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PrEggNut Study: protocol for a randomised controlled trial investigating the effect of a maternal diet rich in eggs and peanuts from <23 weeks' gestation during pregnancy to 4 months' lactation on infant IgE-mediated egg and peanut allergy outcomes

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ABSTRACT

Introduction Clinical studies supported by immunological data indicate early life intervention strategies to be promising in reducing the growing global burden of food allergies. The events that predispose to food allergy, including the induction of allergen-specific immune responses, appear to be initiated early in development. Early exposure to food allergens in utero and via breast milk is likely to be important in initiating oral tolerance. We aim to determine the effectiveness of higher maternal food allergen consumption during pregnancy and lactation on infant food allergy outcomes.

Methods and analysis This is a multisite, parallel, two-arm (1:1 allocation), single-blinded (outcome assessors, statistical analyst and investigators), randomised controlled trial. Pregnant women (<23 weeks' gestation) whose (unborn) infants have at least two biological family members (mother, father or siblings) with medically diagnosed allergic disease are eligible to participate. After obtaining written informed consent, pregnant women are randomised to either a high egg and peanut diet (at least 6 eggs and 60 peanuts per week) or standard (low) egg and peanut diet (no more than 3 eggs and 30 peanuts per week). The women are asked to follow their allocated diet from <23 weeks' gestation to 4 months' lactation. The primary outcome is food challenge proven IgE-mediated egg and/or peanut allergy in the infants at 12 months of age. Key secondary outcomes include infant sensitisation to egg and/or peanut and infant eczema. Our target sample size is 2136 women. Analyses will be performed on an intention-to-treat basis according to a pre-specified statistical analysis plan.

Ethics and dissemination Ethical approval has been granted from the Women's and Children's Health Network Human Research Ethics Committee (approval number HREC/18/WCHN/42). Trial results will be presented at scientific conferences and published in peer-reviewed journals.

Trial registration number Australian New Zealand Clinical Trials Registry ACTRN12618000937213.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This study is a large randomised controlled trial with adequate power that is designed to assess the effect of higher maternal egg and peanut consumption during pregnancy and lactation on infant egg and peanut allergy outcomes.
- ⇒ The use of whole egg and peanut containing foods for the maternal intervention, rather than specific powders or supplements.
- ⇒ Although this study is single blinded due to the use of whole egg and peanut containing foods for the intervention, the outcome assessors, statistical analyst and investigators are all blinded to diet group allocation.

INTRODUCTION

Randomised controlled trials have shown that regular inclusion of traditionally allergenic foods, such as egg and peanut, with solid foods from mid-late infancy reduces the risk of developing egg and peanut allergies in

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some infants.[1 2] However, some infants may have allergic reactions, including anaphylaxis,[3] on first introduction of egg in solid foods as early as 4 months of age. Susceptible infants appear to be already on the pathway to food allergy before commencing solid foods. We have previously reported that food allergen (egg) specific immune responses are established prior to infants eating any egg allergen in solid foods.[3 4] Furthermore, these responses were not altered by early introduction of egg in the infant diet.[4] In other words, it may be too late for many susceptible infants who are already sensitised/allergic at the time these allergenic foods are introduced around 4–6 months of age. Hence, earlier intervention strategies are needed during critical early periods of immune development in pregnancy and lactation when the pathways to food allergy appear to be initiated.

Food allergens can cross the placenta and can be detected in amniotic fluid,[5] where they reach the fetal gastrointestinal tract after fetal swallowing (oral exposure). Allergens are also detectable in placental tissue and in the fetal circulation.[6 7] Human fetal T cells are responsive to allergens[8] as early as 22 weeks' gestation.[9] The fetus develops regulatory immune responses to both self-antigens and to exogenous allergens[10] that cross the placenta. This is consistent with the recognised fetal predisposition for 'active tolerance',[11] where immune tolerance appears to be the default response to maternally derived antigens including allergens.[12]

