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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Signed:

Merryn J Netting
Date:

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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.