size: aOR and 95% CI (except where stated otherwise)	
Prospective and Retrospective cohort studies			
At 2 years of age			
Oien 2010 (7) Retrospective (n = 3086; 183 with asthma) Diet during pregnancy PACT, NORWAY Doctor Diagnosed asthma Moderate-high risk of bias	Fish	never or < once a week (n = 964) OR 1.00 ≥ once per week (n = 2061) OR 0.99 (0.72 to 1.37)	p=0.96
	Vegetables	≤ once a week (n=275) OR 1.00 1 - 5 times / week (n= 1510) OR 0.98 (0.55 to 1.69) almost daily (n= 992) OR 1.02 (0.58 to 1.81)	p=0.94 p=0.94
At 3 years of age			
Camargo 2007 (20) PROJECT VIVA, USA Diet during pregnancy (n= 416) Recurrent wheeze (positive asthma predictive index (≥ 2 wheezing attacks among children with a personal diagnosis of eczema or a parental diagnosis of asthma); Low-moderate risk of bias	Cow's milk (Vit D Fortified) Low milk (<1 cup/day) Moderate (1-1.9 cups/day) High (≥ 2 cups/day)	Recurrent wheeze in children at 3 yo None 1.00 Low 0.57 (0.27 to 1.24) Moderate **0.35 (0.16 to 0.80)** High 0.45 (0.20 to 1.02)	**p 0.046**
	Adjusted for child's sex, birthweight, income, maternal age, maternal prepregnancy BMI, passive smoke exposure, breastfeeding duration at 1 year, presence of children < 12 years of age at home, maternal history of asthma, paternal history of asthma, maternal intake of fruits and vegetables.		
Fitzsimon 2007 (21) (n = 631) Diet during pregnancy Life-ways Cross-Generation cohort study, Galway, IRELAND Retrospective cohort Asthma defined as doctor diagnosed asthma (in GP notes) Moderate risk of bias	Fruit Vegetables	62/605 cases (10.2%) serves FV/day (median, range) Q1: 2.3 (0 to 3.4) 146/631 (23%) 1.00 Q2: 4.1 (3.4 to 5.0) 166/631 (26%) 1.1 (0.53 to 2.3) Q3: 6.0 (5.0 to 7.1) 146/631 (23%) 0.89 (0.39 to 2.0) Q4: 8.9 (> 7.1) 173/631 (27%) 0.50 (0.21 to 1.2)	P-value for Q4 versus Q1+2+3 = 0.07
	Fruit: (apples, pears, satsumas and mandarins, grapefruit, bananas, grapes, melons, peaches, plums and apricots, strawberries, raspberries and kiwifruit); Vegetables (carrots, spinach, broccoli, spring greens and kale, brussel sprouts, cabbage, peas, green beans and runner beans, marrow and courgettes, cauliflower, parsnips, turnips, leeks, onions, garlic, mushrooms, sweet peppers, bean sprouts, green salad and lettuce, cucumber, celery, watercress, tomatoes, sweet corn, beetroot, coleslaw, avocado) *Adjusted for maternal age, maternal BMI, maternal smoking during pregnancy, exposure to smoke in the home, mould or damp in home, problems with pollution in the local area, maternal university education, GMS status (medical card for low-income women), income, maternal parity, child's birthweight, child's gender, breastfeeding, fat, oily fish*		
Lange 2010 (12) (n = 1376; 21% with asthma) Diet during pregnancy PROJECT VIVA, USA ASTHMA defined as a parental report of	Mediterranean diet score (0 to 9 points)	1.01 (0.94 to 1.09) per 1 point increase in score (one point if above median consumption for dairy, fish, fruit, legumes, nuts, unsaturated-to-saturated fat ratio, vegetables and whole grains; and one point if intake of red and processed meats was below the median value)	
	AHEI-P (90 point scale)	1.07 (0.92 to 1.25) per 10 points (each of the following nine components can have 10 possible points: vegetables, fruit; ratio of white to red meat; fibre;	

TABLE E 2 MATERNAL DIET AND ASTHMA IN CHILDREN (cont)

a doctor's diagnosis of asthma in the child at any time Low risk of bias		trans fat; ratio of polyunsaturated to saturated fatty acids; and folate, calcium and iron from foods)	
	Prudent pattern	1.08 (0.93 to 1.26) (fruits, tomatoes, cabbages, green leafy vegetables, poultry and fish)	
	Western pattern	0.89 (0.76 to 1.04) (red meat, processed meat, refined grains, snacks, sweets, desserts, French fries, and pizza)	
	Adjusted for child's sex, maternal race, maternal education level, household income, maternal and paternal history of asthma, presence of children < 12 years of age at home, maternal prepregnancy BMI, breastfeeding duration, and passive smoke exposure		
At 5 years of age			
Salam 2005 (22) (n = 279 cases; 412 controls) Diet during pregnancy Children's Health Study, California, USA (nested case control study) ASTHMA defined as parental report of doctor-diagnosed asthma Low-moderate risk of bias	Oily fish	Any asthma (n=279): Never 1.00 Rarely 1.01 (0.54 to 1.89) At least monthly 0.80 (0.47 to 1.36) Early transient asthma (47/279, 16.8%): (diagnosis before 3 y, no asthma symptoms in previous 12 months or after first grade) Never 1.00* Rarely 0.68 (0.17 to 2.67) At least monthly 0.99 (0.34 to 2.87) Early persistent asthma (166/279, 59.5%): (diagnosis before 3 y & at least one episode of asthma or wheeze or asthma medication use in the 12 months before study entry or after starting 1st grade) Never 1.00 Rarely 1.07 (0.53 to 2.17) At least monthly 0.45 (0.23 to 0.91) Late-onset asthma (66/279, 23.7%): (diagnosis after 3 y) Never 1.00 Rarely 0.80 (0.26 to 3.09) At least monthly 0.84 (0.33 to 2.12)	Ptrend = 0.40 Ptrend = 0.92 Ptrend = 0.04 Ptrend = 0.66
	Fish sticks (fingers)	Any asthma(n=279): Never 1.00 Rarely 1.15 (0.66 to 2.01) At least monthly 2.04 (1.18 to 3.51) Early transient asthma (47/279, 16.8%): (diagnosis before 3 y, no asthma symptoms in previous 12 months or after first grade) Never 1.00 Rarely 0.74 (0.24 to 2.27) At least monthly 2.26 (0.67 to 7.58) Early persistent asthma (166/279, 59.5%): (diagnosis before 3 y & at least one episode of asthma or wheeze or asthma medication use in the 12 months before study entry or after starting 1st grade) Never 1.00 Rarely 1.51 (0.75 to 3.04) At least monthly 2.46 (1.26 to 4.80) Late-onset asthma (66/279, 23.7%): (diagnosis after 3 y) Never 1.00 Rarely 0.98 (0.34 to 2.89) At least monthly 3.05 (1.04 to 8.93)	Ptrend = 0.01 Ptrend = 0.30 Ptrend = 0.01 Ptrend = 0.07

TABLE E 2 MATERNAL DIET AND ASTHMA IN CHILDREN (cont)

	Joint effects of maternal asthma and oily fish	Any asthma: NO MATERNAL ASTHMA Never 1.00 Rarely 1.31 (0.65 to 2.67) At least monthly 1.09 (0.61 to 1.94) MATERNAL ASTHMA Never 3.97 (2.07 to 7.63) Rarely 1.78 (0.54 to 5.90) At least monthly 0.81 (0.29 to 2.28) Early transient asthma: NO MATERNAL ASTHMA Never 1.00 Rarely 0.70 (0.16 to 3.11) At least monthly 1.38 (0.42 to 4.61) MATERNAL ASTHMA Never 3.89 (0.83 to 18.18) Rarely 1.95 (0.10 to 40.01) At least monthly 0.98 (0.12 to 7.82) Early persistent asthma: NO MATERNAL ASTHMA Never 1.00 Rarely 1.55 (0.68 to 3.52) At least monthly 0.62 (0.29 to 1.31) MATERNAL ASTHMA Never 5.58 (2.52 to 12.33) Rarely 2.13 (0.57 to 7.99) At least monthly 0.63 (0.16 to 2.56) Late-onset asthma: NO MATERNAL ASTHMA Never 1.00 Rarely 1.31 (0.65 to 2.67) At least monthly 1.09 (0.61 to 1.94) MATERNAL ASTHMA Never 6.47 (1.92 to 21.81) Rarely 8.11 (0.47 to 14.71) At least monthly 0.33 (0.04 to 2.76)	Ptrend = 0.70 Ptrend = 0.006 Pinter-action = 0.02 Ptrend = 0.67 Ptrend = 0.31 Pinter-action = 0.50 Ptrend = 0.35 Ptrend = 0.006 Pinter-action = 0.06 Ptrend = 0.42 Ptrend = 0.01 Pinter-action = 0.01
	Adjusted for maternal asthma, race/ethnicity, maternal age, maternal education, gestational age, number of siblings, exclusive breastfeeding, and mutually adjusted for the other fish variable ** Adjusted for maternal asthma, race/ethnicity, gestational age, number of siblings, exclusive breastfeeding, and mutually adjusted for the other fish variable*		
Willers 2007 (14) (n = 1253) Diet during pregnancy Aberdeen, SCOTLAND *ASTHMA defined as parental response to ISAAC questionnaire* *Low risk of bias*	Apples (tertiles)	Asthma and wheeze in last 12 months: 107/998 (8.5%) T1 (0-1/week); n = 398 1.00 T2 (1-4/week): n = 427 1.03 (0.59 to 1.80) T3 (>4/week): n = 384 0.60 (0.31 to 1.16) Doctor confirmed asthma: 145/998 (11.6%) T1 (0-1/week); n = 398 1.00 T2 (1-4/week): n = 427 0.83 (0.52 to 1.32) T3 (>4/week): n = 384 **0.47 (0.27 to 0.82)** Ever had asthma: 156/998 (12.5%) T1 (0-1/week); n = 398 1.00 T2 (1-4/week): n = 427 0.86 (0.54 to 1.36) T3 (>4/week): n = 384 **0.54 (0.32 to 0.92)**	Ptrend = 0.149 Ptrend = 0.008 Ptrend = 0.026
	"No consistent linear associations were found between maternal intake of total fruit, citrus/kiwi fruit, total vegetables, green leafy vegetables, fruit juice, whole grain products, fat from dairy products or butter versus margarine/low fat spread use and respiratory or atopic outcomes in the 5-year old children"		
	Adjusted for energy intake, maternal age, paternal social class, maternal age of leaving fulltime education,		

TABLE E 2 MATERNAL DIET AND ASTHMA IN CHILDREN (cont)

	maternal smoking during pregnancy, maternal atopy, child's birthweight, child's sex, presence of older siblings, breastfeeding, smoking in the child's home		
Erkkola 2009 (23) (n = 1669; 6% asthma) Diet during pregnancy FINLAND ASTHMA defined as persistent asthma diagnosed by a doctor, with either wheezing or asthma medication during the previous 12 months Low risk of bias	Vitamin D from food	Q1: (< 0.31µg/MJ) aHR 1.64 (1.02 to 2.64) Q2/3: (0.31-0.54 µg/MJ) aHR 1.00 Q4: (> 0.54 µg/MJ) aHR 0.81 (0.45 to 1.47) <u>Absolute intake (log-transformed)</u>: aHR 0.80 (0.64 to 0.99) aHR 0.73 (0.55 to 0.95)*	
	Adjusted for sex, area of birth, duration of gestation, maternal age, maternal basic education, ,maternal smoking during pregnancy, number of siblings, parental asthma, parental allergic rhinitis, and pets inside the house before the age of 1 year **additionally adjusted for energy-adjusted maternal intake of fruits and vegetables, vitamin C, vitamin E, selenium and zinc*		
Lumia 2011 (24) (n = 2679; 158 cases of asthma) Diet during pregnancy (intake in 8th month) Turku, FINLAND ASTHMA - defined as any wheezing symptom or use of asthma medication during the preceding 12 months plus doctor diagnosed asthma validated against purchase of at least one anti-asthmatic medication during a 12 month period Similar results when allergic (1183) and non-allergic (1403) mothers were analysed separately Low risk of bias	Milk and milk products g/day	Lowest quarter (< 546.2) aHR 1.17 (0.78 to 1.77) Midhalf (546.62 to 1099.84) aHR 1 Highest quarter (> 10.99.84) aHR 0.73 (0.44 to 1.21)	Ptrend = 0.282 (aHR)
	Oils g/day	Lowest quarter (< 7.32) aHR 1.07 (0.69 to 1.65) Mid-half (7.32 to 16.60) aHR 1 Highest quarter (> 16.60) aHR 1.26 (0.82 to 1.94)	Ptrend = 0.576 (aHR)
	Margarines	User (> 15.00) aHR 0.82 (0.58 to 1.17) Non-user (0) aHR 1.00	Ptrend = 0.274 (aHR)
	Butter and butter-oil mixes g/day	Lowest quarter (< 4.63) aHR 1.41 (0.91 to 2.19) mid-half (4.63-19.78) aHR 1 highest quarter (> 19.78) aHR 1.47 (0.96-2.23)	Ptrend = 0.126 (aHR)
	Fish g/day	Lowest quarter (< 10.67) aHR 1.12 (0.74 to 1.70) mid-half (10.67-31.99) aHR 1 highest quarter (> 31.99) aHR 1.06 (0.68 to 1.65)	Ptrend = 0.861 (aHR)
	Red meat and meat products g/day	lowest quarter (< 88.49) aHR 0.76 (0.47 to 1.22) mid-half (88.49-156.44) aHR 1 highest quarter (> 156.44) aHR 1.06 (0.67 to 1.67)	Ptrend = 0.469 (aHR)
	Industrial fat mixes and animal fats g/day	lowest quarter (< 11.44) aHR 1.40 (0.91 to 2.16) mid-half (11.44- 29.70) aHR 1 highest quarter (> 29.60) aHR 1.09 (0.70 to 1.70)	Ptrend = 0.309 (aHR)
	Adjusted for maternal age, mode of delivery, duration of gestation, number of earlier deliveries, birth wt, sex of child, area of birth, maternal smoking during pregnancy, parental asthma or allergic rhinitis, maternal vocational education, pets at home, farming, contact with cow stable during first year of life & duration of total breast feeding. Also adjusted for twins & siblings in the study.		
Grandjean 2010 (16) (n = 464) Faroe Islands, Denmark ASTHMA determined by paediatric assessment. Moderate risk of bias	Fish (as a source of PCBs and mercury)	No allergy* asthma N = 378 n = 35 PCB (µg/g serum lipid) geometric mean, IQR Antenatal 1.24 (0.83-2.0) 1.46 (0.80-2.6) 5 years 1.17 (0.73-1.96) 1.40 (0.63-2.7) Methylmercury (µg/L blood) geometric mean, IQR Antenatal 12.8 (7.2-21.1) 14.7 (7.6-29.2) 0.35 5 years 2.5 (1.34-4.7) 3.5 (1.69-10.2) 0.06 *Unadjusted analyses (adjusted analyses gave similar results)	p-value 0.17 0.20 0.35 0.06
At 6 to 7.5 years			
De Batlle 2008 (25) (n = 1476)	Mediterranean diet scores (high score =	Asthma (ever) n assumed to be 1326	

TABLE E 2 MATERIAL DIET AND ASTHMA IN CHILDREN (cont)

Diet during pregnancy MEXICO Retrospective study Asthma defined as parental response to ISAAC questionnaire on respiratory and allergic symptoms Moderate –high risk of bias	high vegetables, legumes, fruits and nuts, cereals and fish; and low dairy products, meat, junk food and fat); High versus lower score (1st tertile versus 2nd & 3rd tertiles)	1.03 (0.67 to 1.56);	
	Adjusted for gender of child, child's physical exercise, current tobacco smoking at home, maternal education, maternal asthma, maternal rhinitis		
Shaheen 2009 (11) (n = 7625; 12% of children at 7.5 years) Diet during pregnancy ALSPAC, UK Asthma defined as current doctor-diagnosed asthma if mothers responded positively to the question "Has a doctor ever actually said you're your study child has asthma?" and positively to one or both questions on wheezing and asthma in the past 12 months. Moderate risk of bias	Health conscious: salad, fruit, fruit juices, rice, pasta, oat/bran based breakfast cereals, fish pulses, cheese, non-white bread	0.95 (0.86 to 1.04) per standard deviation of dietary pattern score	p-value = 0.27
	Traditional: vegetables, red meat, poultry	0.96 (0.89 to 1.04) per standard deviation of dietary pattern score	p-value = 0.35
	Processed: meat pies, sausages, burgers, fried foods, pizza, chips, crisps, white bread, eggs, baked beans	0.98 (0.90 to 1.07) per standard deviation of dietary pattern score	p-value = 0.68
	Confectionery: chocolate, sweets, biscuits, cakes, pudding	1.00 (0.91 to 1.08) per standard deviation of dietary pattern score	p-value = 0.93
	Vegetarian: meat substitutes, pulses, nuts, herbal tea	1.02 (0.95 to 1.09) per standard deviation of dietary pattern score	p-value = 0.62
	Adjusted for energy intake, maximum smoked, infections, antibiotics and paracetamol use during pregnancy; maternal educational level, housing tenure, financial difficulties, pre-pregnancy body mass index, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine, sex of child, gestational age, breastfed in first 6 months, day care at 8 months, multiple pregnancy, pets in infancy, damp/condensation/mould, child exposed to environmental tobacco smoke at weekends, season of birth, season of food frequency questionnaire completion, birthweight, head circumference, birth length, number of younger siblings and child's BMI at age 7.		
Grandjean 2010 (16) (n = 464) Faroe Islands, Denmark ASTHMA determined by paediatric assessment. Moderate risk of bias	Fish (as a source of PCBs and mercury)	No allergy* N = 378 / asthma n = 35 PCB (µg/g serum lipid) geometric mean, IQR Antenatal 1.24 (0.83-2.0) 1.46 (0.80-2.6) 7 years 0.77 (0.44-1.39) 0.89 (0.49-1.70) Methylmercury (µg/L blood) geometric mean, IQR Antenatal 12.8 (7.2-21.1) 14.7 (7.6-29.2) 0.35 7 years 2.0 (1.00-4.6) 2.1 (1.06-3.9) 0.91 *Unadjusted analyses (adjusted analyses gave similar results)	p-value 0.17 0.33 0.35 0.91
At 8 years			
Willers 2008 (26) (n=2 832) Diet during pregnancy Netherlands Prospective cohort (longitudinal)	Vegetables	n / Asthma Symptoms* Daily 2830 1 Regular + Rare 0.98 (0.84 to 1.14)	
	Fruit	Daily 2828 1 Regular + Rarely 0.91 (0.77 to 1.09)	
	Fish	Rarely 2811 1 Daily + Regularly 1.01 (0.85 to 1.20)	
	Egg	Rarely 2818 1	

TABLE E 2 MATERNAL DIET AND ASTHMA IN CHILDREN (cont)

Part of the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study Low risk of bias		Daily + Regularly	1.03 (0.88 to 1.20)	
	Dairy	Daily 2788	1	
		Regular + Rare	0.92 (0.74 to 1.15)	
	Nuts	Rare 3909	1	
		Daily + Regular	1.00 (0.86 to 1.17)	
	Nut Products	Regular v Rare	0.98 (0.84 to 1.14)	
		Daily v Rare	**1.47 (1.08 to 1.99)**	
	**composite of previous three outcomes – wheeze, dyspnoea & steroid use* *The child's dietary data on fruit, vegetables, fish, eggs, full cream milk, butter and peanut butter consumption at 2 years of age were used to check for potential confounding by the child's diet.* *Results were adjusted for by sex, maternal education, parental allergy, maternal smoking during pregnancy, smoking in the home at 8 years of age, breastfeeding, presence of older siblings, birthweight, maternal overweight 1 year after pregnancy, maternal supplement use during pregnancy, region and study arm (intervention or natural history arm).*		

TABLE E 3 MATERNAL DIET AND WHEEZE IN CHILDREN

Study Details	Maternal dietary pattern / component	Effect Size (aOR and 95% CI unless otherwise stated)	P value
Prospective and Retrospective Cohorts			
Dietary associations – multiple food groups or multiple individual foods			
At 6 months to 1 year of age			
Shaheen 2009 (11) **n=**14,062, 8886 with wheeze. ALSPAC, UK Diet during pregnancy Moderate risk of bias	Health conscious: salad, fruit, fruit juices, rice, pasta, oat/bran based breakfast cereals, fish pulses, cheese, non-white bread	Transient infant wheeze at 6 months (n = 8886) 0.98 (0.90 to 1.06) Later onset wheeze at 6 months (n = 8886) 0.93 (0.84 to 1.03) Persistent wheeze at 6 months (n = 8886) 1.00 (0.86 to 1.16)	0.57 0.19 0.99
	Traditional: vegetables, red meat, poultry	Transient infant wheeze at 6 months (n = 8886) 0.95 (0.89 to 1.02) Later onset wheeze at 6 months (n = 8886) 1.00 (0.92 to 1.09) Persistent wheeze at 6 months (n = 8886) 0.96 (0.86 to 1.08)	0.16 0.95 0.51
	Processed: meat pies, sausages, burgers, fried foods, pizza, chips, crisps, white bread, eggs, baked beans	Transient infant wheeze at 6 months (n = 8886) 0.99 (0.91 to 1.08) Later onset wheeze at 6 months (n = 8886) 1.03 (0.93 to 1.13) Persistent wheeze at 6 months (n = 8886) 1.00 (0.88 to 1.13)	0.87 0.61 0.98
	Confectionery: chocolate, sweets, biscuits, cakes, pudding	Transient infant wheeze at 6 months (n = 8886) 1.03 (0.95 to 1.10) Later onset wheeze at 6 months (n = 8886) 0.96 (0.87 to 1.06) Persistent wheeze at 6 months (n = 8886) 1.02 (0.90 to 1.16)	0.51 0.41 0.72
	Vegetarian: meat substitutes, pulses, nuts, herbal tea	Transient infant wheeze at 6 months (n = 8886) 1.00 (0.94 to 1.06) Later onset wheeze at 6 months (n = 8886) 0.92 (0.85 to 1.00) Persistent wheeze at 6 months (n = 8886) 1.06 (0.96 to 1.16)	0.91 0.06 0.27
** Adjusted for Maternal factors during pregnancy (energy intake, smoking, infections, antibiotics and paracetamol); other maternal factors (educational level, housing tenure, financial difficulties, pre-pregnancy BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine); sex of child, gestational age, breast fed in first 6 months, day care at 8 months, multiple pregnancy, pets in infancy, damp/condensation/mould, child exposed to environmental tobacco smoke at weekends, season of birth, season of FFQ completion, birth weight, head circumference, birth length. Also number of younger siblings and child's BMI at 7 years for later childhood outcomes.*			
Castro-Rodriguez 2010 (27) n=1409 Spain EISL (International Study of Wheezing in Infants) Wheezing during first year of life. (Using ISAAC III questionnaire) Diet during pregnancy Moderate risk of bias	General diet style (Mediteranean diet score) "Pro Mediteranean' foods (fruit, fish, vegetables, legumes, cereal, pasta, rice, potato) rated positively "Anti Mediteranean' (meat, milk, fast foods) foods rated inversely	Wheeze (n=594) no wheeze (n=815) mean Mediterranean diet score in pregnancy, 13.1 [SD2.3] 13.3 [SD1.9]	0.036
	Olive Oil Use	Wheeze no wheeze aOR * 468/594 (78.8%) 694/815 (85.2%) **0.57 (0.4 to 0.9)**	**0.002**
	Mediteranean Diet with no olive oil	aOR=0.96 (0.8 to 1.1)	0.53
	**Adjusted for male gender, exclusive breastfeeding, maternal asthma, day care attendance, number of people in house, number of siblings, mould stains on household walls, maternal smoking during pregnancy*		