In the postnatal period, food allergens secreted in breast milk are also likely to be an important early source of oral food allergen exposure. In animal studies, allergen exposure through maternal milk has been shown to induce oral tolerance.[13] Allergens detected in maternal milk have also been shown to have tolerogenic effects by forming allergen–IgG complexes, which induce antigen-specific T-regulatory cells in newborn animals,[14] and are also found in human milk.[15] In previous studies,[16–18] we have demonstrated that the amount of maternal consumption of egg during lactation influences egg protein (ovalbumin) detection and concentration in human breast milk. Higher maternal dietary intakes of common food allergens would thus increase infant oral exposure to these allergens via breast milk prior to solid food introduction. Induction of oral tolerance is likely to be dose dependent, requiring higher early life exposure to food allergens. In support of this, the consumption of 2 g/week of peanut or egg protein by infants has been associated with a significantly lower prevalence of these food allergies compared with less consumption.[19] Additionally, a large observational study (n=8205) in the USA[20] reported higher maternal nut consumption was associated with fewer nut allergic children, irrespective of age at first introduction of nuts into the child's diet. However, due to a current lack of randomised controlled trials, there is very limited high-quality evidence available to guide maternal dietary recommendations around food allergen consumption especially in pregnancy.

The hypothesis generated from previous studies is that higher maternal dietary intakes of common allergenic foods, such as eggs and nuts, during pregnancy and lactation may reduce offspring food allergy outcomes. Thus, as the next logical step, we aim to investigate in a randomised controlled trial the effectiveness of higher regular egg and peanut maternal dietary intakes during pregnancy and lactation as a strategy to prevent food allergy in infants.

METHODS AND ANALYSIS

Trial design and study setting

This is a multisite, parallel, two-arm (1:1 allocation), single-blinded (outcome assessors, statistical analyst and investigators), randomised controlled trial, known as the PrEggNut Study. The rationale for this trial design is because the dietary intervention uses real whole foods, it is not possible to blind the participants; however, research staff undertaking the outcome assessments are blinded to group allocation, and recruitment/intervention and outcome assessment study teams are separated at each site. Recruitment of pregnant women for participation will occur in the Australian cities of Adelaide, Perth, Sydney and Melbourne. The final infant outcome assessments, including food challenges, will occur at major paediatric hospitals in each city: Women's and Children's Hospital (Adelaide), Perth Children's Hospital (Perth), Children's Hospital at Westmead (Sydney) and Royal Children's Hospital (Melbourne).

Participant eligibility criteria

Participants are pregnant women enrolled <23 weeks' gestation. The inclusion criteria include: women able to give informed consent, a singleton pregnancy and women who are planning to breast feed for at least 4 months. The fetus is to have at least two biological family members (mother, father or siblings) with medically diagnosed allergic disease (asthma, eczema, hay fever or IgE-mediated food allergy). The exclusion criteria are women with egg or peanut allergies, as they would be unable to safely follow the intervention without allergic reactions.

Interventions

The participating women are randomised to either a high egg and peanut diet group or a standard (low) egg and peanut diet group.

- The high egg and peanut diet group: regular maternal consumption of at least 6 eggs and 60 peanuts per week from <23 weeks' gestation until 4 months' postnatal infant age.
- The standard (low) egg and peanut diet group: maternal consumption of no more than 3 eggs and 30 peanuts per week from <23 weeks' gestation until 4 months' postnatal infant age.

The standard (low) egg and peanut diet group is designed to reflect consumption of no more than the average usual maternal intake of eggs and peanuts, based

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on findings from an observational birth cohort at the Nepean Hospital in New South Wales, Australia (one of the recruitment sites for this trial), where 899 postpartum women were found to eat on average 2.5 eggs and 20 peanuts per week. Both of these dietary groups are designed to fit within the Australian Dietary Guidelines for pregnant and breastfeeding women, which recommend 2.5–3.5 serves/day of protein-rich foods such as lean meat, poultry, fish, eggs, nuts, seeds and legumes. One serve is equivalent to 65 g cooked lean meat, 80 g cooked lean chicken, 2 large eggs or 30 g nuts. Participating women can include all forms of egg and peanut, and egg and peanut containing foods, towards their weekly target of egg and peanut ingestion. They are provided with a conversion table showing the amount present in common egg or peanut foods, for example, peanut butter, or egg in quiche, meatballs or in baked goods such as cake and muffins.

The intervention period commences <23 weeks' gestation, as this timepoint corresponds to our knowledge of when immune cells are responsive to allergens. We chose to cease the intervention at 4 months of age as this appears to be a critical period for primary prevention prior to the development of food allergy.[3 4] If the participating women cease breast feeding prior to 4 months of age, the allocated intervention group maternal diet recommendations are no longer required to be followed; however, the infants continue to be studied on an intention-to-treat basis.

The participant group allocation and corresponding dietary advice are provided by a research staff member not involved in any of the outcome assessments. The participant dietary advice is provided at the time of randomisation to group allocation prior to 23 weeks' gestation. Research assistants provide standardised advice, adjusted for group allocation and individual suggestions of specific foods are also made based on the participant likes/dislikes and their maternal egg/peanut baseline data collection (prior to randomisation). The participating women are also encouraged to recontact the study staff at any stage during the intervention period if they require any further dietary adherence suggestions. Research staff at each site who provide the dietary group allocation advice to participants are trained by the national study co-ordinator, and the research staff's intervention advice is monitored on a 6 monthly basis throughout the trial.

To monitor dietary group adherence, participants complete a brief four-question assessment of their egg and peanut intakes each month during the intervention period, these questions can be found in online supplemental file 1. In the postnatal period, one additional question on breastfeeding status is also collected each month along with the egg and peanut intake questions. The same questions are completed by both intervention groups, and were designed to be quickly completed via their mobile phone to encourage dietary compliance as well as capturing adherence. These dietary group adherence assessments cease at 4 months' postpartum or prior if the participating woman ceases to breast feed. For the promotion of breast feeding, a lactation consultant can assist and provide advice to the participants with establishing and maintaining breast feeding until at least 4 months' postnatal.

Outcomes

The primary outcome for this trial is infant food challenge proven IgE-mediated egg and/or peanut allergy at 12 months of age. This is considered the gold standard test for IgE-mediated food allergy and is carried out on all infants with a positive skin prick test (allergic sensitisation) to egg or peanut at 12 months of age, unless an infant has had a previous anaphylaxis to egg/peanut, or a medical decision has been made not to proceed with the food challenge due to a previous allergic reaction consistent with IgE-mediated egg/peanut allergy, those infants are then classified as having IgE-mediated egg and/or peanut allergy. The in hospital medically supervised food challenge will follow the Australasian Society of Clinical Immunology and Allergy (ASCIA) standardised food challenge protocols for egg (lightly cooked scrambled egg) and peanut (peanut butter), with internationally standardised scoring and stopping criteria.[21]

Secondary clinical outcomes (all participants)

- IgE-mediated egg allergy at 12 months of age (defined as above).
- IgE-mediated peanut allergy at 12 months of age (defined as above).
- Infant allergic sensitisation to egg and/or peanut at 12 months of age. The participating infants have skin prick testing using standard single-prick lancets (Entaco distributed by Stallergenes) on the forearm, to determine allergen sensitisation to egg and peanut, with histamine and control solutions, in accordance with standard clinical methods ASCIA Skin Prick Testing for the Diagnosis of Allergic Disease. All assessment sites are using the same commercially available skin prick testing extracts of egg white (Greer Laboratories, USA), peanut (Greer Laboratories, USA), positive control histamine (HollisterStier, USA) and negative control 50% glycerin (Greer Laboratories, USA). Sensitisation is defined as a positive skin prick test with mean weal diameter≥3 mm above the control weal size.
- Infant medical diagnosis of eczema by 12 months of age. In addition, eczema extent and severity will be measured using the standardised and validated SCORing Atopic Dermatitis (SCORAD) clinical tool assessment method[22] at 4 and 12 months of age. Use of any emollients and/or eczema treatments are also recorded.