	WHEEZING (N=594)			NON WHEEZING (N=815)			P Trend
Frequency of other dietary components	*Never / occ*	*1-2/wk*	*≥3/wk*	*Never /occ*	*1-2/wk*	*≥3/wk*	
Meat	1.6%	36.0%	62.4%	2.9%	33.6%	63.5%	0.22
Hamburgers, homemade	74.1%	23.4%	2.5%	80.2%	18.1%	1.7%	**0.03**
Fast food:							
- Pizzas	63.3%	33.6%	3.1%	63.6%	33.6%	2.8%	0.95
- Hamburgers/hotdogs	84.3%	13.7%	2.0%	86.0%	13.0%	1.0%	0.24
- Precooked fried food	58.3%	33.8%	7.9%	62.9%	33.2%	4.0%	**0.009**
White fish	16.1%	65.0%	18.9%	12.2%	69.0%	18.8%	0.11
Oily fish	26.4%	56.4%	17.2%	23.8%	60.6%	15.6%	0.32
Fruit, natural juice	7.0%	17.9%	75.2%	4.2%	13.8%	82.0%	**0.007**
Fresh vegetables	10.9%	25.9%	63.1%	7.0%	23.9%	69.2%	**0.015**
salads	7.2%	21.4%	71.4%	4.5%	17.7%	77.9%	**0.013**
Cooked vegetables	14.9%	39.1%	46.1%	13.6%	38.3%	48.1%	0.70
Legumes	6.2%	55.2%	38.5%	6.1%	56.1%	37.9%	0.96

TABLE E 3 MATERNAL DIET AND WHEEZE IN CHILDREN (cont)

	Cereals (incl bread)	6.2%	21.4%	72.4%	5.6%	18.3%	76.1%	0.31
	Pasta	9.1%	65.8%	25.1%	6.3%	70.1%	23.5%	0.10
	rice	9.5%	68.8%	21.8%	7.2%	74.9%	17.9%	**0.04**
	butter	64.9%	25.0%	10.1%	69.1%	22.2%	8.7%	0.27
	margarine	71.4%	20.0%	8.5%	70.2%	21.9%	7.9%	0.70
	Nuts, peanuts	60.3%	30.0%	9.7%	57.2%	33.2%	9.6%	0.47
	potatoes	11.7%	50.0%	38.3%	12.6%	50.4%	37%	0.83
	snacks	56.0%	31.7%	12.3%	57.3%	30.5%	12.2%	0.88
	milk	6.4%	8.9%	84.6%	4.1%	7.7%	88.2%	0.09
	yoghurt	10.4%	26.5%	63.2%	11.0%	22.7%	66.4%	0.28
	eggs	7.8%	68.1%	24.1%	7.3%	69.4%	23.3%	0.87
	'industrial' pastry	37.3%	36.4%	26.3%	40.7%	37.5%	21.8%	0.15
	alcohol	95.3%	4.3%	0.4%	97.3%	1.7%	1.0%	**0.006**
	Soft drinks	46.3%	34.0%	19.7%	48.5%	35.0%	16.5%	0.32
At 1-2 years								
Miyake 2009 (follow-up of Saito 2009) (6) n=763 Diet during pregnancy Wheeze in infants aged 16-24 months (ISAAC definitions) Moderate risk of bias	Meat Quartiles of maternal meat consumption during pregnancy (medians in g/day adjusted energy intake	**Infant wheeze at 16-24 months (n=763)** cases Q1 (33.8 g/day) 54/763 (7.1%) 1.00 Q2 (49.0 g/day) 37/763 (4.8%) 0.67 (0.40 to 1.11) Q3 (63.6 g/day) 35/763 (4.6%) **0.57 (0.33 to 0.95)** Q4 (90.8 g/day) 43/763 (5.6%) 0.77 (0.47 to 1.27)						P trend 0.22
Miyake et al 2010a (8) N=763 OMCHS, Japan Diet during pregnancy Wheeze in infants (ISAAC definitions) Moderate risk of bias	Total dairy products Total dairy product intake (sum of milk, yoghurt & cheese), (medians in g/day, adjusted for energy intake)	Cases of wheeze Q1 (43.6 g/day) 50/763 (6.6%) 1 Q2 (120.8 g/day) 50/763 (6.6%) 1.04 (0.64-1.70) Q3 (184.5 g/day) 44/763 (5.8%) 0.85 (0.52-1.40) Q4 (280.7 g/day) 25/763 (3.3%) **0.45 (0.25-0.79)**						**0.007**
	Milk	Q1 (16.1 g/day) 46/763 (6.0%) 1 Q2 (77.9 g/day) 51/763 (6.7%) 1.28 (0.79-2.10) Q3 (144.7 g/day) 47/763 (6.2%) 1.10 (0.67-1.82) Q4 (194.0 g/day) 25/763 (3.3%) **0.50 (0.28-0.87)**						**0.02**
	Yoghurt	Q1 (4.4 g/day) 39/763 (5.1%) 1 Q2 (16.9 g/day) 39/763 (5.1%) 1.01 (0.60-1.70) Q3 (40.3 g/day) 51/763 (6.7%) **1.76 (1.05-2.98)** Q4 (94.6 g/day) 40 /763 (5.2%) 1.26 (0.74-2.16)						0.15
	Cheese	Q1 (0.0 g/day) 58/763 (7.6%) 1 Q2 (2.2 g/day) 33/763 (4.3%) **0.47 (0.28-0.78)** Q3 (4.5 g/d)ay 43/763 (5.6%) **0.61 (0.37-1.00)** Q4 (12.5 g/day) 35/763 (4.6%) **0.51 (0.31-0.85)**						**0.02**
	Adjusted for maternal age, gestation at baseline, residential municipality at baseline, family income, maternal and paternal education, maternal and paternal history of asthma, atopic eczema and allergic rhinitis, changes in maternal diet in the previous 1 month, season when data at baseline were collected, maternal smoking during pregnancy, older siblings, sex, birthweight, household smoking in same room, breastfeeding duration and age at third survey								
Miyake et al 2011b (10) N=763 OMCHS, Japan Diet during pregnancy Wheeze in infants (ISAAC definitions) *Moderate risk of bias*	Maternal Dietary Patterns Healthy Pattern (high intake of green and yellow vegetables, seaweed, mushrooms, white vegetables, pulses, potatoes, fish, sea products, fruit and shellfish; low intake of confectionery and soft drinks)	Q1 48/190 (25%) 1.00 Q2 42/191 (22%) 0.80 (0.49-1.32) Q3 39/191 (20%) 0.72 (0.42-1.21) Q4 40/191 (21%) 0.68 (0.40-1.15)						0.14
	Western (high intake of vegetable oil, salt-containing seasonings, beef and pork, processed meat, eggs, chickens and white vegetables and low	Q1 51/190 (27%) 1.00 Q2 45/191 (24%) 0.72 (0.44-1.17) Q3 33/191 (17%) **0.52 (0.31-0.87)** Q4 40/191 (21%) **0.59 (0.35-0.98)**						**0.02**

TABLE E 3 MATERNAL DIET AND WHEEZE IN CHILDREN (cont)

	intake of fruit, soft drinks and confectionery)		
	Japanese (high intake of rice, miso soup, sea products, and fish)	Q1 41/190 (22%) 1.00 Q2 36/191 (19%) 0.77 (0.45-1.30) Q3 41/191 (22%) 0.94 (0.56-1.59) Q4 51/191 (27%) 1.41 (0.86-2.35)	0.12
	Further control for maternal α-linolenic acid and vitamin E intake during pregnancy removed the inverse relationship between the maternal western pattern and childhood wheeze. (some numeric results were reported)		
	Adjusted for maternal age, gestation at baseline, residential municipality, family income, maternal and paternal education, maternal and paternal history of asthma, atopic eczema, allergic rhinitis, changes in maternal diet in the previous 1 month, season when data at baseline were collected, maternal smoking during pregnancy, baby's older siblings, baby's sex, baby's birthweight, household smoking in the same room as the infant, breastfeeding duration, and time of delivery before the third survey		
At 3 years			
Lange 2010 (12) n=1376 Diet during pregnancy PROJECT VIVA, USA Recurrent wheeze at 3 years (compared with no wheeze at all in the first 3 years of life); *Low risk of bias*	Mediterranean diet scores	**<u>Recurrent wheeze</u>:** aOR 0.98 (0.89 to 1.08) per 1 point increase in score Mediterranean diet score (high v low): OR 0.64 (0.43 to 0.95) (one point if above median consumption for dairy, fish, fruit, legumes, nuts, unsaturated-to-saturated fat ratio, vegetables and whole grains, intake of red and processed meats below the median median value – low (0-3) v middle (4-5) v high (6-9) score.)	
	Alternate Healthy Eating Index modified for pregnancy (AHEI-P)	aOR 1.07 (0.87 to 1.30) per 10 points AHEI-P (highest v lowest quartile): OR 0.87 (0.55 to 1.37) (10 possible points for each of vegetables, fruit, ratio of white to red meat, fibre, trans fat; ratio of polyunsaturated to saturated fatty acids, folate, calcium and iron from foods – quartiles compared.)	
	Principal components analysis Prudent diet: Western diet:	Prudent: aOR 1.02 (0.83 to 1.26) (fruits, tomatoes, cabbages, leafy green vegetables, poultry, fish) Western: aOR 0.98 (0.81 to 1.19) (red meat, processed meat, refined grains, snacks, sweets, desserts, French fries and pizza.)	
	Adjusted for child's sex, maternal race, maternal education level, household income, maternal and paternal history of eczema, presence of children < 12 years of age at home, maternal prepregnancy BMI, breastfeeding duration, and passive smoke exposure		
Shaheen 2009 (11) **n=**14,062, 8886 with wheeze. ALSPAC, UK Diet during pregnancy Moderate risk of bias	Dietary Patterns: Health conscious	**<u>Wheezing at 3.5 years (n = 8886)</u>** 0.96 (0.88 to 1.05)	0.37
	Traditional	1.00 (0.93 to 1.07)	0.90
	Processed	1.02 (0.94 to 1.10)	0.69
	Confectionery	0.98 (0.91 to 1.06)	0.61
	Vegetarian	0.97 (0.91 to 1.04)	0.42
	** Adjusted for Maternal factors during pregnancy (energy intake, smoking, infections, antibiotics and paracetamol); other maternal factors (educational level, housing tenure, financial difficulties, pre-pregnancy BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine); sex of child, gestational age, breast fed in first 6 months, day care at 8 months, multiple pregnancy, pets in infancy, damp/condensation/mould, child exposed to environmental tobacco smoke at weekends, season of birth, season of FFQ completion, birth weight, head circumference, birth length. Also number of younger siblings and child's BMI at 7 years for later childhood outcomes.*		
Miyake et al 2011a (13) n=582 OMCHS, Japan Diet during pregnancy Wheeze (ISAAC) 29-39 months Moderate risk of bias	Fish intake (Maternal hair mercury levels at 29-39 months postpartum were used as a surrogate for antenatal mercury exposure (fish))	**Risk (%)** Maternal hair mercury (μg/g) ≤ 1.09 33/145 (22.8) 1.00 1.10-1.51 22/139 (15.8 0.77 (0.41-1.44) 1.52-2.10 28/150 (18.7) 0.91 (0.50-1.64) ≥ 2.11 25/148 (16.9) 0.84 (0.45-1.55)	p-trend 0.68
	Adjusted for maternal age, residential municipality at baseline, maternal and paternal education, maternal and paternal history of allergic disorders, maternal energy-adjusted fish intake during pregnancy, maternal smoking during pregnancy, number of child's older siblings, child's sex, household smoking in the same room as the child, breastfeeding and children's		

TABLE E 3 MATERNAL DIET AND WHEEZE IN CHILDREN (cont)

	fish intake		
At 5 years			
Devereux 2007 (28) n= 1924 Scotland Diet during pregnancy Moderate risk of bias	**TOTAL Maternal vitamin D intake (energy-adjusted)** **1 n=213** / **2 n=246** / **3 n=237** / **4 n=261** / **5 n=255** Median energy-adjusted Intake (IU/d)* 77 / 104 / 128 / 157 / 275 5-95th percentile 46-92 / 94-115 / 117-139 / 142-182 / 189-751 Ever wheeze (n = 20.1%) aOR (model 1) 1 / 0.84 (0.51-1.37) / 0.77 (0.46-1.27) / 0.65 (0.39-1.11) / 0.56 (0.31-1.01) aOR (model 2) 1 / 0.86 (0.51-1.47) / 0.70 (0.41-1.20) / 0.57 (0.32-1.01) / **0.48 (0.25-0.91)** Wheeze in previous year (n = 12.8%) aOR (model 1) 1 / 1.09 (0.61-1.95) / 0.98 (0.54-1.80) / 0.80 (0.42-1.52) / **0.45 (0.21-0.97)** aOR (model 2) 1 / 1.27 0.68-2.37) / 0.95 (0.49-1.82) / 0.70 (0.35-1.41) / **0.35 (0.15-0.83)** Persistent wheeze at 2 and 5 y aOR (model 1) 1 / 0.91 (0.44-1.88) / 0.75 (0.35-1.61) / 0.51 (0.22-1.20) / 0.43 (0.16-1.12) aOR (model 2) 1 / 1.10 (0.51-2.35) / 0.63 (0.27-1.45) / 0.43 (0.17-1.11) / **0.33 (0.11-0.98)** Associations for maternal **dietary** intake were "similar" – no further results reported	**p-trend** **0.04** **0.01** **0.04** **0.009** **0.04** **0.01**
	Model 1: Adjusted for maternal atopy, maternal age, maternal smoking, maternal age at termination of fulltime education, paternal social class, deprivation index based on area of residence, breastfeeding, infant sex, infant antibiotic use in first year, birth weight, birth order, season of last menstrual period, maternal intakes of vitamin E, zinc and calcium *Model 2: Additionally adjusted for energy adjusted children's Vit D intake at 5 years.*		
Willers 2007 (14) N=1212 Scotland Diet during pregnancy Low risk of bias	Fat from dairy products	no consistent linear associations with respiratory and atopic outcomes in 5 year old children (exact numbers not reported in the paper)	
At 6 years			
Chatzi 2008 (29) N=482 Menorca Atopic and Non atopic mothers Pregnancy Low risk of bias	Cereal ≤ 11.5 v > 11.5 serves of cereal per week	***Persistent wheeze at 6.5 years **** Low 21 (15.22%) v high 16 (11.27%) ***Atopic wheeze at 6.5 years***** Low 14 (8.14%) v high 6 (3.51%)	pns pns
	**adjusted for firstborn and lower respiratory tract infections at age 1* ***adjusted for birth weight and maternal atopy*		
De Batlle 2008 (30) n=1476 Diet during pregnancy MEXICO Retrospective study ISAAC questionnaire Moderate –high risk of bias	High versus lower Mediterranean diet scores (1st tertile v 2nd and 3rd tertiles)	Age not stated but presumed to be 6-7 years *Wheezing (ever in child):* [n assumed to be 1326] aOR 0.74 (0.55 to 1.01) *Wheezing (currently in child):* [n assumed to be 1326] aOR 1.02 (0.65 to 1.60)	
At 7.5 to 8 years			
Willers 2008 (26) N= 2 832 Diet during pregnancy Netherlands Prospective cohort (longitudinal) Part of the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study Low risk of bias	Vegetables	**n** / **Wheeze from 1-8 years** Daily 2830 1 Regular + Rare 0.97 (0.83 to 1.12)	
	Fruit	Daily 2828 1 Regular + Rarely 0.89 (0.75 to 1.04)	
	Fish	Rarely 2811 1 Daily + Regularly 1.10 (0.94 to 1.29)	
	Egg	Rarely 2818 1 Daily + Regularly 0.96 (0.84 to 1.12)	
	Dairy	Daily 2788 1 Regular + Rare 0.88 (0.71 to 1.09)	
	Nuts	Rare 3909 1 Daily + Regular 0.99 (0.86 to 1.15)	
	Nut Products	Regular v Rare 1.01 (0.88 to1.18) Daily v Rare **1.42 (1.06 to 1.89)**	
	** The child's dietary data on fruit, vegetables, fish, eggs, full cream milk, butter and peanut butter consumption at 2 years of age were used to check for potential confounding by the child's diet.*		

TABLE E 3 MATERNAL DIET AND WHEEZE IN CHILDREN (cont)

	Results were adjusted for by sex, maternal education, parental allergy, maternal smoking during pregnancy, smoking in the home at 8 years of age, breastfeeding, presence of older siblings, birthweight, maternal overweight 1 year after pregnancy, maternal supplement use during pregnancy, region and study arm (intervention or natural history arm).		
Shaheen 2009 (11) **n=**14,062, 7707 with wheeze. ALSPAC, UK Diet during pregnancy Moderate risk of bias	Dietary Patterns: Health conscious	**<u>Wheezing at 7.5 years (n = 7707)</u>** 1.00 (0.91 to 1.11)	0.94
	Traditional	1.00 (0.92 to 1.08)	0.99
	Processed	0.92 (0.84 to 1.01)	0.098
	Confectionery	1.02 (0.93 to 1.12)	0.70
	Vegetarian	1.02 (0.95 to 1.10)	0.62
	** Adjusted for Maternal factors during pregnancy (energy intake, smoking, infections, antibiotics and paracetamol); other maternal factors (educational level, housing tenure, financial difficulties, pre-pregnancy BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine); sex of child, gestational age, breast fed in first 6 months, day care at 8 months, multiple pregnancy, pets in infancy, damp/condensation/mould, child exposed to environmental tobacco smoke at weekends, season of birth, season of FFQ completion, birth weight, head circumference, birth length. Also number of younger siblings and child's BMI at 7 years for later childhood outcomes.*		

TABLE E 4 MATERNAL DIET AND HAY FEVER OR RHINITIS IN CHILDREN

Study Details	Maternal dietary pattern / component	Effect Size (aOR and 95% CI unless otherwise stated)	P value
Trials - Avoidance Studies			
First year of life / at 1 year			
Zeiger 1989 (31); Zeiger 1995 (32) N=288 USA RCT Pregnancy Lactation Infant: when breastfeeding was supplemented or stopped Parental atopy Moderate risk of bias	Avoidance of Cow's milk Egg Peanut Concentrated soy Limited wheat Casein hydrolysate Delayed and staged introduction to solid foods Versus Standard diet in pregnancy Solid foods from 4-6 months of age Delayed milk introduction.	**Allergic rhinitis:** no significant differences between dietary avoidance and control groups at 4, 12 and 24 months of age **4 year follow-up:** Cumulative reduction in food allergy in infancy by maternal/infant food allergen avoidance at 4 years, but the current prevalence at 4 years was similar (about 5% in each group) and failed to affect respiratory allergy development from birth to 4 years **7 year follow-up:** No significant differences between groups for food allergy, atopic dermatitis, allergic rhinitis, asthma, any atopic disease, lung function or aeroallergen sensitization.	
At 5 years			
Falth-Magnusson 1987 (33) 1987a (34), 1987b(35), 1992 (36) N= 212 Sweden RCT Pregnancy (28 weeks to birth) Moderate risk of bias	Dairy foods (cow's milk) and egg Cow's milk and egg elimination from 28 weeks gestation to birth (and partially during early lactation) (n = 104 randomized) versus usual diet (typically 0.5 L milk/day and 3-5 eggs/week) (n = 108 randomized); (elimination group also had extra calcium and casein hydrolysate)	**<u>Allergic rhinoconjunctivitis</u>** (at five years); diet group 13 non-diet group 14	**pns**

TABLE E 4 MATERNAL DIET AND HAY FEVER OR RHINITIS IN CHILDREN (cont)

Study Details	Maternal dietary pattern / component	Effect Size (aOR and 95% CI unless otherwise stated)	P value
Prospective Cohorts. Dietary associations – multiple food groups or multiple individual foods			
At 5 years			
Willers 2007 (14) N=1212 Scotland Pregnancy – last trimester Low risk of bias	Cereals (wholegrain products)	5 year old children Whole grain products – no consistent linear associations with respiratory and atopic outcomes (exact numbers not reported in the paper).	
Adjusted for maternal age, paternal social class, maternal education, maternal smoking during pregnancy, smoking in the child's home at 5 years, energy intake, maternal asthma, maternal atopy, child's birth weight, child's sex, presence of older siblings, and breastfeeding			
Erkkola 2009 (15) N= 1669 Finland DIPP Study Pregnancy – 8th month Low risk of bias	Vitamin D from food	**Allergic rhinitis HR (95% CI)** Absolute intake (log-transformed) **0.74 (0.57-0.96)*** Energy-adjusted **0.85 (0.75-0.96)#** Energy-adjusted; Model 1 **0.85 (0.75-0.97)*** Model 2 **0.84 (0.73-0.96)*** Model 3 **0.79 (0.66-0.94)*** Model 1 quartiles 1st q (< 0.31µg/MJ) **1.53 (1.14-2.06)#** 2nd & 3rd q (0.31-0.54 µg/MJ) 1 4th q (> 0.54 µg/MJ 0.98 (0.69-1.37)	 p < 0.05 p < 0.01 p < 0.05 p < 0.05 p < 0.05 p < 0.01
	(no use versus use of vitamin D supplements did not show significant differences for asthma, allergic rhinitis, or atopic eczema – adjusted for energy-adjusted maternal intake of vitamin D from food)		
At 6 -7 years of age			
De Batlle 2008 (30) N assumed to be 1326 Mexico Retrospective cohort study Pregnancy Moderate-high risk of bias	Mediterranean diet scores (high score = high vegetables, legumes, fruits and nuts, cereals and fish; and low dairy products, meat, junk food and fat); (1st tertile v 2nd and 3rd tertiles)	Age not stated but presumed to be 6-7 years [n assumed to be 1326] Rhinitis (ever in child): 0.64 (0.36 to 1.15) Rhinitis (currently in child): 0.87 (0.65 to 1.18) Sneezing (currently in child): **0.71 (0.53 to 0.97)** Itchy-watery eyes (currently in child): 0.96 (0.64 to 1.45)	
adjusted for gender of child, physical exercise of child, current tobacco smoking at home, maternal education, maternal asthma, maternal rhinitis			
Shaheen 2009 (11) UK N=14 062 Pregnancy Moderate risk of bias	Health conscious' salad, fruit, fruit juices, rice, pasta, oat/bran based breakfast cereals, fish, pulses, cheese, non-white bread;	**Hay fever at 7.5 years** (673/7674) 1.00 (0.91 to 1.11) per standard deviation of dietary pattern score	
	'Traditional' vegetables, red meat, poultry;	1.04 (0.96 to 1.13) per standard deviation of dietary pattern score	
	'Processed' meat pies, sausages, burgers, fried foods, pizza, chips, crisps, white bread, eggs, baked beans;	0.93 (0.83 to 1.04) per standard deviation of dietary pattern score	
	'Confectionery' chocolate, sweets, biscuits, cakes, puddings.	1.01 (0.92 to 1.11) per standard deviation of dietary pattern score	
	'Vegetarian' meat substitutes, pulses, nuts, herbal tea;	0.97 (0.89 to 1.06) per standard deviation of dietary pattern score	
Adjusted for Maternal factors during pregnancy (energy intake, smoking, infections, antibiotics and paracetamol); other maternal factors (educational level, housing tenure, financial difficulties, pre-pregnancy BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine); sex of child, gestational age, breast fed in first 6 months, day care at 8 months, multiple pregnancy, pets in infancy, damp/condensation/mould, child exposed to environmental tobacco smoke at weekends, season of birth, season of FFQ completion, birth weight, head circumference, birth length. Also number of younger siblings and child's BMI at 7 years for later childhood outcomes.			

TABLE E 5 MATERNAL DIET AND SENSITIZATION IN CHILDREN

Study Details	Maternal dietary pattern / component	Effect size: aOR and 95% CI (except where stated otherwise)	P value
Randomized Controlled Trials - Avoidance of allergens			
Appelt 2004 (37) N=497 High risk families* Trimester 3 & BF Infants given partially hydrolyzed formula if not BF * one first degree relative with asthma or two with IgE mediated allergy Risk of bias unclear: no reporting about randomization, blinding or losses to follow up	(Avoidance of..) Peanut Nut Fish (partial avoidance of..) Cow's milk & egg	Sensitization at 1 year: Intervention Control Milk 16/251 (4%) 11/246 (4%) Egg 48/251 (19%) 36/246 (14%) peanut 23/251 (9%) 16/246 (7%) Sensitization at 2 years Milk 9/242 (4%) 2/231 (1%) **Egg 28/242 (12%) 1/231 (1%)** Peanut 21/242 (9%) 20/231 (9%) Sensitization at 7 years Milk 2/185 (1%) 2/169 (1%) Egg 1/185 (1%) 6/169 (4%) peanut 21/185 (11%) 12/169 (7%)	 **P=0.037** P=0.06 P=0.06
Arshad (1992) (38) **Hide (1994)** [2yo f/u] **(39)** **Hide (1996)** [4 yr f/u] **(40)** **Arshad (2007)** [8yo f/u] **(41)** Isle of Wight study N=58 (prophylaxis) N=62 (control) High risk families Lactation *family history of atopy (allergic disease in both parents, one parent and one sibling, or two siblings) and high (> 0.5 kU/L) concentrations of IgE Moderate risk of bias	Cow's milk Egg Nuts Fish Delayed introduction to infants of cow's milk, soy, egg, wheat, nuts & fish. Dust mite control	**Food Allergy**: At 12 months: prophylaxis control: OR (95% CI) N=58 N=62 2 (3%) 7(11%) 3.29 (0.6 to 17.3) 2 years: 7 (12.1%) 11 (17.7%) 4 years: 3 (5%) 7 (11%) (not influenced by any of the risk factors) **Any Food allergy diagnosis during the first 8 years of life:** **11 (19.0%) 26 (41.9%) 0.32 0.14 to 0.74** **Any IgE-mediated Food allergy diagnosis during first 8 yrs:** **5/55 (9.1%) 16 (25.8%) 0.29 0.10 to 0.85** Up to 8 years Food allergy – repeated measures analysis: aOR 0.75 95% CI 0.27 to 2.10; Food allergy (IgE-mediated) – repeated measures analysis: aOR 0.41 95% CI 0.11-1.53; **Allergic sensitization** (A positive skin prick test to HDM, mould, grass pollen, cat, dog, cow's milk, egg, wheat or fish)) prophylaxis control: OR (95% CI) N=58 N=62 **2 years: 3 (5.2%) 16 (25.8%)** **Single allergen 3.45 16.13** > 2 allergens 1.72% 9.68%; Significant differences between groups for **HDM & cat dander**, but not dog dander, moulds; cow's milk, egg or wheat) 4 years: 8(13.8%) 21(33.9%); **3.7 (1.3 to 10.00)** **(3.0 (1.2 to 7.9)*** ***when adjusted for male sex**; **NS when adjusted for maternal allergy)** Significant differences between groups for HDM and moulds, but not grass pollen, cat, dog, milk, egg, wheat or fish. Sensitization to HDM increased with age in both groups, but the gap widened between the groups as the children reached 8 years of age. Food sensitization increased in early childhood and then decreased by 8 years of age, with the difference between the groups persisting so that no child in the prophylactic group was sensitized to food at 8 years compared with 5 in the control group; Overall 37 (59.7%) children were sensitized to 1 or more	 pns **p = 0.005** **p = 0.02** p = 0.59^ p = 0.18^ **p < 0.005** **p < 0.05** P < 0.1 **p < 0.02**

TABLE E 5 MATERNAL DIET AND SENSITIZATION IN CHILDREN (cont)

<table>
<tr>
<td></td>
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<td>allergens at any time in the control group and 14 (25.2%) in the prophylactic group (OR 0.23 95% CI 0.11 to 0.51; p < 0.001. Repeated measurement analysis confirmed significant differences: foods aOR 0.15 95% CI 0.03 to 0.80; HDM 0.07 (0.02 to 0.23) and any sensitization 0.13 (0.05 to 0.32).

Any sensitization during the first 8 years of life:
37 (59.7%) 14 (25.2%) 0.23 (0.11 to 0.51)</td>
<td>p < 0.001.</td>
</tr>
<tr>
<td>Lilja 1988 (42)
N=165
Sweden

RCT

Maternal atopy

Pregnancy 3rd trimester

Moderate risk of bias</td>
<td>Cow’s Milk
Egg

Four diet types*
‘free’; no milk or eggs during the last three months of pregnancy (n = 37)
‘reduced’; no apparent intake, but diet not completely free of milk and eggs (n = 32)
‘normal’; about 0.5 L cows’ milk daily and three hens’ eggs weekly (n = 39)
‘high’; about one L milk daily and one egg daily (n = 57)</td>
<td>Cord blood specific IgE (ovomucoid & betalactoglobulin)
Cord blood specific IgG(ovalbumin, ovomucoid & betalactoglobulin)

No significant differences between the four different maternal diet groups at birth and cord blood IgE antibodies or IgG antibodies to ovomucoid, ovalbumin and betalactoglobulin.

No cord blood sIgE (ovomucoid & betalactoglobulin) was detected</td>
<td></td>
</tr>
<tr>
<td>Lilja 1989 (43) , 1991 (44)
N=162
Sweden

RCT

High risk families

Pregnancy 3rd trimester
Lactation

Moderate risk of bias</td>
<td>Cow’s Milk
Egg

Two diet types in pregnancy*
‘reduced; no apparent intake, but diet not completely free of milk and eggs (n = 79)
‘daily’; about one L milk daily and one egg daily (n = 83)

After delivery ‘Reduced’ group was divided into two for lactation:
Reduced ‘A group” continued with intervention (n=25)
Reduced ‘B group’ normal diet (n=54)

Breast fed or soy formula until 6 months. Solids not until 3-4 months, No citrus, other fruits, meat, cereals and tomatoes b4 6 months. No egg or fish until 9-12 months</td>
<td>At 6, 12 and 18 months.

SPT results (Ovalbumin, Ovomucoid, Betalactoglobulin and Cow’s milk)
- No difference between reduced ‘A and B’ groups.

Serum specific IgE levels Ovomucoid, Betalactoglobulin (IgE-Ab (≥ PRU/ml)):

<table>
<tr><td></td><td colspan="2">BF > 6 months</td><td colspan="2">BF 2- 6 months + soy formula</td><td colspan="2">BF 2- 6 months + soy formula/ cow’s milk</td><td colspan="2">BF <2months + soy formula</td></tr>
<tr><td></td><td>R N=43</td><td>H N=45</td><td>R N=16</td><td>H N=17</td><td>R N=8</td><td>H N=5</td><td>R N=14</td><td>H N=15</td></tr>
<tr><td>6 months</td><td>3/38</td><td>4/45</td><td>1/14</td><td>2/17</td><td>0/7</td><td>0/5</td><td>0/13</td><td>1/13</td></tr>
<tr><td>18 months</td><td>9/42*</td><td>4/42</td><td>1/15</td><td>2/17</td><td>0/8</td><td>0/5</td><td>0/14</td><td>2/15</td></tr>
</table>
R= Reduced group H = High group BF = Breast fed *P<0.05 compared to infants BF for <6 months in reduced group.</td>
<td></td>
</tr>
<tr>
<td>Zeiger 1989 (31), 1995 (32)
N=288
USA

RCT
High risk families

Pregnancy 3rd trimester
Lactation</td>
<td>Cow’s Milk
Egg
Peanut
Limited soy
Limited wheat

Infant - casein hydrolyzate until 12 months.</td>
<td>Food allergy (includes atopic dermatitis, urticaria / angioedema, and/or GI disease with specific food IgE)
<table>
<tr><td></td><td>N</td><td>dietary avoidance</td><td>N</td><td>control</td></tr>
<tr><td>Food allergy (12 mo):</td><td>99</td><td></td><td>177</td><td></td></tr>
<tr><td>- Definite</td><td></td><td>2.0%</td><td></td><td>7.9%</td></tr>
<tr><td>- Probable</td><td></td><td>3.0%</td><td></td><td>8.5%</td></tr>
<tr><td>- TOTAL</td><td></td><td>5.0%</td><td></td><td>16.4%</td></tr>
</table>

<table>
<tr><td></td><td>N</td><td>dietary avoidance</td><td>N</td><td>control</td></tr>
</table></td>
<td>p-value

0.059
0.021
0.007</td>
</tr>
</table>

TABLE E 5 MATERNAL DIET AND SENSITIZATION IN CHILDREN (cont)

Moderate risk of bias	Solid foods at 4 months of age. Cow's milk, wheat and soy delayed to 12 months Egg delayed to 24 months Fish delayed to 36 months	**Food allergy (24 mo)**: 97 169 definite 4.1% 8.9% 0.216 **probable 3.1% 11.2% 0.021** **TOTAL 7.2% 20.1% 0.005** Foods associated with positive challenges in the control group included egg (n = 8), milk (n = 4), peanut (n = 1) and soy (n = 1). In the dietary avoidance group, peanut (n = 2) and egg (n = 1) caused positive food challenges. **4 year follow up:** Cumulative reduction in food allergy in infancy by maternal / infant food allergen avoidance at 4 years, but the current prevalence at 4 years was similar (about 5% in each group and failed to affect respiratory allergy development from birth to 4 years. **7 year follow up:** No significant differences between groups for food allergy, atopic dermatitis, allergic rhinitis, asthma, any atopic disease, lung function or aeroallergen sensitizations.	
Falth-Magnusson 1987a(33), 1987b(34), 1992 (36) N=212 women from families with at least one allergic family member Pregnancy RCT Moderate risk of bias	Cow's milk and egg elimination from 28 weeks gestation to birth (and partially during early lactation) (n = 104 randomized) v usual diet (typically 0.5 L milk/day and 3-5 eggs/week) n = 108 randomized; (elimination group also had extra calcium and casein hydrolyzate)	Children at five years of age **Cord blood IgE** No sig. differences between groups (but babies of atopic mothers had higher IgG levels than babies of non-atopic mothers regardless of diet) **Positive skin prick tests for egg and milk in infants at 6 and 18 months**: **Intolerance to any food item** (at five years): 16/84 in the diet group and 20/114 non-diet **Allergic disease** (at five years) – probable or definite; 35 in the diet group and 37 in the non-diet group	pns pns pns

Other Trials - Avoidance of allergens			
Hattevig 1989 (45) (and Hattevig 1999 – 10 year follow-up) (46) N= 115 Sweden Non randomized concurrent comparison Lactation Solid foods 6 months Breast fed or casein hydrolyzate formula Moderate risk of bias	Cow's milk Eggs Fish Diet group (n = 65 infants): Non diet group (n = 50):	**Adverse reactions to:** **Age (months)** 0-3 0-6 0-9 0-12 0-18 **Eggs** Diet 0 1.5% 1.5% 1.5% 7.7% No diet 2.0% 2.0% 2.0% 2.0% 6.0% **Cows' milk** Diet 0 3.1% 10.8% 10.8% 10.8% No diet 4.0% 6.0% 14.0% 14.0% 14.0% **Fish** Diet 0 3.1% 3.1% 3.1% 3.1% No Diet 0 0 0 3.1% 3.1% **Adverse reactions to food (eggs, cow's milk or fish) in children at 10 years of age:** 7/50 in diet group versus 8/65 in non-diet group,	pns
Herrmann et al 1996 (47) N=150 Germany Patient preference trial High risk families* Pregnancy 3rd trimester +/- Lactation Staged introduction to solid foods from 18 weeks	Cow's Milk* Egg	**IgE** **A** **B** **C** **HF **** Cow's milk sensitization At 6 months 0/27 0/28 1/36 1/39 At 12 months 1/24 0/21 1/30 0/24 Egg sensitization At 6 months 1/27 1/29 3/36 3/29 At 12 months 3/24 2/21 3/30 2/24	
	*Goat milk & sheep milk allowed as cow's milk substitutes. Looked for sensitization to these proteins, but specific IgE Negative. **Group A (n=30) Cow's milk and egg free diet during 3rd trimester and while exclusively breast feeding		

TABLE E 5 MATERNAL DIET AND SENSITIZATION IN CHILDREN (cont)

High risk of bias	Group B (n=33) Cow's milk and egg free diet while exclusively breast feeding Group C (n=41) 'Prudent' diet. At least 1000ml cow's milk and 1 egg daily during pregnancy and lactation HF (n=34) Infants who were not breast fed received partially hydrolyzed (n=19) or extensively hydrolyzed (n=15) formula.

TABLE E 5 MATERNAL DIET AND SENSITIZATION IN CHILDREN (cont)

Study Details	Maternal dietary pattern / component	Effect size: aOR and 95% CI (except where stated otherwise)	P-value
Dietary associations – multiple food groups or multiple individual foods **Prospective Cohorts**			
At 5 years			
Nwaru 2011 (48) n=652 Finland DIPP Nutrition Study Atopic and Non atopic mothers Lactation Low risk of bias	Chocolate & sweets Intake by grams	***Atopy*** (Measured as serum IgE sensitization to birch, cat, timothy grass, cow's milk, egg, wheat) **Milk allergen** 1.11 (0.82 to 1.50) **Egg allergen** 1.22 (0.85 to 1.76) **Wheat allergen** 1.51 (0.98 to 2.32) **Birch allergen** 1.03 (0.75 to 1.41) **Cat allergen** 0.82 (0.51 to 1.31) **Timothy grass allergen** 0.73 (0.46 to 1.17)	
	Adjusted for gender, place of birth (southern or northern Finland), duration of gestation, maternal smoking during pregnancy, mode of delivery, parental asthma, parental allergic rhinitis, atopic eczema at 6 months of age, and exclusive breast feeding.		
At 6.5 years			
Chatzi 2008 (49) n=482 Menorca Atopic and Non atopic mothers Pregnancy *Low risk of bias*	Mediterranean diet score (0 = minimal adherence to 7 = maximal adherence): high Mediterranean diet quality (4-7) v low Mediterranean diet quality (≤ 3) [dairy assumed to be protective for pregnant women; alcohol consumption not included in the score]	***Atopy (*** sensitization to at least one aeroallergen on SPT) Results from 468/482 children Low vs High diet score **0.55 (0.32 to 0.97)** Low vs High diet score additionally adjusted for child's adherence to a Mediterranean diet **0.55 (0.31 to 0.97)**	
	Adjusted for gender, maternal and paternal asthma, maternal social class and education, BMI at age 6.5 years, total energy intake at 6.5 years, birthweight and maternal atopy		
At 7 years			
Shaheen 2009 (50) N=14062 UK ALSPAC Pregnancy Moderate risk of bias		**Log total serum IgE (n = 4819/14062)**	p value
	Health conscious: salad, fruit, fruit juices, rice, pasta, oat/bran based breakfast cereals, fish pulses, cheese, non-white bread	**1.07 (1.00 to 1.14)** GM ratio per SD total diet score	0.041
	Traditional: vegetables, red meat, poultry	**0.96 (0.91 to 1.00)** GM ratio per SD total diet score	0.075
	Processed: meat pies, sausages, burgers, fried foods, pizza, chips, crisps, white bread, eggs, baked beans	0.97 (0.91 to 1.04) GM ratio per SD total diet score	0.39
	Confectionery: chocolate, sweets, biscuits, cakes, pudding	1.00 (0.94 to 1.06) GM ratio per SD total diet score	0.98
	Vegetarian: meat substitutes, pulses, nuts, herbal tea	**1.07 (1.02 to 1.12)** GM ratio per SD total diet score	0.051*
		Atopy (n=6085/14062) (positive SPT to dermatophagiodes pteronyssinius, cat or grass.	
	Health conscious	0.95 (0.88 to 1.04)	0.26
	Traditional	0.98 (0.91 to 1.05)	0.54
	Processed	0.93 (0.85 to 1.01)	0.08
	Confectionery	1.07 (0.99 to 1.15)	0.09
	Vegetarian	1.02 (0.96 to 1.09)	0.44
	Vegetarian	1.02 (0.96 to 1.09)	0.44
	Adjusted for energy intake, maximum smoked, infections, antibiotics and paracetamol use during pregnancy; maternal educational level, housing tenure, financial difficulties, pre-pregnancy body mass index, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine, sex of child, gestational age, breastfed in first 6 months, day care at 8 months, multiple pregnancy, pets in infancy, damp/condensation/mould, child exposed to environmental tobacco smoke at weekends, season of birth, season of food frequency questionnaire completion, birthweight, head circumference, birth length, number of younger siblings and child's BMI at age 7. ** Reported as 0.0051 in paper*		

TABLE E 5 MATERNAL DIET AND SENSITIZATION IN CHILDREN (cont)

Dietary associations – single foods			
At 1 year			
Dietary associations – cow's milk			
Erkkola 2005 (23) N=112 Pregnancy and lactation Low risk of bias	Milk and dairy products Low intake group as reference group compared with high intake group: Milk high intake = "more than sometimes" Yoghurt high intake = "more than sometimes" Cheese high intake = ≥ 4 times/week Cream high intake = 3-4 times/week	Recorded IgA & IgG levels. *No record of IgE levels to these allergens, and no clinical data re allergic status of children.	
Venter 2009 (51) N=969 Portsmouth Pregnancy and lactation Moderate to high risk of bias	Cow's milk No (< 1% of women) versus moderate (10%) versus frequent (89%) versus uncertain (1%) consumption of milk during pregnancy	*Maternal Intake* Milk Never Moderate Frequently Uncertain Total FHS Positive 1/2 (4) 0/97 (0) 18/831 (82) 3/7 (14) 22 Negative 1/2 (<1) 97/97(11) 813/831 (89) 3/7 (<1) 914 Values are expressed as (n%) FHS- Food Hypersensitivity	
Dietary associations – fruits and vegetables			
Hoppu 2005 (2) N=34 Finland Lactation Atopy in infants defined as occurrence of both atopic eczema in first year, and a positive SPT at the age of 12 months.	Fruit and vegetables (specifically recommended an abundant intake of fresh fruits, berries and vegetables during breastfeeding)	**OR 0.30 (0.09 to 0.94)** (with increased vitamin C in breast milk – attributed to maternal intake of fruits and vegetables.)	P=0.038
Dietary associations – cereal			
Venter 2009 (51) N=969 Portsmouth Pregnancy and lactation Moderate to high risk of bias	Cereal: wheat No (< 1% of women) versus moderate (8%) versus frequent (92%) versus uncertain (< 1%) consumption of wheat during pregnancy	*Maternal Intake* Wheat Never Moderate Frequently Uncertain Total FHS Positive 0/1 (0) 0/75 (0) 4/857 (100) 0/4 (0) 4 Negative 1/1 (<1) 75/75 (80) 853/857 (91) 4/4 (<1) 933 FHS- Food Hypersensitivity	
At 2 years			
Sausenthaler 2007 (5) N= 2641 LISA birth cohort, Germany Pregnancy Allergic sensitization defined as specific serum IgE ≥ 0.34kU/L to egg / cow's milk, wheat, peanut, soy, codfish, house dust, cat dander, mixed mould, seasonal pollen allergens.	**low vs high intake**	**Any Allergen sensitization N (%)** / **Food Allergens** / **Inhalant allergens**	
	Fish high intake = 1-2 times/week	177/1458 (12.1) / 133/1464 (9.1) / 70/1457 (4.8) 86/663 (12.8) / 66/664 (9.9) / 32/663 (4.8)	
		1.02 (0.72 to 1.43) / 1.01 (0.69 to 1.48) / 0.94 (0.56 to 1.57)	
	Butter	96/753 (12.7) / 73/756 (9.7) / 40/752 (5.3) 163/1350 (12.1) / 122/1354 (9.0) / 63/1350 (4.7)	
		0.97 (0.66 to 1.42) / 0.93(0.60 to 1.43) / 0.86 (0.48 to 1.53)	
	Margarine	171/1378 (12.4) / 131/1382 (9.5) / 66/1377 (4.8) 80/653 (12.3) / 62/694 (8.9) / 32/653 (4.8)	
		0.85 (0.56 to 1.27) / 0.80 (0.50 to 1.27) / 0.93 (0.50 to 1.73)	
	Vegetable oils	182/1465 (12.4) / 138/1470 (9.4) / 71/1578 (4.9) 80/653 (12.3) / 60/655 (9.2) / 32/653 (4.8)	
		0.88 (0.63 to 1.25) / 0.91 (0.61 to 1.34) / 0.89 (0.53 to 1.51)	
	Deep frying veg. fat	161/1353 (11.9) / 127/1357 (9.4) / 56/1352 (4.1) 98/739 (13.3) / 69/742 (9.3) / 45/739 (6.1)	
		1.25 (0.92 to 1.70) / 1.12 (0.79 to 1.58) / **1.61 (1.02 to 2.54)**	P<0.05
	Milk	92/689 (13.4) / 70/691 (10.1) / 36/688 (5.2) 171/1445 (11.8) / 129/1450 (8.9) / 67/1445 (4.6)	
		0.93 (0.67 to 1.28) / 0.95 (0.66 to 1.37) / 0.95 (0.58 to 1.57)	
	Yoghurt	132/981 (13.5) / 96/986 (9.7) / 55/981 (5.6) 132/1154 (11.4) / 104/1156 (9.0) / 48/1153 (4.2)	
		0.81 (0.59 to 1.10) / 0.89 (0.62 to 1.27) / 0.69 (0.43 to 1.12)	

TABLE E 5 MATERNAL DIET AND SENSITIZATION IN CHILDREN (cont)