Exploratory laboratory outcomes (subset of Perth and Sydney site participants only)

Blood samples will be collected on up to 400 mother and infant pairs and processed using the ImmunoCAP 250

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	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					Completion
TIMEPOINT	<23 weeks gestation	<23 weeks gestation	t_1	t_2	t_3	t_4	t_5	t_6
ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
Allocation		X						
INTERVENTION:								
High egg and peanut diet			—	—	—			
OR Low egg and peanut diet			—	—	—			
ASSESSMENTS:								
Baseline data	X							
Maternal egg and peanut intake	X		X	X	X	X		
Maternal anthropometry	X					X		
Birth data				X				
Infant feeding details				X	X	X	X	X
Infant anthropometry				X		X		X
Infant egg and peanut intake							X	X
Infant allergy symptoms						X	X	X
Egg and peanut skin prick testing								X
Egg and/or peanut food challenge								X

Figure 1 Participants (maternal and infant) schedule of enrolment, intervention and assessments. t_1=22 weeks' gestation to birth; t_2=birth; t_3=birth to 4 months' postnatal; t_4=infant 4 months of age; t_5=infant 8 months of age; t_6=infant 12 months of age.

system (Phadia AB, Uppsala, Sweden) to measure the following antibody concentration outcomes:

- Egg and peanut-specific-IgG4 in maternal blood (at randomisation, 34–38 weeks' gestation and 4 months' postnatal) and infant blood (at 4 and 12 months of age).
- Egg and peanut-specific-IgE in infant blood (at 4 and 12 months of age).

Participant timeline

Figure 1 illustrates the participants (maternal and infant) schedule of activities and assessments. Before 23 weeks' gestation, informed consent including a screening form checklist is completed. Baseline data are recorded, including basic demographic information, family history of allergic disease, maternal ethnicity, parity, maternal education, household smoking and pets. Usual dietary intakes of all forms of egg and peanut containing foods for all members of the household are collected. Current maternal weight and height is measured, and prepregnancy weight recorded. Each month during the intervention period, participants complete a four-question assessment of their egg and peanut intakes. This is completed via a link sent to their mobile phone. In the postnatal period, one additional question on breastfeeding status is also collected via the same mobile phone link.

Two weeks after their estimated date of delivery, participants are telephoned to collect birth data, including infant details on date of birth, sex, birth weight, gestational age and mode of delivery. Breastfeeding status and any episodes of mastitis are recorded. The mothers are asked whether there is any aspect of breast feeding for which they would like support, and if they would like to be referred to a lactation consultant for assessment and advice.

At 4 months' postnatal age, participants are asked about infant introduction of any solid foods, infant introduction of egg and peanut, breast feeding, infant formula use, any episodes of mastitis and any hospitalisations. The maternal weight is measured, as well as the infant's weight, length and head circumference. The infants are assessed for any clinical allergic disease symptoms (eczema and wheeze). All participants (both groups) are provided with the current ASCIA infant feeding and allergy prevention guidelines,[23] which provide advice on the introduction of solid foods at around 6 months of age. This includes the recommendation that all infants should be given allergenic foods including peanut butter, cooked egg, dairy and wheat in the first year of life. All families are provided with education on recognising the signs and symptoms of an allergic reaction and advice of what to do in such circumstances, consistent with the information provided in the ASCIA Action Plan for Allergic Reactions.

At 8 months' postnatal age, participants are asked about introduction of any solid foods, introduction of egg and peanut foods, breast feeding, infant formula use, any allergic disease symptoms and any hospitalisations.

At 12–15 months' postnatal age, the final study appointment occurs where an infant clinical allergic disease symptom assessment is undertaken. The infant's weight, length and head circumference is measured. Participants are asked about infant consumption of egg and peanut containing foods, breast feeding, infant formula use and any hospitalisations. All participating infants have skin prick testing (as described above in the Outcomes section), and if required an egg and/or peanut food challenge (as described above in the Outcomes section).

Sample size

The expected prevalence of IgE-mediated egg and/or peanut food allergy at 12 months of age (primary outcome) in a population of infants with at least two family members with medically diagnosed allergic disease in Australia is 16%.[24 25] Our previous trials investigating regular inclusion of egg in infant diets have reported a reduced egg allergy risk of 25%–35%.[3 26] In this PrEggNut Study with an earlier intervention in pregnancy and lactation, we expect a minimum reduced effect of 30% on infant egg and peanut allergies. Such a relative reduction in the diagnosis of food allergy will lead to changes in food allergy prevention guidelines, as has been the case for the regular inclusion of allergenic solids in infant diet trial results. This level is also importantly meaningful to families and will be associated with significant healthcare

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savings and improved quality of life. To detect a reduction in food allergy from 16% to 11.2%, (relative reduction of 30%) with 85% power and overall two-sided alpha 0.05 (0.049 at the final analysis) and to allow for 10% loss to follow-up, we require 1068 women per group, thus aim to recruit a total of 2136 women.