	Cheese	166/1312 (12.7) 98/824 (11.9)	126/1315 (9.6) 74/828 (8.9)	66/1311 (5.0) 37/824 (4.5)	
		0.99 (0.72 to 1.36)	0.97 (0.68 to 1.39)	0.93 (0.57 to 1.53)	
	Eggs	71/563 (12.6) 192/1568 (12.2)	51/566 (9.0) 148/1572 (9.4)	30/563 (5.3) 73/1567 (4.7)	
		0.91 (0.56 to 1.28)	0.93 (0.63 to 1.38)	0.90 (0.53 to 1.53)	
	Seeds	203/1578 (12.9) 58/525 (11.0)	155/1585 (9.8) 43/525 (8.2)	77/1578 (4.9) 24/524 (4.6)	
		0.78 (0.53 to 1.14)	0.72 (0.47 to 1.12)	0.75 (0.42 to 1.33)	
	Nuts	209/1668 (12.5) 51/447 (11.5)	157/1673 (9.4) 41/449 (9.1)	81/1668 (4.9) 20/446 (4.5)	
		0.92 (0.62 to 1.34)	1.10 (0.72 to1.67)	0.84 (0.46 to 1.53)	
	Raw Carrots	83/615 (13.5) 180/1511 (11.9)	60/618 (9.7) 140/1515 (9.2)	31/615 (5.0) 71/1510 (4.7)	
		0.85 (0.61 to 1.18)	1.02 (0.69 to 1.49)	0.77 (0.47 to 1.28)	
	Spinach	107/861 (12.4) 156/1252 (12.5)	85/867 (9.8) 115/1253 (9.2)	39/860 (4.5) 63/1252 (5.0)	
		0.97 (0.71 to 1.32)	0.82 (0.58 to 1.17)	1.18 (0.73 to 1.91)	
	Cabbage	164/1295 (12.7) 100/832 (12.0)	126/1300 (9.7) 74/834 (8.9)	60/1295 (4.6) 43/831 (5.2)	
		0.92 (0.66 to 1.28)	0.84 (0.58 to 1.22)	1.16 (0.71 to 1.90)	
	Celery	209/1739 (12.0) 52/340 (15.3)	154/1746 (8.8) 43/340 (12.6)	82/1738 (4.7) 21/340 (6.2)	
		1.61 (1.07 to 2.41)	**1.85 (1.18 to 2.89)**	1.39 (0.74 to 2.58)	P<0.05
	Raw tomatoes	189/1476 (12.8) 75/647 (11.6)	147/1482) (9.9) 53/648 (8.2)	68/1475 (4.6) 35/647 (5.4)	
		0.81 (0.57 to 1.16)	0.74 (0.49 to 1.11)	1.05 (0.62 to 1.77)	
	Raw sweet pepper	65/637 (10.2) 196/1481 (13.2)	55/642 (8.6) 143/1483 (9.6)	18/637 (2.8) 83/1480 (5.6)	
		1.45 (1.03 to 2.06)	1.16 (0.79 to 1.69)	**2.16 (1.2 to 3.9)**	P<0.05
	Salad	181/1450 (12.5) 82/677 (12.1)	136/1457 (9.3) 64/677 (9.5)	72/1449 (5.0) 30/677 (4.4)	
		1.09 (0.76 to 1.57)	1.14 (0.76 to 1.72)	0.92 (0.52 to 1.62)	
	Vegetable juice	212/1622 (13.1) 49/482 (10.2)	158/1629 (9.7) 39/482 (8.1)	84/1621 (5.2) 19/482 (3.9)	
		0.78 (0.53 to 1.16)	0.85 (0.56 to 1.31)	0.85 (0.46 to 1.56)	
	Citrus fruit	150/1355 (11.1) 109/757 (14.4)	110/1359 (8.1) 86/759 (11.3)	64/1355 (4.7) 38/756 (5.0)	
		1.82 (1.29 to 2.56)	**1.73 (1.18 to 2.53)**	**1.72 (1.02 to 2.92)**	P<0.05
	Apples	170/1397 (12.2) 92/734 (12.5)	127/1402 (9.1) 71/736 (9.6)	72/1396 (5.2) 30/734 (4.1)	
		1.07 (0.77 to 1.07)	1.01 (0.70 to 1.46)	0.87 (0.52 to 1.47)	
	Exotic Fruit	97/740 (13.1) 162/1373 (11.8)	70/745 (9.4) 126/1375 (9.2)	41/739 (5.5) 61/1373 (4.4)	
		0.77 (0.55 to 1.07)	0.84 (0.58 to 1.23)	0.64 (0.39 to 1.07)	
	Bananas	53/484 (11.0) 208/1643 (12.7)	39/488 (8.0) 159/1646 (9.7)	20/484 (4.1) 82/1642 (5.0)	
		1.08 (0.75 to 1.55)	1.14 (0.75 to 1.72)	1.10 (0.63 to 1.93)	
	Strawberries	168/1412 (11.9) 94/708 (13.3)	132/1418 (9.3) 67/709 (9.4)	59/1411 (4.2) 43/708 (6.1)	
		1.06 (0.75 to 1.51)	0.90 (0.60 to 1.34)	1.46 (0.87 to 2.47)	
	Fruit Juices	73/621 (11.8) 190/1507 (12.6)	54/625 (8.6) 145/1510 (9.6)	33/624 (5.3) 70/1506 (4.6)	
		1.03 (0.73 to 1.46)	1.12 (0.76 to 1.65)	0.78 (0.47 to 1.30)	
Adjusted for study area, sex, maternal age, maternal smoking, level of parental education, exclusive breastfeeding ≥ 4 months, parental history of atopic diseases, season of birth and all dietary variables					
At 3 years					
Dietary associations – cow's milk					
Venter 2009 (51) N=969 Portsmouth Pregnancy and lactation Moderate to high risk of bias	Cow's milk No (< 1% of women) versus moderate (10%) versus frequent (89%) versus uncertain (1%) consumption of milk during pregnancy	*Maternal Intake* Milk Never Moderate Frequently Uncertain Total FHS Positive 1/2 (4) 2/97 (8) 20/831 (80) 2/7 (8) 25 Negative 1/2 (<1) 95/97(10) 810/831 (89) 5/7 (<1) 933 Values are expressed as (n%) FHS- Food Hypersensitivity		

TABLE E 5 MATERNAL DIET AND SENSITIZATION IN CHILDREN (cont)

Dietary associations – cereal			
Venter 2009 (51) N=969 Portsmouth Pregnancy and lactation Moderate to high risk of bias	Cereal: wheat No (< 1% of women) versus moderate (8%) versus frequent (92%) versus uncertain (< 1%) consumption of wheat during pregnancy	*Maternal Intake* Wheat Never Moderate Frequently Uncertain Total FHS Positive 0/1 (<1) 0/75 4/857 (100) 0/4 4 Negative 1/1 (<1) 75/75 (80) 853/857 (91) 4/4 (<1) 933 Values are expressed as (n%) FHS- Food Hypersensitivity	
Dietary associations – fish			
Venter 2009 (51) N=969 Portsmouth Pregnancy and lactation Moderate to high risk of bias	White Fish No versus moderate versus frequent versus uncertain consumption during pregnancy	*Maternal Intake* Fish Never Moderate Frequently Uncertain Total FHS Positive 0/107(<1) 1/107 0/107 0/107 1 Negative 107/107(11) 781/107(83) 44/107(5) 4/107 (<1) 936 Values are expressed as (n%) FHS- Food Hypersensitivity	
At 4 years			
Romieu 2007 (3) N=458 Menorca Pregnancy Atopy (at least one positive SPT to **aeroallergen** Dust mite, cat, grass, olive, mixed graminae, perietaria) Low risk of bias	Fish Fish intake as portions per week: 0, 1/52 (once per 52 weeks), ¼ (once per month), 1 (once per week) and 7 (once per day)	Specific IgE to any at 4 years 0.93 (0.59 to 1.47) (per unit increase of log transformed weekly fish consumption) Specific IgE to HDM at 4 years 1.00 (0.62 to 1.62) (per unit increase of log transformed weekly fish consumption)	0.768 0.984
	Adjusted for maternal asthma, type of fish, smoking during pregnancy		
At 5 years			
Dietary associations – cow's milk			
Nwaru 2011 (52) n=652 Finland DIPP Nutrition Study Atopic and Non atopic mothers Lactation Low risk of bias	Dairy foods Average Intake by grams	***Atopy*** Measured as serum IgE sensitization to birch, cat, timothy grass, cow's milk, egg, wheat) **Milk allergen** 1.07 (0.76 to 1.50) **Egg allergen** 0.96 (0.67 to 1.38) **Wheat allergen** 0.93 (0.57 to 1.50) **Birch allergen** **0.68 (0.48** to **0.95)** **Cat allergen** 1.04 (0.69 to 1.60) **Timothy grass allergen** 1.07 (0.73 to 1.57)	p < 0.05
	Adjusted for gender, place of birth (southern or northern Finland), duration of gestation, maternal smoking during pregnancy, mode of delivery, parental asthma, parental allergic rhinitis, atopic eczema at 6 months of age, and exclusive breast feeding.		
Dietary associations – cereals			
Nwaru 2010 (48) N=931 Finland DIPP Nutrition Study Pregnancy Lactation Low risk of bias	Total Cereals (rye, wheat, oats, barley, rice, pasta, macaroni, starches and other grains) Average intake by grams	***Food allergens (***sensitization to egg, cow's milk, fish, wheat) 1.26 (0.66 to 2.43) ***Inhalant allergens*** (house dust mite, cat, timothy grass, birch) 0.94 (0.53 to 1.66)	
	Wheat	***Food allergens (***sensitization to egg, cow's milk, fish, wheat) 1.20 (0.75 to 1.93) ***Inhalant allergens*** (house dust mite, cat, timothy grass, birch) 1.16 (0.77 to 1.74)	
Nwaru 2011 (52) n=652 Finland DIPP Nutrition Study Atopic and Non atopic mothers Lactation Low risk of bias	cereals Average intake by grams	***Atopy*** (serum IgE sensitization to birch, cat, timothy grass, cows milk, egg, wheat) **Milk allergen** 0.77 (0.48 to 1.23) **Egg allergen** 0.73 (0.42 to 1.26) **Wheat allergen** 0.68 (0.32 to 1.47) **Birch allergen** 1.02 (0.67 to 1.56) **Cat allergen** 0.70 (0.38 to 1.27) **Timothy grass allergen** 1.06 (0.63 to 1.79)	
	Adjusted for gender, place of birth (southern or northern Finland), duration of gestation, maternal smoking during pregnancy, mode of delivery, parental asthma, parental allergic rhinitis, atopic eczema at 6 months of age, and exclusive breast feeding.		

TABLE E 5 MATERNAL DIET AND SENSITIZATION IN CHILDREN (cont)

Dietary associations – fats			
Calvani 2006 (53) N=988 (N=295 Allergic & N=693 Non Allergic mothers) Italy APAL study Pregnancy Moderate risk of bias	Butter ≤ 1 serve/month (reference) v 1 serve/week v ≥ 2-3 serves/week	**Food Sensitization** (positive skin prick test for raw cow's milk and egg-white or other foods if indicated by clinical history) **Allergic mothers n/N(%) aOR (95%CI)*** ≤ 1 serve/month 19/156 (12.2%) 1 1 serve/week 6/72 (8%) 0.49 (0.16 to 1.43) ≥2-3 serves/ week 6/49 (12.2%) 0.84 (0.26 to 2.71) **Non Allergic mothers aOR (95%CI)**** ≤ 1 serve/month 29/373 (7.9%) 1 1 serve/week 11/168 (6.5%) 0.91 (0.37 to 2.25) ≥2-3 serves/ week 5/86 (5.8%) 0.92 (0.27 to 3.13)	ptrend 0.80 0.46
		Inhalant sensitization (positive skin prick test for a range of allergens) **Allergic mothers n/N(%) aOR (95%CI)***** ≤ 1 serve/month 76/156 (48.7%) 1 1 serve/week 25/72 (34.7%) **0.27 (0.10 to 0.73)** ≥2-3 serves/ week 28/49 (57.1%) 1.59 (0.51 to 4.97) **Non Allergic mothers aOR (95%CI)****** ≤ 1 serve/month 150/373 (40.2%) 1 1 serve/week 88/168 (52.4%) **1.73 (1.00 to 2.99)** ≥2-3 serves/ week 37/86 (43%) 0.81 (0.38 to 1.70)	 0.77 0.15
	Margarine ≤ 1 serve/month (reference) v 1 serve/week v ≥ 2-3 serves/week	**Food Sensitization** (positive skin prick test for raw cow's milk and egg-white or other foods if indicated by clinical history) **Allergic mothers n/N(%) aOR (95%CI)*** ≤ 1 serve/month 24/214 (11.2%) 1 1 serve/week 2/34 (5.9%) 0.26 (0.02 to 2.54) ≥2-3 serves/ week 4/22 (18.2%) 2.24 (0.59 to 8.49) **Non Allergic mothers n/N(%) aOR(95%CI)**** ≤ 1 serve/month 39/528 (7.4%) 1 1 serve/week 3/43 (7.0%) 1.63 (0.38 to 6.87) ≥2-3 serves/ week 2/47 (4.3%) 0.51 (0.06 to 4.32)	ptrend 0.67 0.45
		Inhalant sensitization (positive skin prick test for a range of aeroallergens) **Allergic mothers n/N(%) aOR(95%CI)***** ≤ 1 serve/month 100/214 (46.7%) 1 1 serve/week 13/34 (38.2%) 0.39 (0.10 to 1.48) ≥2-3 serves/ week 12/22 (54.5%) 3.02 (0.52 to 17.2) **Non Allergic mothers aOR(95%CI)****** ≤ 1 serve/month 229/528 (43.4%) 1 1 serve/week 25/43 (58.1%) 1.28 (0.53 to 3.07) ≥2-3 serves/ week 20/47 (42.6%) 0.52 (0.19 to 1.43)	 0.85 0.54
	*adjusted for maternal age, occupation and eczema **adjusted for age, gestation age, maternal occupation, oculorhinitis and eczema ***adjusted for age, allergy clinics, maternal age, preterm labour, occupation, asthma, oculorhinitis and eczema ****adjusted for age, gender, number of older siblings, allergy clinics, maternal ae, number of pregnany, maternal occupation, paternal atopy, asthma, oculorhinitis.		
Nwaru 2011 (52) n=652 Finland DIPP Nutrition Study Atopic and Non atopic mothers Lactation Low risk of bias	Dietary Fats Average Intake by grams	***Atopy*** Measured as serum IgE sensitization to birch, cat, timothy grass, cow's milk, egg, wheat) Milk allergen 0.92 (0.60 to 1.39) Egg allergen 1.42 (0.87 to 2.32) Wheat allergen 1.59 (0.83 to 3.05) Birch allergen 0.72 (0.47 to 1.11) Cat allergen 0.77 (0.46 to 1.30) Timothy grass allergen 1.18 (0.73 to 1.92)	
	Butter and Butter spreads Average Intake by grams	Milk allergen 0.92 (0.67 to 1.26) Egg allergen 1.37 (0.98 to 1.90) Wheat allergen **2.06 (1.26** to **3.12)** Birch allergen 1.05 (0.79 to 1.40) Cat allergen 1.01 (0.70 to 1.45) Timothy grass allergen 1.23 (0.89 to 1.69)	 p < 0.01
	Margarine and low fat spreads Average Intake by grams	Milk allergen 0.96 (0.70 to 1.31) Egg allergen 0.80 (0.51 to 1.26) Wheat allergen **0.41 (0.18** to **0.93)** Birch allergen **0.68 (0.47** to **0.99)**	 p < 0.05 p < 0.05

TABLE E 5 MATERNAL DIET AND SENSITIZATION IN CHILDREN (cont)

		Cat allergen 0.69 (0.43 to 1.12) Timothy grass allergen 0.96 (0.66 to 1.40)	
	Adjusted for gender, place of birth (southern or northern Finland), duration of gestation, maternal smoking during pregnancy, mode of delivery, parental asthma, parental allergic rhinitis, atopic eczema at 6 months of age, and exclusive breast feeding.		
Dietary associations – fish			
Calvani 2006 (53) N=988 (N=295 Allergic & N=693 Non Allergic mothers) Italy APAL study Pregnancy Moderate risk of bias	Fish ≤ 1 serve/month (reference) v 1 serve/week v ≥ 2-3 serves/week	**Food Sensitization** (positive skin prick test for raw cow's milk and egg-white or other foods if indicated by clinical history) **Allergic mothers n/N(%) aOR (95%CI)*** ≤ 1 serve/month 7/62 (11.3%) 1 1 serve/week 16/138 (11.6%) 1.15 (0.38 to 3.47) ≥2-3 serves/ week 8/38 (9.6%) 1.13 (0.31 to 4.1) **Non Allergic mothers aOR (95%CI)**** ≤ 1 serve/month 20/136 (14.7%) 1 1 serve/week 16/330 (4.8%) **0.22 (0.08 to 0.55)** ≥2-3 serves/ week 10/197 (5.1%) **0.23 (0.08 to 0.69)**	ptrend 0.72 **0.002**
		Inhalant sensitization (positive skin prick test to one of 8 aeroallergens *Dermatophapgoides pteronissinus, Alternaria tenuis, Aspergillus fumigatus,* mixed grass pollen, *Artemisia vulgaris, Parietaria officinalis, Olea europea*, cat dander) **Allergic mothers n/N(%) aOR (95%CI)***** ≤ 1 serve/month 27/62 (43.5%) 1 1 serve/week 63/138 (50.7%) 0.89 (0.30 to 2.60) ≥2-3 serves/ week 48/83 (42.2%) 0.74 (0.23 to 2.37) **Non Allergic mothers aOR (95%CI)****** ≤ 1 serve/month 69/136 (50.7%) 1 1 serve/week 137/330 (41.5%) 1.73 (0.70 to 1.30) ≥2-3 serves/ week 92/197 (43%) 0.55 (0.28 to 1.08)	0.76 0.62
	*adjusted for maternal age, occupation and eczema **adjusted for age, gestation age, maternal occupation, oculorhinitis and eczema ***adjusted for age, allergy clinics, maternal age, preterm labour, occupation, asthma, oculorhinitis and eczema ****adjusted for age, gender, number of older siblings, allergy clinics, maternal ae, number of pregnany, maternal occupation, paternal atopy, asthma, oculorhinitis.		
Nwaru 2011 (52) n=652 Finland DIPP Nutrition Study Atopic and Non atopic mothers Lactation ***Atopy*** Measured as serum IgE sensitization to birch, cat, timothy grass, cow's milk, egg, wheat) Low risk of bias	Fish and Fish Products Average Intake by grams	**Milk allergen** 0.80 (0.58 to 1.12) **Egg allergen** 0.97 (0.69 to 1.37) **Wheat allergen** 0.77 (0.46 to 1.31) **Birch allergen** 0.79 (0.58 to 1.09) **Cat allergen** 1.04 (0.72 to 1.51) **Timothy grass allergen** 0.73 (0.50 to 1.09)	
	Adjusted for gender, place of birth (southern or northern Finland), duration of gestation, maternal smoking during pregnancy, mode of delivery, parental asthma, parental allergic rhinitis, atopic eczema at 6 months of age, and exclusive breast feeding.		
Grandjean 2010 (16) N=464 Faroe Island birth cohort No indication of maternal atopy status Pregnancy Moderate risk of bias	Seafood Fish (as a source of PCBs and mercury);	Se total IgE Conc(kUA/l) Low (<13.6) Medium(13.6-47.7) High (>47.7) PCB (µg/g serum lipid) geometric mean, IQR Antenatal 1.16 (0.73-1.79) 1.17(0.76-1.97) 1.29 (0.83-2.3) 5 years 1.00 (0.54-1.85) 1.19(0.76-1.91) 1.27 (0.81-2.2) Methylmercury (ug/L blood) geometric mean, IQR Antenatal 12.1 (6.6-19.4) 13.5(7.8-21.7) 13.3 (7.6 -24.7) 5 years 2.5 (1.26-4.9) 2.8(1.41-6.1) 2.5 (1.41-4.4) Serum Grass specific IgE concentration (kUA/l) low (≤0.35 n=425) high(>0.35 n=39) PCB (µg/g serum lipid) geometric mean, IQR Antenatal 1.22 (0.79-2.01) 1.12(0.54-2.3) 5 years 1.14 (0.69-1.94) 1.15(0.81-2.3) Methylmercury (ug/L blood) Antenatal 13.3 (7.6-22.5) 9.6 (6.2-12.2) 5 years 2.7 (1.35-5.2) 2.0 (1.29-3.0)	0.30 0.01 0.06 0.44 0.47 0.95 0.02 0.08

TABLE E 5 MATERNAL DIET AND SENSITIZATION IN CHILDREN (cont)

Dietary associations – fruits and vegetables				
Nwaru 2010 (48) n=931 Finland DIPP Nutrition Study Pregnancy and first 3 months of lactation Allergic sensitization in offspring by 5 years: food allergens (egg, cow's milk, fish, wheat); inhalant allergens (house dust mite, cat, timothy grass, birch) Results for 931/1175 (79.2%) children Low risk of bias	Total fruits Fruit (apple, peach, plum, prune, orange, lemon, grapefruit, mandarin, canned fruits, melons, pineapple, grapes, banana, kiwi-fruit, avocado, dried fruits, berries) and fruit and berry juices	*Food allergens* *Inhalant allergens*	0.97 (0.77 to 1.23) **1.36 (1.09 to 1.70)**	
	Malaceous fruits	*Food allergens* *Inhalant allergens*	0.97 (0.84 to 1.13) 1.00 (0.87 to 1.14)	
	Citrus fruits	*Food allergens* *Inhalant allergens*	1.00 (0.92 to 1.09) **1.14 (1.05 to 1.25)**	
	Berries	*Food allergens* *Inhalant allergens*	1.07 (0.92 to 1.25) 1.12 (0.88 to 1.28)	
	Juices	*Food allergens* *Inhalant allergens*	0.99 (0.90 to 1.08) 0.98 (0.90 to 1.06)	
	Adjusted for energy intake, place of birth, season of birth, sex of the child, number of siblings, gestational age at birth, parental asthma, parental allergic rhinitis, maternal age at birth, maternal smoking during pregnancy, maternal education			
Nwaru 2011 (52) n=652 Finland DIPP Nutrition Study Lactation Atopic and Non atopic mothers ***Atopy*** Measured as serum IgE sensitization to birch, cat, timothy grass, cow's milk, egg, wheat) Low risk of bias	Fruits Average Intake by grams	**Milk allergen** **Egg allergen** **Wheat allergen** **Birch allergen** **Cat allergen** **Timothy grass allergen**	1.19 (0.87 to 1.63) 1.11 (0.76 to 1.63) 1.06 (0.62 to 1.83) 1.27 (0.96 to 1.69) 1.18 (0.80 to 1.74) 0.90 (0.61 to 1.32)	
	Berries	**Milk allergen** **Egg allergen** **Wheat allergen** **Birch allergen** **Cat allergen** **Timothy grass allergen**	1.12 (0.86 to 1.46) 1.05 (0.74 to 1.50) 1.22 (0.81 to 1.85) 0.74 (0.48 to 1.13) 0.70 (0.38 to 1.27) 0.98 (0.68 to 1.41)	
	Veg and roots	**Milk allergen** **Egg allergen** **Wheat allergen** **Birch allergen** **Cat allergen** **Timothy grass allergen**	0.75 (0.51 to 1.12) 1.09 (0.77 to 1.54) 0.72 (0.39 to 1.32) 0.76 (0.54 to 1.08) 0.80 (0.50 to 1.28) 0.68 (0.43 to 1.08)	
	Potatoes	**Milk allergen** **Egg allergen** **Wheat allergen** **Birch allergen** **Cat allergen** **Timothy grass allergen**	0.77 (0.53 to 1.10) 1.11 (0.80 to 1.55) 1.01 (0.63 to 1.63) **0.65 (0.45** to **0.94)*** **0.55 (0.36** to **0.91)*** 1.03 (0.73 to 1.46)	 p < 0.05 p < 0.05
	Adjusted for gender, place of birth (southern or northern Finland), duration of gestation, maternal smoking during pregnancy, mode of delivery, parental asthma, parental allergic rhinitis, atopic eczema at 6 months of age, and exclusive breast feeding.			
At 6 to 6.5 years				
Dietary associations – cow's milk				
Chatzi 2008 (49) n=482 MENORCA Atopic and Non atopic mothers Pregnancy Low risk of bias	Dairy Food ≤ 23 v > 23 serves of nuts per week	***Atopy n=70 (***sensitization to at least one aeroallergen on SPT) Low 34 /70 (16.83%) High 36/70 (17.22%)		pns
	Adjusted for gender, maternal and paternal asthma, maternal social class and education, BMI at age 6.5 years, total energy intake at 6.5 years, birth weight and maternal atopy			
Dietary associations – cereal				
Chatzi 2008 (49) n=482 MENORCA	Cereal ≤ 11.5 v > 11.5 serves of cereal per week	***Atopy n=70*** (sensitization to at least one aeroallergen on SPT) Low 34/70 (17.00%) high 36/70 (17.06%)		pns

TABLE E 5 MATERNAL DIET AND SENSITIZATION IN CHILDREN (cont)

Atopic and Non atopic mothers Pregnancy Low risk of bias	*Adjusted for gender, maternal and paternal asthma, maternal social class and education, BMI at age 6.5 years, total energy intake at 6.5 years, birth weight and maternal atopy*		
Chatzi 2008 (49) n=482 MENORCA Atopic and Non atopic mothers Pregnancy Low risk of bias	Nuts ≤ 1 v > 1 serves of nuts per week	***Atopy n=70*** (sensitization to at least one aeroallergen on SPT) Low 37 /70 (16.09%) High 33/70 (18.23%)	pns
	Adjusted for gender, maternal and paternal asthma, maternal social class and education, BMI at age 6.5 years, total energy intake at 6.5 years, birth weight and maternal atopy		
Dietary associations – fish			
Romieu 2007 (3) N=401 Pregnancy Atopy (at least one positive SPT to **aeroallergen** Dust mite, cat, grass, olive, mixed graminae, perietaria) Low risk of bias	Fish Fish intake as portions per week: 0, 1/52 (once per 52 weeks), ¼ (once per month), 1 (once per week) and 7 (once per day)	SPT to House dust mite (HDM), Fel d 1, grass pollen, olive tree, mixed graminae, parietaria. Specific SPT to any at 6 years 0.74 (0.5 to 1.09) (per unit increase of log transformed weekly fish consumption) Specific SPT to HDM at 6 years 0.68 (0.46 to 1.01 (per unit increase of log transformed weekly fish consumption)	0.123 0.058
	Adjusted for maternal asthma, type of fish, smoking during pregnancy		
Chatzi 2008 (49) n=482 MENORCA Atopic and Non atopic mothers Pregnancy *Low risk of bias*	Fish ≤ 2.5 v > 2.5 serves of fish per week	***Atopy n=70*** (sensitization to at least one aeroallergen on SPT) Low 36 /70 (16.59%) high 34/70 (17.53%)	pns
	Adjusted for gender, maternal and paternal asthma, maternal social class and education, BMI at age 6.5 years, total energy intake at 6.5 years, birth weight and maternal atopy		
Dietary associations – meat			
Chatzi 2008 (49) n=482 MENORCA Atopic and Non atopic mothers Pregnancy *Low risk of bias*	Red Meat < 3.25 v ≥ 3.25 serves per week	***Atopy n=70*** (sensitization to at least one aeroallergen on SPT) Low 47 /70 (17.41%) High 23/70 (16.31%)	pns
	White Meat < 2.5 v ≥ 2.5 serves per week	Low 49 /70 (16.78%) High 21/70 (17.65%)	pns
	Adjusted for gender, maternal and paternal asthma, maternal social class and education, BMI at age 6.5 years, total energy intake at 6.5 years, birth weight and maternal atopy		
Dietary Associations- Fruits and Vegetables			
Romieu 2007 (3) N=401 Pregnancy Atopy defined as one positive SPT (HDM, cat, grass pollen, olive tree, mixed graminae, parietaria) Low risk of bias	Fruits and Vegetables	No observations with any association with atopy and fruit & vegetable intake. Numerical results not reported in paper	
	Adjusted for maternal asthma, type of fish, smoking during pregnancy		
Chatzi 2008 (49) n=482 MENORCA Atopic and Non atopic mothers	Fruits ≤ 14 v > 14 serves of fruit per week	Low 45 /70 (17.65%) high 25/70 (11.98%)	pns
	Vegetables ≤ 8 v > 8 serves of vegetables per week	**Low 47/70 (21.46%)** **high 23 /70 (16.23%)** **OR 0.40 (0.22-0.72)**	**p<0.01**

TABLE E 5 MATERIAL DIET AND SENSITIZATION IN CHILDREN (cont)

Pregnancy ***Atopy n=70 (***sensitization to at least one aeroallergen on SPT) *Low risk of bias*	Legumes ≤ 1v > 1 serves of legumes per week	Low 49 /70 (18.35%) high 21 /70 (14.58%)	pns
	Adjusted for gender, maternal and paternal asthma, maternal social class and education, BMI at age 6.5 years, total energy intake at 6.5 years, birth weight and maternal atopy		
At 7 years			
Grandjean 2010 (16) N=464 Faroe Island birth cohort No indication of maternal atopy status Pregnancy Moderate risk of bias	Seafood Fish (as a source of PCBs and mercury);	Se total IgE Conc (kUA/l) Low (<13.6) Medium(13.6-47.7) High (>47.7) PCB (µg/g serum lipid) geometric mean, IQR 7 years 0.66 (0.35-1.30) 0.74(0.46-1.26) 0.88 (0.56-1.47) Methylmercury (ug/L) 7 years 2.1 (0.95-4.5) 2.1(1.07-4.2) 2.2 (1.06-4.9) Serum Grass specific IgE concentration (kUA/l) low (≤0.35 n=425) high(>0.35 n=39) PCB (µg/g serum lipid) geometric mean, IQR 7 years 0.75 (0.43-1.39) 0.82(0.61-1.43) Methylmercury (ug/L) 7 years 2.2 (1.05-4.5) 1.7 (0.76-3.0)	 0.005 0.37 0.57 0.19

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APPENDIX 3

SEARCH TERMS USED FOR SYSTEMATIC REVIEWS IN CHAPTER 2

(egg hypersensitivit*[tw] OR egg allerg*[tw] OR (egg proteins, dietary[mh] OR egg white*[tw] OR egg yolk*[tw] OR egg protein*[tw] OR conalbumin[tw] OR ovotransferrin[tw] OR ovalbumin[tw] OR serpin b14[tw] OR avidin[tw] OR ovomuc*[tw] OR phosphovit*[tw]) AND (food hypersensitivity[mh:noexp] OR hypersensitivit*[tw] OR allerg*[tw])) AND (cook*[tw] OR uncooked[tw] OR raw[tw] OR pasteuri*[tw] OR baked[tw]).

APPENDIX 4

RANDOMISED CONTROLLED TRIAL (CAKE STUDY) PROTOCOL

Does Consumption of Baked Egg Hasten Development of Tolerance to Raw Egg in Children with Egg Allergy?

– A Randomised, Controlled Trial.

Simplified title: **Can Egg Allergic Kids Eat Baked Egg? The CAKE Study.**

FINANCIALLY SUPPORTED BY:
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Original Study protocol:	**Date: 29/11/2011**
Amendment 1:	**Date: 19/12/11**
Approval to collect data on household consumption of egg	
Amendment 2:	**Date: 7/3/2012**
Approval to include IgE testing for peanuts. Approval of revised Information sheet (Version 6 Feb 2012)	
Amendment 3:	**Date: 18/4/2012**
Approval of letter to be sent to patients of the WCH Allergy Unit on the waiting list at for a baked egg challenge, alerting them of the CAKE study	
Amendment 4:	**Date: 7/8/2014**
Approval to modify raw egg oral food challenge protocol for children at risk of anaphylaxis Approval of revised information sheet (Version 9 August 2014)	

STUDY SYNOPSIS

STUDY DESIGN:
Randomised controlled double-blind trial.

AIM:
To determine whether allergy to raw egg is better resolved by regular consumption of baked egg (intervention group, baked egg exposure) compared with the standard practice of an egg free diet (control group, egg avoidance).

STUDY PRODUCTS:
- Intervention: egg containing bread, biscuit and muffin (cow's milk and nut free).
- Control: egg free bread, biscuit and muffin (cow's milk and nut free).

INCLUSION CRITERIA:
- 6 months to 5 years of age.
- Definite diagnosis of IgE mediated raw egg allergy as demonstrated by one of the following:
  - Definite clinical reaction to egg in the past 12 months and positive SPT or egg serum specific IgE.
  - Positive oral food challenge to pasteurised raw egg.
  - Highly predictive biomarkers (SPT or egg specific IgE > 95% predictive value).
- Tolerance to baked egg demonstrated by a negative oral food challenge to egg in baked goods.

EXCLUSION CRITERIA:
- Older than 5 years of age.
- Inability to provide informed consent.
- Children with non-IgE mediated egg allergy (those reacting to egg, but with negative skin prick tests to egg).
- Children who are already including baked egg in their diet.
- Children with Food Protein Induced Enterocolitis Syndrome (FPIES)
- Children with any congenital or acquired disease or developmental disorder (including wheat allergy) likely to affect ability to undergo an oral egg challenge or to consume intervention products.

STUDY INTERVENTION:
Children will follow an egg free diet and will be asked to consume 2-3 serves of the study product each week for six months. The intervention group will consume the equivalent of 10 g egg (1.3g egg protein) per serve.

OUTCOME ASSESSMENTS:

Primary Outcome:
The primary outcome will be tolerance to pasteurised raw egg as determined by the oral food challenge one month after the end of a six month intervention.

Secondary Outcome (Clinical):
Individuals consuming baked egg will have fewer reactions to accidental exposure to egg (because of improved tolerance) compared to an egg free diet. Families will be asked to keep a diary recording any accidental exposure to egg, and any allergic symptoms.

For children with eczema, the effect of the intervention on eczema will be monitored by regular SCORADS.

Secondary Outcomes (Laboratory):

Regular exposure to baked egg will lead to an increased level of immune markers indicative of development of tolerance compared to an egg free diet.

STUDY DURATION:

Children will be followed for around seven months, or until their final raw egg challenge.

SAMPLE SIZE:

110 children aged six months to five years who have allergy to raw egg, but who tolerate baked egg will be enrolled in this study.

OUTCOMES AND SIGNIFICANCE:

The strength of this study is the focus on one patient group, ensuring that the trial outcome will be specific and able to be translated into practice.

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LIST OF ABBREVIATIONS

AE	Adverse Event
AIs	Associate Investigators
ASCIA	Australasian Society of Clinical Immunology and Allergy
CI	Confidence Intervals
CIs	Chief Investigators
CRF	Case Report Form
CTN	Clinical Trial Notification
DMC	Data Monitoring Committee
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HREC	Human Research Ethics Committee
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
LEAP	Learning Early About Peanut
NHMRC	National Health and Medical Research Council
RCT	Randomised Controlled Trial
RR	Relative Risk
SAE	Serious Adverse Event
SPT	Skin Prick Test
TGA	Therapeutics Goods Administration
WHO	World Health Organisation

BACKGROUND

Significance

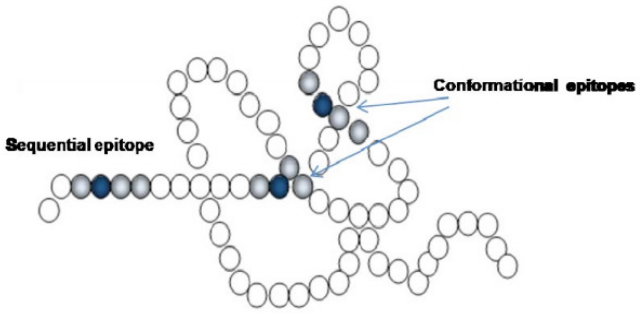
The rate of food allergy has escalated in recent years, with a five fold increase in food anaphylaxis in Australian children under 4 years of age (1). Food allergies are one of the first manifestations of allergic disease and have been shown to significantly impact on general health perception, parental emotional distress and family activities (2). Egg allergy is the most common IgE mediated allergy in Australian children with an incidence estimated to be around 9% (3). Approximately 50% of children with egg allergy go on to develop respiratory (asthma, allergic rhinitis) allergic disease in childhood (4) magnifying the burden to the individual, the family and the health care system. In 2007 allergic disease cost the Australian economy $7.8 billion and the estimated cost due to reduced quality of life (the burden of disease) to Australians who suffer from allergy was $21.5 billion, approximately double the figure for arthritis ($11.7 billion) (www.allergy.org.au).

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 (5).

Heat Treatment Affects the Allergenicity of a Protein

Proteins are three dimensional molecules held together by electrostatic charge. IgE binding sites (or epitopes) may be sequential (several amino acids in a row) or conformational (part of the shape of the protein) – see figure 1(6). Differences in allergenicity are, in part, due to changes in the structure of the proteins when heated, which affects the specific conformational IgE binding sites on the protein molecule. It is recognised that heat treatment of a protein can result in conformational changes in epitopes by disrupting hydrogen bonds within the protein and thus affecting the ability of the IgE molecule to bind (7).

Figure 1 (from Alessandri et al) (6)



Other means of food processing (eg whipping, mincing) can also disrupt protein structure and also affect the allergenicity of proteins.

Allergenicity of Different Forms of Egg

Not all forms of egg have the same allergenicity, and it is recognised that the allergenicity of baked egg differs to raw egg (7). This is thought to be related to effects of heat on the different egg allergy epitopes (6). It could also be related to differences in digestibility of the egg proteins when heated affecting their relative allergenicity (8). Clarke et al (5) in a British longitudinal study followed a group of children with egg allergies given yearly egg challenges found that the median age for tolerance to well cooked egg (sponge cake) was 67 months (5.6 yrs) & 127 months (10.3yrs) for uncooked egg.

Specific Oral Tolerance induction (SOTI)

There is interest in using an immunotherapy approach in the treatment of food allergies. In SOTI, food allergens are given to an individual with a food allergy to eat, initially starting with tiny doses and gradually increasing the amount (9). This approach to managing food allergies is still experimental in nature and requires hospitalisation of the individual to establish a safe dose of the allergen to commence at home. There is also concern that the child may lose tolerance to the allergen if it is not consumed on a daily basis.

Kurihara (9) reported 12 cases of egg allergic children, aged 6-12 years given raw egg SOTI. This study used raw egg and a 'rush induction' protocol where individuals were admitted to hospital and given minute and increasing doses of raw egg. All children gained tolerance to cooked egg. Kurihara discussed the potential of baked egg as a vehicle to promote tolerance to raw egg and in another review of food immunotherapy Kamdar (14) also suggested heat denatured proteins should be considered as potential therapeutic agents.

Heated Egg Studies

Lemon Mule et al (10) were the first to report that up to 70% of children with diagnosed egg allergy tolerated heated (baked) egg in the form of a muffin or a waffle. Children who tolerated baked egg were advised to include baked egg in their diet and were followed up at 3, 6 and 12 months. Regular consumption of heated egg was associated with decreasing Skin Prick Test (SPT) weal sizes to egg white and increasing ovalbumin and ovomucoid specific IgG4 levels. Markers of intestinal permeability were no different between children consuming baked egg compared to children on an egg free diet. The children consuming baked egg continued to grow well.

The authors note that they did not perform challenges to raw egg white as raw egg is not usually encountered by children in their diet. In Australia, challenges to raw egg are routine in many centres – The Allergy Unit at the Royal Children's Hospital, Melbourne uses raw egg white challenges routinely (3, 11), and the Allergy Unit at the WCH routinely uses pasteurised raw whole egg challenges. An oral food challenge using raw whole egg gives an indication of the overall egg allergy status of the child. If a child passes a raw egg challenge they will tolerate other forms of egg. This is in contrast to oral challenge with cooked egg in a baked product, as children may tolerate the 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 (for example an omelette or a soft boiled egg).

Heated Cow's Milk Studies

Support for the concept that feeding allergic children a cooked form of the allergen will induce tolerance comes from the work of Nowak-Wegrzyn et al (12) who challenged 100 cow's milk allergic children (average age 7.5 years, range 2.1-17.3 yrs) with cow's milk baked in waffles or muffins. 75% of the cow's milk allergic children tolerated baked milk. Those who tolerated baked milk were asked to consume products containing baked milk at home for 3 months and then were re evaluated. Immune response (specific IgE & IgG4), growth and intestinal permeability were also monitored. After 3 months children consuming baked milk products had significantly smaller SPT and higher casein – specific IgG4 compared with baseline. On repeat challenges with uncooked cow's milk, the group of children who tolerated heated cow's milk outgrew their milk allergy quicker than the group who did not tolerate heated cow's milk in their diet (13).

Although both the baked egg and milk studies by Lemon Mule et al and Nowak- Wezgryn et al (10, 12) monitored IgE and IgG levels they have not answered the question 'does consuming baked egg or milk protein hasten the time taken to outgrow allergies to raw egg or unheated milk'? It is possible that tolerance to heat treated proteins selects a group of children already growing out of their allergy and that exposure to these foods is not itself causal in the subjects' earlier tolerance when compared with those not tolerating baked egg. The authors make the point that "further studies are required to determine whether ingesting heated egg affects the natural history of egg allergy when compared with strict avoidance and that until such studies are completed, there remains insufficient evidence to routinely advise the introduction of heated egg into the diet of those undertaking strict avoidance" (10).

The anticipated impact of this study:

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

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

STUDY RESEARCH PLAN:

1.1.1 AIM

The primary aim of this study is to determine whether allergy to raw egg is better resolved by regular consumption of baked egg (intervention group, baked egg exposure) compared with the standard practice of an egg free diet (control group, egg avoidance).

1.1.2 STUDY DESIGN

Randomised controlled double-blind trial.

1.1.3 STUDY SITE

The hospital involved is the: Women's & Children's Hospital, Adelaide, SA, Australia

The Coordinating Centre is the Women's and Children's Health Research Institute, based at the Women's and Children's Hospital, Adelaide.

1.1.4 ELIGIBILITY CRITERIA

INCLUSION CRITERIA

- 6 months to 5 years of age.
- Definite diagnosis of IgE mediated raw egg allergy as demonstrated by one of the following:
  - Definite clinical reaction to egg in the past 12 months and positive SPT or egg serum specific IgE. A definite clinical reaction is defined as a history of exposure to egg resulting in any one of the following symptoms within two hours of the ingestion: generalised urticaria (in two body sites eg face and limbs), generalised skin erythema, and forceful or projectile vomiting.

  - Positive oral food challenge to pasteurised raw egg.

  - Highly predictive biomarkers (SPT or egg specific IgE > 95% predictive value).

- Tolerance to baked egg demonstrated by a negative oral food challenge to egg in baked goods.

EXCLUSION CRITERIA

- Older than 5 years of age
- Inability to provide informed consent
- Children with non-IgE mediated egg allergy (those reacting to egg, but with negative skin prick tests to egg).
- Children who are already including baked egg in their diet.
- Children with Food Protein Induced Enterocolitis Syndrome (FPIES)
- Children with wheat allergy
- Children with any congenital or acquired disease or developmental disorder that is likely to affect the ability of the child to undergo an oral egg challenge, or to consume the intervention product (including wheat allergy).

1.1.5 SCREENING FOR PARTICIPANTS

Families with children attending the WCH Allergy Clinic for management of food allergies will be invited to participate. We will also advertise in private allergy rooms to enable involvement of other families.

A screening appointment will be scheduled for all families interested in participating in this trial, at this visit we will:

- Obtain written informed consent.
- Collect basic demographic and health information (parental education, parental smoking, parental history of allergic disease, birth order and sex of the child)
- Measure the child's weight and length / height.
- Ask questions relating to child's symptoms of allergic disease (including eczema SCORAD)
- Assess the child's usual diet to ensure that it is egg free
- Perform Skin Prick Testing (SPT) to egg (whole egg, egg white, egg yolk, ovalbumin and ovomucoid.) Skin Prick Testing will be performed at least 15 minutes prior to commencement of any oral food challenge.

- Collect a venous blood sample (5 ml) which will be used to measure specific IgE to whole egg, peanut, ovalbumin and ovomucoid, IgG4 to whole egg, ovalbumin and ovomucoid, and immune markers of tolerance.

- Tolerance to baked egg will be confirmed by a medically supervised baked egg challenge according to clinical practice in the Medical Day Unit. Children who tolerate the baked egg challenge will be eligible to enter the randomised trial.
  Clinical outcome data of the egg challenge will be collected including dose of challenge reached and symptoms of any positive challenge. A positive reaction to the egg challenge will be defined by the development of symptoms within 2 hours of the egg challenge and include 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 must include circulatory and/or respiratory involvement).

- Existence of an allergy to raw egg will be confirmed, by a medically supervised graded egg oral food challenge to pasteurised raw whole egg powder according to clinical practice in the Medical Day Unit using a protocol adapted from the ASCIA Egg challenge protocol (2009) using pasteurised whole raw egg powder. A raw egg challenge will not be given if the criteria listed below are met. Clinical outcome data of the egg challenge will be collected including dose of challenge reached and symptoms of any positive challenge. A positive reaction to the egg challenge will be defined by the development of symptoms within 2 hours of the egg challenge and include 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 must include circulatory and/or respiratory involvement).
- The raw egg challenge should be given within 4 weeks of the baked egg challenge.
- If a child has a suboptimal food challenge (such as refusal to consume the study product) the challenge will be rebooked for another day. If the repeated challenge is still suboptimal, the child will be excluded from the study.

Criteria for assumption of raw egg allergy without oral food challenge to raw egg and eligibility for the trial:

Children will **not** be given a raw egg challenge if they have:

• a history of anaphylaxis to egg in the previous six months (regardless of SPT size).

• a clear clinical history of a reaction to raw or whole cooked egg in the past three months, are following an egg free diet and have SPT to **egg white** highly predictive of a reaction ie:

- 6 months to 2 years of age: SPT (≥5mm) to egg white

- 2 to 5 years of age: SPT (≥8mm) to egg white

• Sensitization to egg white (SPT≥8mm)

1.1.6 RANDOMISATION

Each participant will be assigned a unique study number according to a randomisation schedule programmed into a secure spread sheet located on the WCHRI server. The randomisation service will be developed by the Data Management and Analysis Centre (DMAC), University of Adelaide and managed by a WCHRI staff member independent to the CAKE Study. The randomisation schedule will be computer-generated and stratified by the age of the child.