Recruitment

Women are approached to enter the PrEggNut Study by research staff at the time of attending their routine visits in antenatal clinics or early pregnancy classes. Pregnant women are also informed about the trial by display of approved advertising material, in hard copy as flyers and posters, as well as online via social media (eg, Facebook advertisements), directing potential participants to contact participating recruitment sites. Following a screening process to ensure inclusion and exclusion criteria are met, a participant information and consent form describing the purpose of the study, the procedures to be followed and the risks and benefits of participation is explained to interested women. The participants are given as much time as they wish to consider participation in the study, have any questions answered, or to discuss taking part with their family, friends and/or antenatal team healthcare professionals. Participants are required to provide written informed consent and are given a copy of their signed consent form.

A record of all women screened and their enrolment status is maintained to adhere to the consolidated standards for the reporting of randomised controlled trials. Women may withdraw their involvement in the trial at any time, without explanation and without prejudice to their future care, and, wherever possible, the reason for withdrawal is recorded. Participants who discontinue or are withdrawn will not be replaced.

Assignment of interventions

Once the consent process has been documented by signing of the written consent form, the participant is randomised by an intervention team research staff member using a secure web-based randomisation service. The randomisation service allocates a group assignment according to a computer-generated randomisation schedule produced by a statistician not otherwise involved in the trial. Randomisation is stratified by city and by first-born or subsequent born child to the mother participant using randomly permuted blocks of varying sizes.

Blinding

Due to the nature of this type of dietary intervention, it is not possible to blind the participants; however, research staff undertaking the outcome assessments are blinded to group allocation. We have designated recruitment staff at each site who provide the maternal group allocation dietary advice and undertake any participant contact phone calls if needed during the intervention period. Different research staff members (research nurses) at each site, who are blinded to group allocation, conduct the outcome measures appointments and phone calls. The trial statistician, all investigators, the trial steering committee and the serious adverse event (SAE) committee are all also blinded to diet group allocation.

Data collection and management

Data are collected by trained research staff at each participating site and entered directly into an electronic case report form with password protection and defined user-level access Research Electronic Data Capture (REDCap) is used to facilitate trial management and data collection. A record of all women successfully screened for eligibility and consented is recorded in real time. Once consented and randomised, REDCap has been designed to automatically calculate study milestones for each participant. This information is readily available for research staff to enable scheduling of appointments and phone calls. The electronic case report form has inbuilt data entry validity checks to ensure immediate resolution of data queries. Data queries are also generated by statisticians during regular blinded reviews of data quality. Electronic data are stored on secure servers with access only granted to authorised study personnel. All data collected will be treated with confidence. Data entered by individual study sites are routinely monitored by the coordinating centre to check protocol adherence and study progress. Summary reports are generated, including screening data, enrolment, appointment attendance, sample collection, SAEs and study completion, and reviewed at monthly trial management committee meetings. Site monitoring to ensure compliance with good clinical practice and the study protocol are conducted at site start-up and then 6 monthly or as required to ensure the integrity of the trial.

Statistical analysis

Analyses will be performed on an intention-to-treat basis (ie, all randomised women analysed as randomised) according to a prespecified statistical analysis plan (see online supplemental file 2). The proportion of infants with food challenge proven IgE-mediated egg and/or peanut allergy will be compared between groups using log binomial regression. Adjustment will be made for variables used to stratify the randomisation and other prespecified baseline prognostic variables, with the difference between groups expressed as an adjusted relative risk with a CI and two-sided p value. Statistical significance will account for a single prespecified interim analysis using the O'Brien-Fleming approach,[27] with the overall type 1 error rate maintained at 0.05. A sensitivity perprotocol analysis of the primary outcome will also be undertaken in women that breast feed to 4 months and adhere to the suggested intake of egg and peanut. Missing outcome data will be addressed using multiple imputation, with imputation performed separately by treatment

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group using fully conditional specification.[28] In planned subgroup analyses of the primary outcome, we will also test for evidence of effect modification by socioeconomic status, firstborn compared with subsequent born children for the mother participant and total household egg and peanut dietary intakes.