1.1.7 DIETARY TREATMENTS AND SCHEDULES

STUDY TRIAL PRODUCTS

Intervention: egg containing bread, biscuit and muffin (cow's milk and nut free).

Control: egg free bread, biscuit and muffin (cow's milk and nut free).

Active and placebo products are identical in colour, odour, texture and taste. Products will be cow's milk and nut free to enable children with allergies to these proteins to participate in this study. See Appendix 7 (of thesis) for standard recipes.

Manufacture

All products will be manufactured according appropriate food handling standards and the products will be packaged and labeled in accordance with Good Manufacturing Procedures (GMP). Egg and egg free products will be manufactured separately to avoid the possibility of cross contamination.

Labeling, packaging and blinding.

An independent research assistant will package and label the baked products. The independent research assistant will not be involved in the dietary group allocation or assessment process, thus keeping the outcome assessments blinded.

Each package of product will be assigned a product identification number and this will be paired to the unique participant randomisation code.

THE ACTIVE (INTERVENTION) EGG GROUP

Children will continue on an egg free diet. They will be asked to consume 2-3 serves of the active study product each week for six months. The intervention group will consume the equivalent of 10 g egg per serve (1.3 g egg protein).

Children will be offered a bread, muffin or biscuit. All children will be encouraged to consume the lower sugar bread product and this will be the only product made available to children under the age of one year.

THE CONTROL (NO EGG) GROUP

Children will continue on an egg free diet. They will be asked to consume 3-4 serves of the placebo study product each week for six months. The control group will consume no egg.

BLINDING OF THE STUDY PRODUCTS

The baked product recipes were developed and tested to allow the trial to be double-blinded. Egg free and egg containing products usually differ in appearance, texture, odour

and taste. The trial products were tested on a consumer group to ensure the blinding was adequate, and parents of participants in the study will be asked to guess which group they were assigned to at the end of the study.

DURATION OF DIETARY INTERVENTION

Treatment will start after randomisation. Parents will be provided with dietary instruction on how to follow an egg-free diet, the study product, and instructions on how to maintain the intake record.

After 6 months the study products will be ceased.

COMPLIANCE

Compliance will be assessed at a review appointment one month into the intervention and by monthly phone calls to check consumption and tolerance to the baked good. Participants will be asked to return unused products. The product returned will also serve as a measure of compliance.

1.1.8 STUDY PROCEDURES

SCREENING

1. Informed consent, including screening form checklist completed.
2. Baseline data, including alternate contact details basic demographic and health information (parental education, parental smoking, parental history of allergic disease, birth order and sex of the child), and the child's weight and length / height will be measured. Questions relating to child's symptoms of allergic disease (including eczema SCORAD) will also be asked.
3. Skin Prick Testing (SPT) to egg (whole egg, egg white, egg yolk, ovalbumin and ovomucoid) will be performed.
4. Child's weight, length and head circumference will be measured.
5. A venous blood sample (5 ml) will be collected and used to measure specific IgE to whole egg, egg white, peanut, ovalbumin and ovomucoid, IgG4 to whole egg, ovalbumin and ovomucoid, and immune markers of tolerance.
6. The child will have a medically supervised oral baked egg food challenge.
7. The child will have a medically supervised oral pasteurised raw whole egg food challenge.
8. Usual diet will be assessed to ensure that it is egg free.

ENROLMENT AND RANDOMISATION

1. Randomisation to treatment assignment. Children will be assigned to treatment groups.
2. Products will be supplied, and the supply inventory completed.
3. Instruction regarding an egg-free diet.
4. Product intake record form will be provided and the family will be instructed in how to complete it.
5. Allergic reaction action plan and record sheet will be provided.
6. CRF will be completed.

ONE MONTH VISIT

A review appointment will be scheduled at one month. At this visit:

1. Measure weight and length / height.
2. Check consumption and tolerance to the baked product (eg no flaring eczema).
3. Check any accidental exposure to egg.
4. Check compliance with the egg free diet.
5. Complete questions relating to child's symptoms of allergic disease (including eczema SCORAD).

MONTHLY TELEPHONE CALLS

1. The participating child's mother/father/guardian will be telephoned each month from the second month of study commencement up to and including the sixth month of intervention. The telephone call should be made within the week that it is due and not prior.
2. The CRF questions will be completed at each of these telephone calls. These questions will prospectively capture information regarding consumption and tolerance to baked egg product, compliance with the egg-free diet, any accidental exposure to egg and document any possible food allergy symptoms including eczema.

6 MONTH TELEPHONE CALL

In addition to the above questions, when the child has been in the study for 6 months, the participating child's mother/father/guardian will be advised to cease the study product and continue an egg free diet.

For children with active eczema, a SCORAD will be performed.

At this phone call the family will be invited to return for the optional blood draw, which will be used to determine IgE to whole egg, peanut, ovalbumin and ovomucoid, IgG4 to whole egg, ovalbumin and ovomucoid, and immune markers of tolerance.

PRIMARY OUTCOME ASSESSMENT AND RAW EGG ORAL FOOD CHALLENGE

1. This appointment should be conducted as close to 1 month after ceasing the baked egg product as possible, at the Women's and Children's Hospital (South Australia).
2. Child's weight, length and head circumference will be measured.
3. A venous blood sample (5 ml) will be collected and used to measure IgE to whole egg, ovalbumin and ovomucoid, IgG4 to whole egg, peanut, ovalbumin and ovomucoid, and immune markers of tolerance.

4. Skin Prick Testing (SPT) to egg (whole egg, egg white, egg yolk, ovalbumin and ovomucoid) will be performed.
5. The CRF questions need to be completed. These questions will include information on allergic symptoms (including SCORAD for eczema), general health and hospitalisations during the study.
6. The child will have a medically supervised oral pasteurised raw whole egg food challenge.

PASTEURISED RAW WHOLE EGG ORAL FOOD CHALLENGE PROTOCOL:

Children will proceed to a graded oral food challenge. Six doses, one drop inside lip, 1 ml (0.1g egg protein), 2 ml, 5 ml, 10ml and 20ml (2.0g egg protein), will be given at 15 minute intervals and then the child will be observed for 2 hours after the last dose.

An allergic reaction to egg will be defined as the development of symptoms within 2 hours of the egg challenge and include 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 must include circulatory and/or respiratory involvement)(14).

Full emergency resuscitation facilities will be available and an age appropriate dose of adrenaline will be immediately available for each child. For any child who experiences an allergic reaction to the egg challenge, the families will be advised to continue the egg-free diet for the child and will be referred back to their local doctor with advice to be referred on for further assessment by a paediatric allergist. Paediatric allergists (Dr Gold or Dr Quinn) will train and monitor the assessments undertaken by the medical practitioners employed by the study to ensure they follow standardised protocols for the skin prick testing, egg challenge and management of acute allergic reactions including anaphylaxis. The nursing staff and medical practitioners supervising the oral food challenges will be blinded to the dietary group allocation.

Unless the child experiences an allergic reaction to egg, the families will be advised to include all forms of egg containing foods in the child's diet.

<u>Challenge protocol for children with a history of anaphylaxis:</u>

For children with a history of anaphylaxis the challenges will be performed in the morning so as to minimise the chance of needing to treat patients into the early evening. The challenge protocol for will be modified to increase the number of doses while keeping the same total dose of egg the same. This will give the opportunity for challenge to be ceased should minor reactions occur at low doses and provide some mitigation against more severe reactions at higher doses. For those children we consider to be at higher risk we will place an IV line prior to the food challenge – this will be used to take the planned blood sample instead of a venepuncture. We will re-consent children for this IV.

TRIAL MANAGEMENT

A trial Steering Committee will consist of all Chief Investigators (CIs) and will be chaired by Mrs Merryn Netting and deputy chair Professor Maria Makrides. A monthly meeting of all Investigators will occur to monitor the progress of the trial and actively consider strategies to maintain the monthly recruitment rate.

An independent blinded Serious Adverse Event Committee will consist of an allergist, paediatrician and a pathologist not involved with the trial. This committee will meet 6 monthly (or as required) to review deaths, admissions to level 3 hospital care or anaphylactic reactions. The primary role of the Serious Adverse Event Committee is to review all Serious Adverse Events to determine whether there is any likelihood that involvement in the trial could have contributed to the event. The cause of the event is determined from the autopsy results or other hospital summary of the event by the relevant medical personnel.

The study will be registered with the Australian Clinical Trials Registry.

STATISTICAL CONSIDERATIONS

1. SAMPLE SIZE CALCULATIONS AND CLINICAL SIGNIFICANCE OF PROPOSED EFFECT SIZE

Based on the known natural history of egg allergy we expect that after six months of treatment with an egg free diet that 90% of children will still be egg allergic (5). We hypothesise that regular exposure to baked egg will result in **30% absolute reduction** (ie from 90% to 60%) of egg allergy. To detect such a difference with **90% power** and p=0.05, we will require **49 children per group** (total n=98). We will aim to recruit 55 children to each group to allow for withdrawals from the study.

Feasibility of attaining the required sample size: We plan to recruit 4 children per week. Recruitment is to commence in February 2011 and will end in December 2011.

2. DATA ANALYSIS

All analyses will be on an intention-to-treat basis. For the primary analysis, the proportion of children reacting to the second pasteurised raw egg challenge will be compared between treatment groups using an identity binomial generalized linear model, adjusting for the pre-specified prognostic stratification variables.

In secondary analyses we will compare the IgE, IgG4 and IgE/IgG4 ratios taken at the various time points between treatment groups.

Finally, we will examine for differences in immune markers of tolerance between treatment groups.

SERIOUS ADVERSE EVENTS AND ADVERSE EVENT REPORTING

SERIOUS ADVERSE EVENTS

A serious adverse event (SAE) is any untoward medical experience in an child that suggests a significant hazard. A SAE is any event which:

results in death;

is life-threatening (such as admission to level 3 hospital care or anaphylactic reactions);

Serious adverse events that are life-threatening or result in death should be notified immediately to the Chair of the Steering Committee, Merryn Netting. Any event related to the study protocol that in the opinion of a Chief Investigator may be of immediate or potential concern for a child's health or well being will also be reported immediately to the Chair of the Ethics Committee.

The independent Serious Adverse Event Committee will review all deaths and SAEs. If a child dies any post-mortem findings, including histopathology, must be provided to the Coordinating Centre to allow full independent review.

EMERGENCY CONTACT DETAILS

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The Investigator, or nominee, will also be responsible for reporting any serious adverse events to their HREC as soon as they have been reviewed by the Serious Adverse Event Committee. In agreeing to the provisions of the protocol, these responsibilities are accepted by the Investigator, or nominee.

ADVERSE EVENTS

An adverse event (AE) is any untoward medical experience in a child during a clinical study, whether or not considered related to the study products. Minor AEs will be recorded in the CRF with particular focus on allergic reaction symptoms. Hospitalisations (of more than 24 hours) will be recorded in the CRF including the length of hospital stay and the primary diagnoses.

In the case of children who appear to have worsening of their allergy symptoms on the study product, the child will have their eczema assessed by SCORAD and then the

product will be ceased for 2 weeks. The eczema will be re scored. The product will then be reintroduced (in the MDU).

STUDY APPROVAL AND CONDUCT

REGULATORY APPROVAL

The requirement for the conduct of clinical trials in accordance with the Clinical Trial Notification (CTN) scheme of the Australian Therapeutic Goods Administration (TGA) will be met before commencement of this study.

HUMAN RESEARCH ETHICS COMMITTEE (HREC) APPROVAL

Approval in writing from the Human Research Ethics Committees (HREC) shall be granted prior to the initiation of the study. Any study amendments to the protocol shall have written approval by the HREC.

ETHICAL CONSIDERATIONS

This study will be carried out in accordance with the Principles of International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) (as adopted in Australia) which builds upon the ethical codes contained in the current version of the Declaration of Helsinki and the Australian National Statement on Ethical Conduct in Research Involving Humans.

All data collected will be treated with confidence and parents/guardians will be free to withdraw their child from the study at any time, without explanation and without prejudice to their future care.

WRITTEN INFORMED CONSENT

A parent/guardian will have the study explained by a Chief Investigator or nominee. They will receive a full explanation, in lay terms, of the aims of the study as well as the discomfort, risks and benefits of participation. This explanation shall include insurance and other procedures for compensation in case of injury. The study is for research purposes and any therapeutic benefit to the individual is unknown. The parent/guardian's right to withdraw their child from the study at any time without prejudice will be confirmed.

The parent/guardian will be required to provide written informed consent and be given a copy of the signed Consent Form.

EMERGENCY CONTACT WITH INVESTIGATORS

Mrs Merryn Netting and Prof Maria Makrides will ensure they remain contactable in the event of an emergency.

EMERGENCY CONTACT DETAILS

Merryn Netting
Women's and Children's Health Research Institute
Women's & Children's Hospital
72 King William Road
North Adelaide
South Australia 5006
Telephone: 08 8161 6067
Facsimile: 08 8239 0267
Mobile:

Prof Maria Makrides
Women's and Children's Health Research Institute
Women's & Children's Hospital
72 King William Road
North Adelaide
South Australia 5006
Telephone: 08 8161 6067
Facsimile: 08 8239 0267
Mobile:

WITHDRAWALS

Parents/guardians will be advised that they are free to withdraw their child from the study at any time. The reasons for withdrawal will be recorded on the CRF and included in the final report. Childs who discontinue or are withdrawn will not be replaced.

STUDY DOCUMENTATION

Original/copies of study documents listed below will be retained at both the Coordinating Centre and the Study Site or in archives. Retention of documents shall either be for at least 21 years after completion of the study.

The material to be stored shall include the following:

1. Signed and dated copy of the final study protocol and any amendments.
2. Signed and dated letter of HREC approval, letter of constitution of the HREC and copies of any other correspondence relevant to the study with the HREC or regulatory authorities.
3. The HREC approved Study Information Form/Informed Consent Form.
4. Current curriculum vitae (signed and dated) of the Investigators.
5. Blank CRF.
6. Signed subject informed consent forms.
7. The completed CTN Application Form.
8. CRFs, treatment accountability forms, and dispensing records, etc.

ACCESS TO SOURCE DOCUMENTS

In order to ensure the accuracy of data collected on the CRFs, representatives from the Coordinating Centre and regulatory authorities will have access to source documents (i.e. child's medical records). Confidentiality will be maintained at all times.

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APPENDIX 5

RANDOMISED CONTROLLED TRIAL (CAKE STUDY) INFORMATION SHEET

Can Egg Allergic Kids Eat Baked Egg? The CAKE Study

INFORMATION SHEET

You are invited to participate in a study that aims to find out if eating products that contain baked egg helps raw egg allergic children outgrow their allergy to raw egg.

Allergies are common and result in a significant burden to families and the health care system. Children with egg allergies were traditionally advised to avoid all forms of egg in their diet, even though they could tolerate products that contain baked egg (eg cake, biscuits or muffins). Recent research indicates that up to 70% of children with raw egg allergy can eat egg baked in a cake or muffin without apparent reaction, and this is associated with changes in the immune system as it moves from reacting to egg protein to ignoring it. This study will help to find out if eating baked egg helps the development of tolerance to raw egg in children with allergy to raw egg.

WHO CAN PARTICIPATE IN THIS STUDY?

- Children between 6 months and 5 years of age with;
  - A known egg allergy because they have eaten egg and had an immediate reaction or
  - Suspected egg allergy because they have had a documented positive skin test or blood test to egg yet have not eaten egg and had a reaction
- **All children need to be on an egg free diet**
- Children who are free of illnesses likely to affect their ability to have a food challenge.

WHAT WILL HAPPEN BEFORE THE STUDY STARTS?

Finding out which children still have an egg allergy

The first part of the study involves finding out if your child is still allergic to egg and if they are if they can tolerate egg in baked goods. This will be determined by performing two oral food challenges, one to baked egg, and if passed, another to pasteurised whole raw egg to confirm a raw egg allergy. The challenges will be done at the Women's and Children's Hospital, in the medical day unit.

The children who will be included in the study are those who tolerate baked egg and who don't tolerate raw egg.

WHAT WILL HAPPEN DURING THE STUDY?

How these children with an egg allergy will be studied

All children will continue on an egg free diet. This means that we will ask that you continue to avoid giving your child any egg for the duration of the study (6 months). We will provide free of charge baked products (biscuits, muffin or bread) and ask that your child be encouraged to eat a serve of this every second day for six months.

Children in the study will be divided into two groups and both groups will follow an egg free diet as outlined above. For one group the baked products we supply will contain egg and the other group will be given products that look and taste identical, but are egg free. We will provide you with a simple tick box chart to record which days the study product was given to your child during the study. Neither you nor the research team will be able to choose which group your child is in and you will not know which group your child is in until after the study is completed.

After six months the product will be stopped, and your child will come back to the medical day unit for skin prick testing and another pasteurised raw egg challenge. If your child does not pass the pasteurised raw egg challenge at 6 months you will be offered a repeat egg in baked goods challenge to ensure that you can offer your child commercial and home prepared baked items containing egg.

All of the children in the study will have two blood tests (after each of the raw egg challenges) to measure their levels of antibodies to egg and peanut, and to look at some other immune measurements of allergy, and there is the option of a third blood test when the baked egg product is stopped. We will test for peanut allergy as many children with egg allergy also have peanut allergy.

Clinic Visit

- At the routine Allergy clinic visit you will be asked some questions about any symptoms of allergies.
- At the Allergy clinic visit your child will have a skin prick test to determine whether s/he has a reaction to egg. In this test, a liquid containing tiny amounts of these allergens is placed on your child's back and then the skin is gently scratched inside the droplet. If your child has an allergic response a small bump (or weal) will develop. This may cause some temporary discomfort such as itching. The weal should subside within 2 hours.

Oral Food Challenges to Baked Egg and Pasteurised Raw Egg

- Children with egg allergy and skin prick test results to egg with a weal greater than 3 mm will attend the medical day unit for two, three or four oral food challenges depending on their circumstances. All children will have a baked egg challenge to start. If after this and from the testing it looks like you child might not be allergic to egg, your child will have a second challenge, this time with pasteurised egg. Children who pass this second challenge will not continue in the study as they are no longer allergic to egg. All children who continue in the study will have a pasteurised egg challenge after 7 months. Those children who react to this 7 month challenge will have a further egg in baked goods challenge to prove they are still tolerant to egg in this form even though they have reacted to the pasteurised egg after 7 months.
- The food challenges will take 5 to 6 hours each.

- During a food challenge your child will be given either a muffin containing egg or pasteurised raw whole egg powder (mixed in apple puree or custard) to eat and you will be asked to wait for 4 hours to watch for any signs of an allergic reaction
- When your child is given egg during the egg challenge a medical person will be at the appointment should there be an allergic reaction such as hives, generalised skin rash or severe reactions like anaphylaxis (this may include breathing difficulties and/or low blood pressure). If an allergic reaction occurs your child will be treated immediately. Serious allergic reactions like anaphylaxis may occur but are more likely if your child has had a previous anaphylaxis. If your child has a previous history of anaphylaxis to egg an IV line may be inserted prior to the 7 month pasteurised egg challenge, and this will be used to take the blood test (discussed below), and also used in case your child reacts during the egg challenge.
- If your child has been hospitalised, we will need to access your child's medical records to document the reasons for hospitalisation and the treatments given.
- There will be a $20 reimbursement per visit for each of the food challenges to assist with travel costs associated with the hospital visits.
- You will receive a phone call one week after each of the egg challenges to ask if there were any delayed symptoms of food allergy after the food challenge.

Blood Testing

- At the Medical Day Unit visits for the raw egg challenges an experienced nurse will collect a sample of your child's blood. This may cause brief discomfort, which can be minimised by using an anaesthetic cream and by comforting the child. Temporary bruising can occur and infection is possible but extremely rare. This blood sample will be used to measure antibodies to egg and other immune markers (substances like antibodies) that provide information about the immune system.
- If for any reason the blood sample is unable to be taken, your child will still be able to have an egg challenge.
- A third blood test when your child stops consuming the baked egg product is optional, and would provide the research team with information about the development of immune tolerance.

STORAGE OF BLOOD SAMPLES

With your consent we will also store a small portion of the bloods taken, and these may be used for future tests to measure how your child's immune cells react to egg protein. These samples will not be used in any other testing without your consent.

ANY RISKS?

All children with egg allergy will have a food challenge to egg at some stage to see if they have 'outgrown' their egg allergy. In this study we will closely observe all children during this process in a hospital setting and a medical person will be at the appointment should there be an allergic reaction. Possible allergic reactions include hives, generalised skin rash or severe reactions like anaphylaxis. If an allergic reaction occurs, your child will be treated immediately.

YOUR RIGHTS

Your participation in this study is voluntary, and you are free to withdraw from the study at any time without any explanation of why you have chosen to do so and without prejudice to you and your child's future care. All information gathered will be treated with confidence and no information that could identify you or your child will be released to any person not associated directly with the study, except in the case of a legal requirement to pass on personal information to authorised third parties. This requirement is standard and applies to information collected both in research and non-research situations. Such requests to access information are rare; however we have an obligation to inform you of this possibility. These results may eventually be published in medical journals or at professional meetings, but you or your child will not be identified in any way.

ANY QUESTIONS?

If at any time during the study you have any problems regarding appointments or have any other queries, please ring our office on 8161 8045 and leave a message on our answering machine; one of our research staff will return your call as soon as possible. If you have a problem and would like to talk to us immediately please ring 8161 7000 and ask for pager 4670, and one of our research staff will answer your call.

WCHN HUMAN RESEARCH ETHICS COMMITTEE CONTACT DETAILS

This study has been reviewed and approved by the Children, Youth & Women's Health Network Research Ethics Committee. Should you wish to discuss the study with someone not directly involved, in particular in relation to matters concerning policies, information about the conduct of the study or your rights as a participant, or should you wish to make a confidential complaint, you may contact the Secretary of the Research Ethics Committee, Ms Brenda Penny, phone 8161 6521.

APPENDIX 6

RANDOMISED CONTROLLED TRIAL (CAKE STUDY) CONSENT FORM

CHILDREN, YOUTH & WOMEN'S HEALTH NETWORK (CYWHN)
HUMAN RESEARCH ETHICS COMMITTEE (HREC)

CONSENT FORM

Lay Title: Can Egg Allergic Kids Eat Baked Egg? The CAKE Study

SCIENTIFIC TITLE: Does Consumption of Baked Egg Hasten Development of Tolerance to Raw Egg in Children with Egg Allergy? – A Randomised, Controlled Trial.

I, _____

hereby consent to my child's involvement in the research project entitled:

Does Consumption of Baked Egg Hasten Development of Tolerance to Raw Egg in Children with Egg Allergy? – A Randomised, Controlled Trial.

1. The nature and purpose of the research project described on the attached Information Sheet has been explained to me. I understand it and agree to my child taking part.
2. I understand that my child may not directly benefit by taking part in this study.
3. I acknowledge that the possible risks and/or side effects, discomforts and inconveniences, as outlined in the Information Sheet, have been explained to me.
4. I understand that I can withdraw from the study at any stage and that this will not affect medical care or any other aspects of my child's relationship with this healthcare service.
5. I understand that there will be a $20 reimbursement per visit for food challenges to assist with travel costs associated with the hospital visits. There is no reimbursement for the initial clinic visit.
6. I have had the opportunity to discuss taking part in this research project with a family member or friend, and/or have had the opportunity to have a family member or friend present whilst the research project was being explained by the researcher.
7. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.

8. I consent to my child having the following procedures:
   - Skin prick testing at the beginning of the study and prior to the final pasteurised raw whole egg challenge.
   - Two blood tests, one before commencing the baked egg product, and one at the final pasteurised raw whole egg challenge.
   - Medically supervised egg challenges to pasteurised raw whole egg and to baked egg.

9. I consent to my child having an optional blood test when she/he ceases consuming the baked egg product. ☐ yes ☐ no

10. I consent to samples of blood being stored for future tests to measure immune cell function. These samples will not be used in any other testing without your consent. ☐ yes ☐ no

11. I understand that study personnel may review my child's medical records at the Women's and Children's Hospital.

12. I understand that my child's information will be kept confidential as explained in the information sheet except where there is a requirement by law for it to be divulged.

Full name: .....

Signed: .....

Full name of participant (child): .....

Dated:.....

I certify that I have explained the study to the parent / guardian of the participant and consider that he/she understands what is involved.

Signed: ..... Title: .....

Dated: .....

APPENDIX 7

MATERIALS AND METHODS

This appendix describes the materials and methods used for investigations into the allergenicity of pasteurised whole raw egg powder (Chapter 3), and the immunophenotyping and cytokine analysis after culture with egg antigens of peripheral blood mononuclear cells from children enrolled in the CAKE study (Chapter 6).

MATERIALS

All materials unless otherwise specified were of analytical reagent grade.

APPENDIX 7 TABLE 1 REAGENTS USED FOR TISSUE CULTURE

AIM-V media (serum free)	Invitrogen, Life Technologies Sydney, Australia
BD Falcon conical tubes	BD Biosciences, USA
Cryo 1°C freezing container "Mr Frosty"	Nalgene, Denmark
Cryovials (2ml barcoded)	Interpath Services, Vic, Australia
Dimethyl sulphoxide (DMSO)	Ajax, Vic, Australia
Fetal Bovine Serum (FBS)	Sigma-Aldrich, Sydney, Australia
Isopropanol	Sigma-Aldrich, Sydney, Australia
Lymphoprep™	Axis- Shield, Oslo, Norway
Nunclon sterile round-bottomed 96 well plates	Thermo scientific Sydney, Australia
Ovalbumin (Albumin from chicken egg white) (OVA)	Sigma-Aldrich, Sydney, Australia
Ovomucoid Type III-0 (OVM)	Sigma-Aldrich, Sydney, Australia
Phytohemagglutinin-L (PHA-L)	Roche Diagnostics, Australia
Phytohemagglutinin-L (PHA-L)	Remel, KS, USA (Kindly donated by Prof A Ferrante)
Roswell Park Memorial Institute Medium (RPMI-1640 Media, RPMI)	Sigma-Aldrich, Sydney, Australia
Trypan Blue Solution 0.4%	Sigma-Aldrich, Sydney, Australia

APPENDIX 7 TABLE 2 REAGENTS USED FOR WESTERN BLOTTING

Acetic acid	Sigma-Aldrich, Sydney, Australia
Bis-tris Methane (Bis Tris)	Sigma-Aldrich, Sydney, Australia
Bromophenol Blue	Sigma-Aldrich, Sydney, Australia
β Mercapto ethanol	Sigma-Aldrich, Sydney, Australia
Calcium Chloride ($CaCl_2$)	Sigma-Aldrich, Sydney, Australia
Colipase from Porcine pancreas	Sigma-Aldrich, Sydney, Australia
Coomassie Brilliant Blue stain	Sigma-Aldrich, Sydney, Australia
Goat Anti-Human IgE HRP	Invitrogen, Life Technologies, Sydney, Australia
Glycerol	Chem-Supply Pty Ltd SA, Australia
Glycine	Amresco, OH, USA
Hydrochloric Acid (HCl)	Sigma-Aldrich, Sydney, Australia
Methanol (MeOH)	Chem-Supply Pty Ltd SA, Australia
Pepsin from Porcine gastric mucosa	Sigma-Aldrich, Sydney, Australia
Phosphate Buffered Saline (PBS)	Sigma-Aldrich, Sydney, Australia
Pierce BCA Protein Assay Kit	Thermo scientific Sydney, Australia
Polyethylene glycol sorbitan monolaurate (Tween 20)	Sigma-Aldrich, Sydney, Australia
Ponceau Red Stain	Sigma-Aldrich, Sydney, Australia
Precision Plus Protein™ Kaleidoscope™ Standards marker	Bio-Rad Laboratories, NSW Australia
4-15% SDS-Polyacrylamide gel (Mini-Protean TGX Precast gel: 4-15% resolving 10 well/ 50µl volume)	Bio-Rad Laboratories, NSW, Australia
Sodium Bicarbonate ($NaHCO_3$)	Sigma-Aldrich, Sydney, Australia
Sodium Chloride (NaCl)	Sigma-Aldrich, Sydney, Australia
Taurocholic acid sodium salt hydrate	Sigma-Aldrich, Sydney, Australia
Trizma base	Sigma-Aldrich, Sydney, Australia
Trizma® hydrochloride (Tris-HCl)	Sigma-Aldrich, Sydney, Australia
Sodium dodecyl sulphate (SDS)	Medicago, Upsala, Sweden
Super Signal West Femto Maximum Sensitivity Substrate Kit	Pearce Biotechnology, Rockford, USA

APPENDIX 7 TABLE 3 REAGENTS AND KITS USED FOR FLOW CYTOMETRIC ANALYSIS

12x75mm polypropylene tubes (FACS tubes)	Evergreen Scientific, CA, USA
BD™ CompBeads Set Anti-Mouse Ig	BD Biosciences, USA
BD Biosciences enhanced sensitivity flex sets: Human IL-4 (#561510), Human IFNg (#561 515) Human IL-5 (#561511)	BD Biosciences, USA
BD Cytometric Bead Array Human Inflammatory Cytokine Kit	BD Biosciences, USA
Bovine Serum Albumin	Sigma-Aldrich, Sydney, Australia
Dynabeads CD4 positive isolation kit	Invitrogen, Life Technologies, Sydney, Australia
Ethylenediamine tetraacetic Acid (EDTA)	Sigma-Aldrich, Sydney, Australia
Human Enhanced Sensitivity Master Buffer Kit (#561523)	BD Biosciences, USA
Paraformaldehyde	Chem-Supply Pty Ltd SA, Australia
Sodium azide	Sigma-Aldrich, Sydney, Australia

APPENDIX 7 TABLE 4 ANTIBODIES USED FOR FLOW CYTOMETRIC ANALYSIS

Target antigen	Clone	Isotype	Conjugate	Manufacturer	Catalogue Number
CD4	SK3	IgG1	Per CP	BD Biosciences, USA	347324
CD4	SK3	IgG1	PE-Cy7	BD Biosciences	348 789
CD8	RPA-T8	IgG1, κ	PE-Cy7	BD Biosciences	557746
CD45RA	HI100	IgG2b, κ	APC	BD Biosciences, USA	550855
CD14	27-35	IgG2b, κ	APC-Cy7	BD Biosciences, USA	557801
CD19	HIB19	IgG1, κ	PE	BD Biosciences, USA	555413
HLA-DR	G46-6	IgG2a, κ	FITC	BD Biosciences, USA	555811
CD45RO	UCHL1	IgG2a, κ	FITC	BD Biosciences, USA	555492
CD69	FN50	IgG1, κ	PE	BD Biosciences, USA	555531
CD197 (CCR7)	FR11-11E8	IgG1	PE	Miltenyi Biotec, USA	130-093-621
CD27	M-T271	IgG1, κ	APC-Cy7	BD Biosciences, USA	560222
CD28	CD28.2	IgG1, κ	FITC	BD Biosciences, USA	555728

BUFFERS AND SOLUTIONS USED FOR WESTERN BLOTTING AND FLOW CYTOMETRIC ANALYSIS

Heat inactivated Fetal Bovine Serum (HI FBS)
Heat inactivated Fetal Bovine Serum (HI FBS) was inactivated for 30 minutes at 56°C. Aliquots stored at -4 °C.

125mM Tris pH6.8		
Compound	Concentration	Quantity
Tris Base MilliQ Water	125mM	1.51 Up to 100ml
Adjust to pH 6.8 prior to addition of DDH20 to 100ml.		

5xSDS Loading Buffer		
Compound	Concentration	Quantity
Tris pH6.8 Glycerol SDS Bromophenol Blue β Mercapto ethanol	125mM 40% (v/v) 5% (w/v) 0.1% (v/v) 0.05% (v/v)	Up to 10ml 4ml 0.5g 10 µl 500 µl
Aliquots stored at -4°C		

Isopropanol fixing solution		
Compound	Concentration	Quantity
isopropanol acetic acid MilliQ Water	25% 10%	250ml 100ml 750ml
Stored at room temperature.		

Running Buffer (25mM Tris; 192mM Glycine; 1% SDS)		
Compound	Concentration	Quantity
Tris-HCl (pH8.3)	0.025M (w/v)	3.3g
glycine	0.192M (w/v)	14.4g
SDS	0.1% (w/v)	10g
MilliQ Water		1000ml
SDS added last. Stored at 4°C.		

Wet Transfer Buffer – Towbins Buffer (25mM Tris; 192mM Glycine; 10% MeOH)		
Compound	Concentration	Quantity
Tris base	25mM	3.3g
Glycine	192mM	14.4g
Methanol	10%	100ml
MilliQ Water		Up to 1000ml
Stored at 4°C.		

Tris-Buffered Saline (TBS)		
Compound	Concentration	Quantity
10x Trizma Base, (pH7.6)	10%	100ml
10x NaCl	10%	100ml
MilliQ Water		800ml
Stored at 4°C.		

TTBS (TBS + 10% Tween 20)		
Compound	Concentration	Quantity
TBS		100ml
10%Tween 20	0.5% (v/v)	0.5ml
Stored at 4°C.		

Blocking solution (12% skim milk powder in TBS)		
Compound	Concentration	Quantity
TBS Skim Milk Powder (SMP)	 12% (w/v)	100ml 12g
Stored at 4°C.		

MACS buffer (0.5% BSA and 2mM EDTA in PBS)		
Compound	Concentration	Quantity
Bovine Serum Albumin EDTA PBS	0.5% (w/v) 2mM 	 500ml
Aliquots stored at -4°C.		

PBS/Azide buffer (0.05% Sodium azide in PBS)		
Compound	Concentration	Quantity
Sodium azide PBS	0.05% (w/v) 	 1000ml
Stored at 4°C.		

FACS fix (2% paraformaldehyde in PBS)		
Compound	Concentration	Quantity
paraformaldehyde PBS	2% (v/v) 	2ml 98ml
Aliquots stored at -4°C.		

METHODS

Centrifugation of all samples was performed in either a Sigma 4K15 or an Eppendorf 5415R bench top centrifuge at room temperature.

PREPARATION AND DETERMINATION OF PROTEIN CONTENT OF EGG SAMPLES

PASTEURISED RAW WHOLE EGG POWDER

The protein content of Farm Pride Whole Pasteurised Raw egg powder (Farm Pride, Keysborough, Victoria, Australia) was assessed. 0.5g pasteurised egg powder was diluted in 5ml of Phosphate Buffered Saline (PBS), centrifuged for 5 minutes at 5000 xg, and the supernatant collected. The protein concentration of the supernatants was determined using a Pierce BCA Protein Assay Kit as per the manufacturer's instructions for a microplate procedure. Briefly, diluted Albumin Standards were prepared. 25ul of each standard or unknown sample was pipetted in triplicate into a 96 well microplate. 200µl of working reagent was added to each well, and the plate mixed thoroughly on a plate shaker for 30min. The plate was then incubated at 37°C for 30 mins. After cooling to room temperature the absorbance was measured at 562nm on a Sunrise™ microplate absorbance reader (Tecan Trading AG, Switzerland). The average 562nm absorbance measures of the blank standard replicates were subtracted from the 562nm measures of all other samples, and a standard curve was generated using Table curve 2D software v4 (Systat Software Inc. CA, USA), and used to determine the protein concentration of the diluted egg samples. The protein content of the supernatant was determined to be 33 +/- 5 mg/ml

FRESH EGG

The protein content of 60g free-range fresh eggs (Coles Pty Ltd) was assessed. It was estimated (from the documented protein content of whole egg {Zealand, 2011 #214} that 1.2g of fresh egg would provide the equivalent amount of protein as 0.5g pasteurised whole egg powder and so 1.2g samples of whole egg, egg white and egg yolk were diluted in 2.5ml PBS and homogenised (using a Heidolph Diax 600 homogeniser). The samples were

centrifuged at 5000 xg and the supernatant collected. The protein content of each supernatant was determined to be 35 +/- 5 mg/ml using a Pierce BCA Protein Assay Kit as per the manufacturer's instructions.

INVITRO DIGESTION OF EGG PROTEIN

The method for *in vitro* digestion of egg protein was adapted from Martos et al {Martos, 2011 #263}. To simulate gastric digestion, samples of pasteurised egg powder, whole egg, egg white and egg yolk were diluted in 1% PBS, adjusted to pH2 with 39% HCl and then incubated for 15 min at 37°C. Pepsin from Porcine gastric mucosa at an enzyme to substrate ratio of 1:20 (wt:wt) was added. The samples were incubated for 60 min at 37°C. The reaction was stopped with 1mol/L NaHCO3 to give a final protein concentration of 5mg/mL and pH7. To simulate duodenal digestion, the gastric digests were adjusted to pH7 with 1mol/L $CaCl_2$ and 0.25mol/L Bis Tris pH6.5. 6.15 mmol/L of Taurocholic acid sodium salt was then added and the samples were incubated at 37°C for 15 min. Colipase from Porcine pancreas, prepared in 35mmol NaCl adjusted to pH7 was then added to an enzyme to substrate ratio of 1:895.

The protein content of the *in vitro* digested supernatants was determined to be 4.8 +/- 0.1 mg/ml using a Pierce BCA Protein Assay Kit, as per the manufacturer's instructions.

SODIUM DODECYL SULPHATE POLYACRYLAMIDE (SDS-PAGE) GEL ELECTROPHORESIS OF EGG PROTEINS

Egg proteins (whole egg, egg white, egg yolk, pasteurised egg, ovalbumin and ovomucoid) were electrophoresed on a 4-15% SDS-Polyacrylamide gel (Mini-Protean TGX Precast gel: 4-15% resolving 10 well/ 50µl volume) as per the manufacturer's instructions. 30µl of each egg sample combined with 6 µl 5xSDS Loading Buffer was loaded into each lane. 5µl of Precision Plus Protein™ Kaleidoscope™ Standards marker was loaded on each gel as a standard. Electrophoresis was carried out in 0.025M (w/v) Tris-HCl (pH8.3), 0.192M (w/v) glycine, and 0.1% (w/v) SDS at 100V for 90 min in the electrophoresis tank. Gels were then fixed with Isopropanol fixing solution and stained with Coomassie Brilliant Blue stain for 90 min. The gel was de-stained overnight with 10% acetic acid solution and then photographed using GeneSnap analysis software program (Syngene, Maryland, USA).

To optimize the method, egg samples were loaded onto the gel at 1, 2, 3, 4 and 5 mg/ml dilutions. For all samples dilution to 2mg/ml, providing 60µg of protein loaded per well, gave optimal results.

POOLED SERA FOR USE IN IMMUNOBLOTTING

Blood was collected from five children (aged 2.6 to 3.9 years) with known egg allergy who had failed an egg in baked goods oral food challenge as part of the screening for the CAKE study (REC2400/9/14). Blood was centrifuged at 850xg for 10 min. Serum was collected and aliquots were stored at -80°C.

WESTERN BLOT ANALYSIS OF EGG PROTEINS USING POOLED SERA FROM EGG ALLERGIC CHILDREN.

To compare the binding of serum IgE from egg allergic children to *in vitro* digested and undigested egg proteins (whole egg, egg white, egg yolk, pasteurised egg, ovalbumin and ovomucoid) a Western Blot Analysis was performed. Concentrations of the pooled serum and the secondary antibody were titrated and optimised prior to the western blot analysis.

The egg proteins separated on the SDS-PAGE gels were transferred onto 0.2µm pore nitrocellulose membranes (Advantec MFS Inc, SA, USA) by the process of Wet Transfer using the Bio-Rad Mini-Protean II Transfer Apparatus (Bio-Rad Laboratories, NSW, Australia) and 1x Wet Transfer Buffer – Towbins Buffer at 100V for 1 hour. The membrane was then stained in Ponceau Red Stain for one minute and de-stained in MilliQ water to check for transfer efficiency. Membranes were rinsed in TBS (2x5 min), and then blocked for non-specific binding with followed by blocking solution for 40 min (12% skim milk powder (SMP) in TBS), rinsed in TBS and TTBS (2x5 min) then incubated with pooled sera from children with known egg allergy (diluted 1:4 with TTBS +0.5% SMP) shaking at 4°C overnight. Membranes were washed in TTBS (2x5 min) and then incubated with the secondary detection antibody Goat Anti-Human IgE HRP (diluted 1:700 with TTBS +0.5% SMP) for 40 min, rinsed with TTBS (2x5 min), then TBS (2x5 min). Membranes were developed using Super Signal West Femto Maximum Sensitivity Substrate Kit (Pearce Biotechnology, Rockford, USA) according to the manufacturer's instructions and the signal captured using GeneSnap analysis software program (Syngene, Maryland, USA).

PERIPHERAL BLOOD SAMPLE COLLECTION

Peripheral blood was collected into Lithium Heparin tubes from all children screened for the CAKE study and at the end of the intervention for children enrolled in the CAKE study. All parents provided written informed consent and research ethics approval was obtained from the Adelaide Women's and Children's Health Network, Human Research Ethics Committee (HREC 2400/9/14). The protocol for the CAKE Study is described in Chapter 6.

ISOLATION AND CRYOPRESERVATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS

Whole blood was centrifuged at 820xg for 10 min. Plasma was collected using a sterile Pasteur pipette and aliquots were stored at -80°C for determination of serum specific IgE / IgG4 levels.

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation over Lymphoprep™: Where collected blood volumes were greater than or equal to 5 ml, the blood was diluted up to 10mL with RPMI and for those ≤ 2.5mL blood was diluted with RPMI to 5ml total volume. 5mL aliquots of the diluted blood was carefully layered on top of 2.5mL Lymphoprep™ in a 15 ml Falcon conical tube and centrifuged at 500xg for 30 min, brake off (deceleration 0). After centrifugation, the interface (containing mononuclear cells) was collected using a sterile Pasteur pipette and cells washed once with 10mL RPMI at 500xg for 10 min, and then twice with RPMI supplemented with 2% HI FBS at 500xg for 7 min. Cell viability was assessed using Trypan blue exclusion. Briefly, the cells were resuspended in 1ml of RPMI, and a 10µl aliquot of the cell suspension was mixed with 90µl RPMI/2% HI FBS. 10µl of this dilution was mixed with a 10µl aliquot of 0.4% Trypan blue, prior to counting on a haemocytometer. The resulting cell suspension was adjusted to 5-20x10^6 cells / ml with RPMI/2% HI FBS. An equivalent volume of freezing medium 20% dimethyl sulphoxide (DMSO) in HI FBS was added slowly to the cell suspension. Cells were transferred into 1.5ml cryovials, placed into a Cryo 1°C freezing container filled with isopropanol, and transferred to a -80°C Freezer. After 24 hours the cryovials were transferred to liquid nitrogen storage.

THAWING OF CRYOGENICALLY STORED PBMCS.

Cryovials containing PBMCs isolated from children randomised into the CAKE study were removed from liquid nitrogen storage and placed in a 37°C water bath until the cells were half defrosted. 1ml of AIM-V serum free growth media was added drop wise to the vial to gently defrost the cells. The cells were transferred to a sterile 10 ml tube and media was added

slowly up to a volume of 10 ml. Cells were pelleted by centrifugation at 300g for 5 min and the supernatant was discarded. Cells were resuspended in 1 ml of AIM-V media, counted as previously described and diluted with AIM-V media to a final concentration of 2 x 10^6 cells/ml.

PERIPHERAL BLOOD MONONUCLEAR CELL CULTURE AND ANTIGENIC STIMULATION.

PBMCs were cultured with egg allergens OVA and OVM for 5 days in AIM-V culture media. 2.5 x 10^5 cells in 125µl of media were plated in sterile round-bottomed 96 well plates and 125µl of ovalbumin (OVA) or ovomucoid (OVM) added at a final concentration of 100µg/ml. Cells cultured with AIM-V medium alone served as a no-antigen control, and phytohemagglutinin-L (PHA-L) (1 ug/ml) was used as the positive control. To prevent evaporation, empty wells were filled with sterile PBS prior to incubation. All cell cultures were incubated at 37°C and 5% CO2.

IMMUNOPHENOTYPING OF PERIPHERAL BLOOD MONONUCLEAR CELLS

The phenotype of cells in culture with OVA and OVM and the untreated cells was assessed at day 5. The phenotype of cells after PHA-L stimulation was assessed at day 3. All available cells were harvested and analysed. Cells were pelleted at 300xg for 5 min and the resulting supernatants were collected and stored at -80°C for cytokine analysis. The cells were washed once with PBS, and after centrifugation at 300xg for 5 min, the cell pellet was equally divided into FACS tubes for incubation with saturating concentrations of mouse anti human conjugated monoclonal antibodies specific for cell surface antigens directly labelled with PE, PE-Cy7, FITC, APC, APC-Cy7 or PerCP flourochromes (see table x).

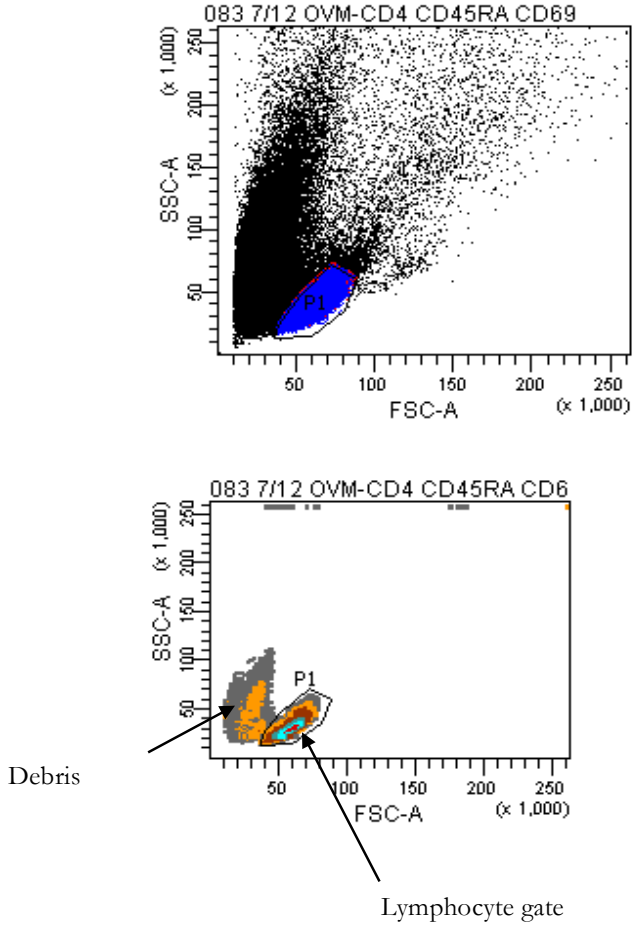
Cells were stained at baseline with cell surface markers for CD4, CD8, CD45RA, CD45RO, CD14, CD19, HLA DR and CD69. After incubation with OVA and OVM cells were assessed for activation markers (CD4, CD45RA and CD69) and memory markers (CD45RO, CCR7, CD27 and CD28).