Data monitoring

The trial steering committee will review and make protocol amendments, be responsible for the statistical analysis plan, monitor overall study progress and make decisions regarding resource allocation at monthly telemeetings. The trial management committee, chaired by the national study coordinator will consist of chief investigators, associate investigators and site trial coordinators and meets via monthly telemeetings. This management committee manages study promotion, recruitment, staff training and adherence to the protocol for all sites. As this management committee consists of both blinded and non-blinded committee members, only blinded data are discussed in these meetings.

An independent data monitoring committee (DMC) will be established to safeguard the interests of trial participants. The DMC will consist of three independent clinicians (an obstetrician, a neonatologist and an allergist) and an independent biostatistician who, collectively, are experienced in the conduct and monitoring of randomised controlled trials. The DMC will meet annually and review general trial progress (recruitment, compliance, loss to follow-up) and protocol modifications suggested by investigators. The DMC will also review results of a single-unblinded interim analysis of the primary outcome once 50% of participants have primary outcome data available. Using O'Brien-Fleming stopping criteria,[27] a two-sided p value of less than 0.0031 at the interim analysis will be taken to provide statistical evidence in support of early stopping.

An independent blinded SAE committee has also been set up to review any SAE and determine if any such events were due to the study intervention and provide reports to be sent to the human research ethics committees at each participating site. The constitution of the SAE committee is three independent clinicians (an obstetrician, a neonatologist and an allergist). The members of the SAE committee are different to those of the DMC. The SAE committee meets annually or more frequently if required.

ETHICS AND DISSEMINATION

Ethics

Ethical approval has been granted from the Women's and Children's Health Network Human Research Ethics Committee (HREC) approval number HREC/18/WCHN/42, as the lead HREC, with governance site approvals at all participating maternity and children's hospital sites. The study will be conducted in compliance with the current approved version of the protocol (Version 2, 11 June 2019). Any change to the protocol document or informed consent form that affects the scientific intent, study design, patient safety or may affect a participant's willingness to continue participation in the study will be considered a major amendment and shall have written approval by the lead HREC and governance at each participating site. Participant confidentiality is strictly held in trust by participating investigators and research staff. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants.

Patient and public involvement statement

A qualitative substudy is being planned to conduct focus groups of completed participants to enable participant feedback and input into the practical dietary considerations needed to enhance future translation of the study findings into allergy prevention recommendations.

Data sharing

Once the primary trial is published, the PrEggNut Study data will be available for data sharing. Data sharing requests will need approval by the trial steering committee. Please send requests to DJP (debbie.palmer@telethonkids.org.au) and MM (maria.makrides@sahmri.com). The Australian National Health and Medical Research Council (NHMRC) supports the sharing of outputs from NHMRC funded research including publications and data. All recipients of NHMRC grants must therefore comply with all elements of the NHMRC Open Access Policy (15 January 2018).

Dissemination

All investigators will be integral in the communication of the results from this PrEggNut Study. The trial findings will be submitted for peer-reviewed publication and for presentation at appropriate local and international conferences, as well as to the general public through various forms of media and public presentations on nutrition and allergy prevention. In addition, the trial findings will be disseminated to participants through a one-page lay summary. The PrEggNut Study has been designed with the translational plan that the outcomes will inform national and international guidelines on food allergy prevention, irrespective of whether the hypothesis is correct.

CURRENT TRIAL STATUS

The first participant was randomised in October 2018. Recruitment for this PrEggNut Study is expected to be completed by October 2022. The final participant primary outcome assessments are expected to be completed by May 2024.

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Contributors DJP, MM, TRS, DEC, RN, PSH and MSG conceived the trial and proposed the trial design. TRS advised on sample size calculations and drafted the statistical analysis plan. DJP, MM, TRS, DEC, RN, PSH and MSG drafted the protocol. DJP, MM, TRS, DEC, RN, PSH, MSG, PQ, MO, RG, JJK, KPP, SLP, MJN and VM all contributed to refinement of the PrEggNut Study Protocol and approved the final manuscript.