The cells were incubated on ice, protected from light, for 30 minutes then washed in cold PBS/Azide buffer (unstained cells and surface markers) or MACS buffer (activation and memory markers) by centrifugation at 300xg for 5 min. The cells were resuspended and fixed with 50 µl 2% paraformaldehyde in PBS and stored at 4°C protected from light until flow cytometric analysis.

Labelled cells were analysed on a BD Biosciences FACS Canto flow cytometer (Becton Dickinson, CA, USA). The data were analysed using BD FacsDiva™ software version 6.1.3 (BD Biosciences, USA). After selecting a lymphocyte gate based on forward and side-scatter characteristics, events within the lymphocyte gate were analysed (Figure 1). Fluorescence compensation was set prior to each run using BD™ CompBeads Set Anti-Mouse Ig to ensure that there was no 'spillover' (physical overlap among the emission spectra of the fluorochromes) from one channel into another thus avoiding false positive or negatives. The absolute numbers of cell subsets were calculated from the flow cytometric relative reading.

APPENDIX 7 FIGURE 1 – LYMPHOCYTE GATING FOR FLOW CYTOMETRY

Scatter plot (A) and density plot (B) indicating lymphocyte population (P1). Lymphocytes were gated based on forward (FSC) and side scatter (SSC) properties, in order to exclude populations such as monocytes and cellular debris from the flow cytometric analysis.



DETERMINATION OF CYTOKINE CONCENTRATION IN SUPERNATANT AFTER CULTURE OF PBMCS WITH EGG ALLERGENS

A BD Cytometric Bead Array Human Inflammatory Cytokine Kit was used to measure concentrations of IL-8, Il-1, IL-6, TNF, IL-12 and IL-10 from supernatants collected on days 3 and 5 after incubation with OVA and OVM as above. . Briefly, 25µl of supernatant was incubated with 25 µl capture beads and 25 µl of Human Inflammatory Cytokine PE Detection Reagent for 3 hours at room temperature, protected from light. 1ml of supplied wash buffer was added and the samples were centrifuged at 200xg for 5 min. The supernatant was discarded, the beads resuspended in 200µl wash buffer and flow cytometric analysis was performed using a BD Biosciences FACS Canto flow cytometer (Becton Dickinson, CA, USA). The data were analysed using BD FacsDiva™ software version 6.1.3 (BD Biosciences, USA). If the concentration of cytokine was above the detection limit of the assay the supernatant was diluted 1 in 4 and the analysis repeated.

Standard curves modelling the protein concentration as a function of the mean fluorescent intensity for each cytokine were generated using standards of known concentration (provided in the BD Cytometric Bead Array Human Inflammatory Cytokine Kit). The mean fluorescence intensities of the serially diluted standard samples were calculated using FacsDiva™ software. Table Curve 2D software v4 (Systat Software Inc. CA, USA) was used to generate the standard curves.

Individual cytokines IL-4, IL-5 and IFNg were measured using the BD Biosciences enhanced sensitivity flex sets for Human IL-4, Human IL-5 and Human IFNg and a BD Cytometric Bead Array (CBA) Human enhanced Sensitivity Master Buffer Kit. Briefly, 25µl of supernatant was incubated with 10ul of mixed capture beads and incubated for 2 hours at room temperature. 10 µl of supplied analyte specific detection reagent (Part A) was added, and the tubes incubated for a further 2 hours at room temperature. Samples were washed with 1 ml wash buffer by centrifugation at 200xg for 5 minutes and the supernatant decanted. 100 µl of the supplied enhanced sensitivity detection reagent (Part B) was added, and the

tubes incubated for 1 hour at room temperature. Samples were washed as above, the supernatant was decanted, and the beads resuspended in 100µl wash buffer prior to flow cytometric analysis.

Cytokine standards were prepared and used to generate standard curves for use in analysis as described above.

SERUM SPECIFIC IGE AND IGG4 LEVELS

Serum egg specific IgE and IgG_4 concentrations in plasma were measured using the Phadia CAP system by the Department of Immunopathology, SA Pathology at the Women's and Children's Hospital, using NATA accredited methodologies as per SA Pathology protocols.

STATISTICAL ANALYSIS

The cytokine arrays were analysed on a BD Biosciences FACS Canto flow cytometer (Becton Dickinson, CA, USA). Data were analysed using FacsDiva software version x (BD Biosciences, USA), and standard curves were generated using Table curve 2D software v4 (Systat Software Inc. CA, USA).

APPENDIX 8

DEVELOPMENT OF INTERVENTION PRODUCTS FOR THE CAKE STUDY

This appendix describes the development and assessment of blinding of the intervention products for the CAKE study. To promote compliance over the six-month intervention period several products containing equivalent amounts of baked egg and an egg free 'placebo' version indistinguishable by appearance, taste and texture were developed for the CAKE study.

PRODUCT SPECIFICATIONS FOR THE CAKE STUDY

The intervention products for the CAKE study were designed to meet the following parameters:

- All products were to be cow's milk and nut free, as many of the participants for the CAKE study also had IgE mediated cow's milk or nut allergies.
- All products needed to be moderate in fat and sugar content and in a suitable serve size for a toddler.
- Active products needed to contain around 10 g egg per serve (1/6 of an egg, approx. 1.3g egg protein) and to be baked at 160 - 180°C for around 30 minutes to achieve adequate heating of the egg.
- The active product should not taste of egg.
- Placebo (egg free) products were to be identical in taste, texture and appearance to the egg containing products.

To meet the product specifications several technical issues needed to be overcome while developing the active and matching placebo products for the CAKE study. The main issues were related to the texture and colour of the placebo product compared with the matching egg containing product – this is outlined below:

TEXTURE OF THE EGG FREE PLACEBO PRODUCT

Eggs contribute structural texture, colour and minimal flavour to baked goods. When combined with wheat flour, egg protein provides rise and structural texture to a cake. Addition of sugar or fat (butter, margarine or oil) interferes with the gluten formation and egg protein coagulation resulting in products with a softer texture.

A commercial 'egg replacer' was used in many of the egg free placebo products to ensure they were of similar appearance and texture when compared to the egg containing products. No Egg™ (Orgran, Australia) is an egg replacer composed of tapioca starch and potato starch. No Egg™ contributes to the structure of a product, and also helps to bind a product together, but does not contribute to the overall protein content or colour of a product. One teaspoon of No Egg™ powder combined with two tablespoons of water is used in place of one egg. Commercial self-raising flour (wheat flour plus a raising agent, usually bicarbonate of soda), additional bicarbonate of soda (calcium carbonate), baking powder (calcium carbonate and potassium bi tartrate 'cream of tartar'), and yeast may all be used to incorporate air into a baked product and contribute to the overall 'lightness' of the finished product.

COLOUR OF THE EGG FREE PLACEBO PRODUCT

Egg yolk provides colour to baked goods. Chickens fed fresh greenery lay eggs with bright orange yolks, and this property is valued in commercial 'Free Range' eggs. There is no difference in taste or macronutrient value between brightly coloured egg yolk and less coloured yolk.

Like egg, butter or margarine also contribute colour to baked products. Most margarine is coloured using annatto (160b), derived from the annatto seed, and a known allergen. A cow's

milk free margarine (Nuttelex™) was used as the fat source in products developed for the CAKE study to ensure they were cow's milk free. Nuttelex™ has no added artificial colour, and is pale in comparison with 'ordinary' milk containing margarine. Raw castor sugar, brown sugar, cinnamon, fruit juice or puree and saffron based food colouring may be used to provide colour to the placebo product, or to mask the colour contributed by the egg in the active products.

SUGAR AND FAT CONTENT

Chopped and pureed fruits and vegetables may be successfully used to substitute for sugar and provide some of the elements that fat provides in a baked product. Fruit puree provides colour, texture and fibre as well as contributing to the overall nutritional value of the product.

MATERIALS

Australian standard Metric cups (250ml) and 20 ml Tablespoon measures were used.

Soehnle Domestic Kitchen scales (0.5g)

Kitchen Aid Bench Top Mixer

Cookie press SAWA 2000 Deluxe Cookie Press

APPENDIX 8 TABLE 1 INGREDIENTS USED FOR INTERVENTION PRODUCTS

All food products were purchased from Coles Supermarket Ltd.

Ingredient	**Brand**
Nuttelex™ margarine	Nuttelex Food Products, Vic, Australia
Caster sugar	CSR, Vic, Australia
Raw caster sugar	CSR, Vic, Australia
Free Range Hens Eggs (60g)	Coles Pty Ltd, Australia
Whole Pasteurised egg powder	Farm Pride, Vic, Australia
Plain flour	White Wings, NSW, Australia
Self-Raising flour	White Wings, NSW, Australia
Vanilla extract	Queen fine Foods, QLD, Australia
Cocoa powder	Bournville, Cadbury, Vic, Australia
'No Egg' egg replacer	Orgran Foods, Vic, Australia
Dried apricots	Angas Park, NSW, Australia
Apricot nectar	SPC Ardmona, Vic, Australia
Dessicated coconut	Coles Pty Ltd, Australia
Apples	Coles Pty Ltd, Australia
Dried Pitted Dates	Coles Pty Ltd, Australia
Bicarbonate of soda	McKenzie's, Vic, Australia
White sugar	CSR, Vic, Australia
Brown sugar	CSR, Vic, Australia
Bananas	Coles Pty Ltd, Australia
Canola oil	Coles Pty Ltd, Australia
So Good Regular soy milk	Sanitarium, NSW, Australia
Ground Cinnamon	Masterfoods, NSW, Australia
Frozen Blueberries	Coles Pty Ltd, Australia
Baking powder	Wards, McKenzie's, Vic, Australia
Frozen raspberries	Coles Pty Ltd, Australia

SUMMARY OF RECIPE DEVELOPMENT FOR THE CAKE STUDY

The original recipes and modifications required to develop the final egg containing and corresponding egg free 'placebo' recipes used in the CAKE study are summarised in Table 2.

In summary, the original recipes were adjusted to make them cow's milk and nut free. The volumes of ingredients in the recipe were modified so that each serve was of an appropriate size and the amount of egg adjusted to give approximately 10g (1/6 of an egg) per serve. For the egg free recipes, a commercial egg replacer was substituted for egg, extra fluid was added and adjustments were made to the type of flour, raising agent, sugar or the addition of spices, fruit and fruit puree to ensure the texture and colour of the egg free product was equivalent to the egg containing recipe. Biscuits usually do not contain egg, and the addition of egg alters their texture. To overcome this issue, a biscuit recipe using half fresh egg and half pasteurised whole egg powder was developed. Each product was tested at least three times in a domestic kitchen and then at least twice by the patisserie supplying the products for the CAKE Study to ensure consistency.

Twelve study products were developed including seven egg containing products and seven egg free placebo products. The products included banana bread, apple and date loaf, apricot and coconut muffin, chocolate biscuit, a raspberry muffin, and a blueberry muffin. The first four products were used as intervention in the CAKE study. Recipes for raspberry and blueberry muffins were developed, but not used due to concerns that the products may not cook evenly potentially affecting the consistency of heating of the egg in the egg-containing product. A vanilla orange cake recipe was tested with the aim of providing an option of a 'plain' cake, but adequate blinding of the placebo product was not achieved after 8 batches. Egg containing and placebo versions of a Date and Carrot loaf were developed but not used as it was thought that it would not be a popular product (in terms of taste) in the subject group. Products such as pancakes and scones are not exposed to high enough heat for long enough to adequately

denature the egg protein and may be under cooked in the middle so were not suitable as products for the study.

TESTING OF BLINDING

Products were tested for blinding in parallel with the recipe development, and modifications were made accordingly.

The final recipes for the banana bread, apple and date loaf, apricot muffins were tested on 8 volunteers to assess visual appearance, texture and taste detection of egg in the egg containing products. 1 of 8 volunteers correctly identified the egg containing product for the banana bread and the apple and date loaf and 0 of 8 correctly identified the egg containing apricot muffin. The chocolate biscuits were tested on 15 volunteers. 5 of 15 volunteers were able to correctly identify the egg containing biscuits.

APPENDIX 8 TABLE 2 SUMMARY OF PRODUCT DEVELOPMENT AND FINAL PRODUCTS FOR CAKE STUDY.

Chocolate Biscuit			
Original Recipe	Modifications Required	**Final Study Recipe** **Egg Containing**	**Final Study Recipe** **Egg Free Placebo**
(source:http://alteredcutlery.blogspot.com.au/2007/12/refrigerator-biscuits-cookies.html Accessed 11/4/2012) **Ingredients** 1 cup butter or margarine 1 ½ cups caster sugar 2 x 60g eggs 3 cups plain flour 1 tsp vanilla extract 2 Tbs melted chocolate **Method** Cream butter and sugar; add eggs one at a time, then flour and vanilla. Roll into 3 long snakes. Wrap each in alfoil (I prefer to use cling wrap) and then freeze. To use: Still frozen, slice cookies off the snake onto a greased tray; bake till very pale gold @ 200C. Remove from tray while still warm. Store in a sealed container when cool.	**For egg containing:** Halved recipe, used Nuttelex™ margarine, and cocoa powder to make cow's milk and nut free. Added 1 Tbs egg powder to maintain egg concentration. Added SR flour to make the product lighter. Increased amount of cocoa to make a darker product. Used biscuit forcer so that biscuits were identical in size and shape. Total versions: 3 **For egg free:** Started with egg containing recipe. Added egg replacer and water to substitute for egg. Added SR flour to make the product lighter. Increased amount of cocoa to make a darker product. Total versions: 2	**Ingredients** 100g Nuttelex™ margarine ¾ cup caster sugar 1 x 60g egg 1 Tbs Farm Pride Pasteurised egg powder 1 cup plain flour + extra if dough looks too sticky. ¼ cup SR flour 1 tsp vanilla extract 1/3 cup cocoa powder 60ml water – approximately **Method** Cream Nuttelex™ margarine and sugar. Add vanilla extract, egg and egg powder mix until combined. Add dry ingredients, and mix. Mix until mixture comes together – should be the consistency of a 'crème patissiere' (a very soft dough). Refrigerate for 10-15 mins. Put through biscuit forcer* Bake 15 mins at around 180C. Should make about 48 biscuits. 12 biscuits = 1 egg. 2 biscuits = 1 serve.	**Ingredients** 100g Nuttelex™ margarine ¾ cup caster sugar 1 cup plain flour ¼ cup SR flour 1 tsp vanilla extract 1/3 cup cocoa powder 1 tsp 'No Egg' egg replacer 60ml water – approximately **Method** Cream Nuttelex™ margarine and sugar. Add vanilla extract, and mix until combined. Add dry ingredients, and mix. Gradually add water until mixture comes together – should be a very soft dough. Refrigerate for 10-15 mins. Put through biscuit forcer – put any misshapen biscuits back into mixture. Bake 15 mins at around 180C. Should make about 48 biscuits.

APPENDIX 8 TABLE 2 (cont) SUMMARY OF PRODUCT DEVELOPMENT AND FINAL PRODUCTS FOR CAKE STUDY.

Apricot and Coconut Muffins			
Original Recipe	Modifications Required	**Final Study Recipe** **Egg Containing**	**Final Study Recipe** **Egg Free Placebo**
(Source: The Australian Women's Weekly Cakes and Slices Cookbook. 1989 Editor: Sue Wendt ISBN 0 949128 10 4) **Ingredients** 1 cup chopped dried apricots 1 cup apricot nectar 125g butter 2/3 cup raw caster sugar 2 eggs 1 ½ cups coconut 1 ½ cups SR Flour ½ cup Choc Bits **Method** Preheat oven to 155°C Combine apricots and nectar in a bowl, stand 1 hour Cream butter and sugar in a bowl. Beat in eggs one at a time. Stir in coconut, then half of the sifted flour and half the apricot mixture. Stir in remaining flour and apricots. Spread into prepared cake tin. Bake in moderate oven for 1 1/4 hours. Dust with icing sugar.	**For egg containing:** Halved recipe. Substituted butter with Nuttelex™ margarine to make cow's milk free. Removed choc bits (cow's milk and nut contamination). Original recipe made 8 muffins, so reduced amount of dry ingredients to make 6 muffins. Total versions: 2 **For egg free:** Started with egg containing recipe. Added egg replacer and water to substitute for egg. Original recipe was too pale, so trialled brown sugar (but this made the product too dark and too moist), so added raw caster sugar to achieve the correct colour and texture. Total versions: 3	**Ingredients** 1/2 cup chopped dried apricots 1/2 cup apricot nectar 50g Nuttelex™ margarine 2 Tbs caster sugar 1 egg 2/3 cup coconut 2/3 cup SR Flour **Method** Preheat oven to 155°C Combine apricots and nectar in a bowl, stand 1 hour Cream margarine and sugar in a bowl. Beat in eggs one at a time. Stir in coconut, then half of the sifted flour and half the apricot mixture. Stir in remaining flour and apricots. Spread into prepared tin or place in muffin tins. Bake in moderate oven for 1 hour. Dust with icing sugar. Makes 6 muffins. 1 muffin is 1 serve.	**Ingredients** 1/2 cup chopped dried apricots 1/2 cup apricot nectar + 2 Tbs water 50g Nuttelex™ margarine 1 Tbs caster sugar + 1 Tbs raw caster sugar 1 tsp egg replacer 2/3 cup coconut 2/3 cup SR Flour **Method** Preheat oven to 155°C Combine apricots and nectar in a bowl, stand 1 hour Cream margarine and sugar in a bowl. Beat in eggs one at a time. Stir in coconut, then half of the sifted flour and half the apricot mixture. Stir in remaining flour and apricots. Spread into prepared tin or place in muffin tins. Bake in moderate oven for 1 hour. Dust with icing sugar Makes 6 muffins. 1 muffin is 1 serve.

APPENDIX 8 TABLE 2 (cont) SUMMARY OF PRODUCT DEVELOPMENT AND FINAL PRODUCTS FOR CAKE STUDY.

Date and Apple Loaf			
Original Recipe	Modifications Required	**Final Study Recipe** **Egg Containing**	**Final Study Recipe** **Egg Free Placebo**
Original Recipe (Source: Modified from Better Homes and Garden's Magazine.) **Ingredients** 2 medium apples 200g dates 1 cup boiling water 1 tsp bicarb soda 125g butter 1 cup castor sugar 1 egg 1 ½ tsp vanilla extract 1 cup plain flour ½ cup SR flour Topping: 120ml milk 90g butter ¾ cup brown sugar 110g flaked almonds 1 tsp vanilla extract **Method** Preheat oven to 155°C. Spray and line a spring form pan. Chop dates, peel, core and chop apple. Soak in boiling water and bicarbonate of soda. Cream butter and sugar. Add eggs. Stir in flour alternately with apple and date mixture. Bake for 1 ¼ hours. For topping, heat milk, butter, add sugar, vanilla and almonds, cook for 5 minutes until slightly thick. When cake is nearly cooked pour topping over the top. Bake for 10-15 minutes	**For egg containing:** Reduced size of original recipe. Doubled number of eggs. Substituted butter with Nuttelex™ margarine to make cow's milk free. Deleted topping (cow's milk and nuts). Increased temperature of oven. Total versions: 2 **For egg free:** Started with egg containing recipe. Added egg replacer and water to substitute for egg. Total versions: 1	**Ingredients** 1 apple 150g dates ¾ cup water ¾ tsp bicarbonate of soda 125g Nuttelex™ margarine 1 cup sugar 1 eggs 1 cup plain flour ½ cup SR flour **Method** Chop dates, peel and chop apple. Soak in ¾ cup boiling water and ¾ tsp bicarbonate of soda for ½ hour. Cream Nuttelex™ margarine and sugar. Add eggs. Stir in flour alternately with apple and date mixture. Divide mixture evenly between two prepared loaf tins. Bake at 180°C for 1 hour or until well cooked Makes two loaves, slice each into 6 slices. Each slice is one serve.	**Ingredients** 1 apple 150g dates 1 cup water ¾ tsp bicarbonate of soda 125g Nuttelex™ margarine 1 cup sugar 2 tsp egg replacer 1 cup plain flour ½ cup SR flour **Method** Chop dates, peel and chop apple. Soak in ¾ cup boiling water and ¾ tsp bicarbonate of soda for ½ hour. Cream Nuttelex™ margarine and sugar. Stir in flour and egg replacer alternately with apple and date mixture. Divide mixture evenly between two prepared loaf tins. Bake at 180°C for 1 hour or until well cooked. Makes two loaves, slice each into 6 slices. Each slice is one serve.

APPENDIX 8 TABLE 2 (cont) SUMMARY OF PRODUCT DEVELOPMENT AND FINAL PRODUCTS FOR CAKE STUDY.

Banana Bread			
Original Recipe	Modifications Required	**Final Study Recipe** **Egg Containing**	**Final Study Recipe** **Egg Free Placebo**
(Source: Family Recipe) ¾ cup SR Flour ¾ cup Wholemeal SR Flour ½ tsp mixed spice 2/3 cup brown sugar 2 large over ripe bananas (200g) 2 eggs 1/3 cup canola oil ¼ cup milk 125g blueberries Heat oven to 180°C. Grease 9 x 22cm tin Sift dry ingredients. Mix wet ingredients together. Gradually combine wet and dry ingredients. Pour into prepared loaf tin. Bake for 1 hour.	**For egg containing:** Substituted cow's milk with soy milk to make cow's milk free. Used only plain SR flour (not wholemeal). Deleted blueberries so that the product was evenly cooked. Total versions: 1 **For egg free:** Started with egg containing recipe. Added egg replacer and water to substitute for egg. Added baking powder to ensure texture was the same as the egg containing version. Total versions: 2	**Ingredients** 1 ½ cup SR Flour ½ tsp mixed spice 2/3 cup brown sugar 2 large over ripe bananas 2 eggs 1/3 cup canola oil ¼ cup soy milk Heat oven to 180C. Sift dry ingredients. Mix wet ingredients together. Gradually combine. Divide mixture evenly between two greased and lined two loaf tins. Bake for 1 hour. Makes two loaves, slice each into 6 slices. Each slice is one serve.	**Ingredients** 1 ½ cup SR Flour 1 tsp baking powder ½ tsp mixed spice 2/3 cup brown sugar 2 large over ripe bananas 2 tsp egg replacer 4 Tbs water 1/3 cup canola oil ¼ cup soy milk Heat oven to 180C. Sift dry ingredients. Mix wet ingredients together. Gradually combine. Divide mixture evenly between two greased and lined two loaf tins. Bake for 1 hour. Makes two loaves, slice each into 6 slices. Each slice is one serve.