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Competing interests DEC reports personal fees from DBV technologies (part-time salary), Allergenis and Westmead Fertility Centre as a member of advisory and governance boards outside the submitted work. VM reports speaker honoraria and advisory panel consultancy outside the submitted work for Nutricia, Abbott and Nestle. KPP is an investigator on industry sponsored studies of investigational products and her institution has received funding from Aravax, DBV Technologies, GSK, Novartis and Novavax outside of the submitted work. All other authors have nothing to disclose.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

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To encourage compliance and monitor dietary group adherence, participants complete the following four-questions each month during the intervention period.

Thinking about the last week (7 days) how much of the following have you eaten:

- Eggs (boiled, fried, scrambled, poached, as an omelette or egg on hamburger/sandwich)?

  □no egg □1 egg □2 eggs □3 eggs □4 eggs □5 eggs □6 eggs □7 or more eggs.

- Serves of any of these egg containing foods: frittata, quiche or french toast?

  □no serves □1 serve □2 serves □3 serves □4 serves □5 serves □6 serves □7 serves □8 serves □9 serves □10 or more serves

- Peanuts (not including peanut butter)?

  □no peanuts □1-10 peanuts □11-20 peanuts □21-30 peanuts □31-40 peanuts □41-50 peanuts □51-60 peanuts □61-70 peanuts □71-80 peanuts □more than 80 peanuts

- Peanut butter?

  □no peanut butter □1 teaspoon peanut butter □2 teaspoons peanut butter □3 teaspoons peanut butter □4 teaspoons=1 tablespoon peanut butter □1 ½ tablespoons peanut butter □2 tablespoons of peanut butter □3 tablespoons of peanut butter □4 tablespoons of peanut butter □5 tablespoons peanut butter □ 6 tablespoons peanut butter □ more than 6 tablespoons peanut butter

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PrEggNut Study
STATISTICAL ANALYSIS PLAN

Study Title: Maternal diet rich in eggs and peanuts to reduce food allergies: a randomised controlled trial [PrEggNut Study]

Trial registration Australian and New Zealand Clinical Trials Registry
ACTRN12618000937213

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1 PREFACE

This Statistical Analysis Plan (SAP) describes the planned analyses and reporting for the PrEggNut randomised controlled trial. The following documents were reviewed in preparation of this SAP:

- PrEggNut case report form (CRF) (20th March 2019);
- PrEggNut NHMRC project grant application (APP1147576);
- PrEggNut trial protocol (version 2, 11th June 2019).

Any deviations from the planned analyses detailed in this SAP will be clearly documented with reasons in a post-analysis version of the SAP. Any post-hoc analyses which are not identified in this SAP but are completed to support planned study analyses will also be clearly identified.

2 INTRODUCTION

2.1 Background and rationale

Australia has the highest reported prevalence of food allergy in children in the world. Recent randomised controlled trials have shown that regularly including traditionally allergenic foods, such as egg and peanut, with solid foods from mid-late infancy reduces the risk of developing egg and peanut allergies in some infants. Australian studies, led by Chief Investigators Palmer, Makrides, Prescott, Campbell and Gold, have made a key contribution to clinical practice internationally in this field. Our findings also demonstrate that a significant proportion of infants have allergic reactions, including anaphylaxis, on first introduction of egg in solid foods as early as 4 months of age. We have recently shown that food-allergen (egg) specific immune responses can be established prior to infants eating any egg-allergen in solid foods. Furthermore, these responses were not altered by early introduction of egg in the infant diet. In other words, it is too late for many infants who are already allergic by the time these foods are introduced around 4-6 months of age.

Food allergens are first encountered before birth and can be detected in amniotic fluid, and in the immediate postnatal period in breastmilk. Intriguingly, the fetal immune system has been indicated to be particularly tolerogenic during this developmental period. Immune tolerance appears to be the default response to allergen encounter. Hence, early allergen exposure is a logical strategy for food allergy prevention. Accordingly, we have recently shown that higher maternal intakes of egg during early lactation can beneficially modify infant egg-specific immune responses. However, it is not known whether these changes are associated with a reduced likelihood of developing egg allergy in

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infants. Hence this randomised controlled trial will investigate higher regular egg and peanut maternal dietary intakes during pregnancy and lactation as a strategy for infant food allergy prevention.

2.2 Objectives

To determine whether the incidence of food allergies in infancy can be reduced by a maternal diet rich in eggs and peanuts during pregnancy and lactation.

3 STUDY METHODS

3.1 Trial design

Multi-centre, parallel, two-arm (1:1 allocation), researcher blinded (outcome data assessors, statistical analyst and investigators), randomised trial. Women with a singleton pregnancy who are planning to breastfeed for at least 4 months will be randomised to follow either a high (at least 6 eggs and 60 peanuts per week) or standard (no more than 3 eggs and 30 peanuts per week) egg and peanut intake diet from 22 weeks gestation until 4 months postnatal infant age. The primary outcome is food challenge proven IgE-mediated egg and/or peanut allergy in the infants at 12 months of age.

3.2 Randomisation

Pregnant women are assigned to a high egg and peanut diet (treatment group) or standard egg and peanut diet (control group) using a secure web-based randomisation service. The randomisation service allocates group assignments according to a computer-generated randomisation schedule, produced by an independent statistician using ralloc.ado version 3.7.6 in Stata version 15.1. Randomisation was stratified by city (Adelaide, Melbourne, Perth, Sydney) and by first or subsequent born child to the mother using randomly permuted blocks of varying sizes.

3.3 Sample size

To detect a reduction in egg and/or peanut allergy from 16% in the control group to 11.2% in the treatment group (absolute reduction 4.8%, relative reduction 30%) with 85% power and two-sided alpha of 0.049 at the final analysis, 961 women per group were required (with a continuity correction applied). Conservatively assuming 10% loss to follow-up, this led to a sample size estimate of 1068 women per group, or 2136 women total. Further details on the assumptions involved in the sample size calculations are provided in the PrEggNut trial protocol (Version 2, Section 5.7).

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3.4 Framework

All comparisons will be undertaken assuming a standard superiority hypothesis testing framework.

3.5 Statistical interim analyses and stopping guidance

An independent statistician will conduct a single interim analysis of the primary outcome once 961 infants (50% of the planned sample size, accounting for loss to follow-up) have primary outcome data available. The analysis will be performed according to the statistical methods described in Section 6. An independent Data Monitoring Committee will review the results of this analysis and employ O'Brien-Fleming stopping criteria (1) to maintain the overall alpha for the primary outcome across interim and final analyses at 0.05. A two-sided p-value of less than 0.0031 at the interim analysis (symmetric stopping boundary of Z = ±2.96259), indicating a large difference between treatment groups, will be taken to provide statistical evidence in support of early stopping. A p-value of 0.0490 (Z = ±1.96857) will be used to indicate statistical significance at the final analysis.

3.6 Timing of final analysis and unblinding

The database will be locked for analysis once data collection and cleaning are complete and the final version of this SAP has been approved. Following the database lock, blinded treatment codes will be made available to the trial statistician and analysis of the listed outcomes will be performed blinded to treatment group. Results of these analyses will be made available to the Trial Steering Committee members, with the blinding broken following a review of results.

3.7 Timing of outcome assessments

The 12-month clinical outcome assessment should occur when infants are between 12 and 15 months of age. Outcome data collected outside this window will still be included in the main intention to treat analyses but excluded from a per-protocol analysis of the primary outcome (see Section 4.3).

4 STATISTICAL PRINCIPLES

4.1 Confidence intervals and p values

For each outcome variable, a 95% confidence interval will be reported to express uncertainty about the estimated treatment effect. The statistical significance of the estimated treatment effect will be assessed at the 0.05 level using a two-sided comparative test, unless otherwise specified.

In describing the effectiveness of the intervention, multiple hypothesis tests will be performed due to multiple secondary outcomes, subgroup analyses and sensitivity analyses for the primary outcome. No multiplicity adjustment will be made for the number of secondary analyses, as these are of less importance than the overall intention to treat (ITT) analysis of the primary outcome. In the absence of a formal procedure for controlling the type-I error rate, less emphasis will be placed on the results of secondary analyses.

4.2 Adherence and protocol deviations

Adherence to the intervention involves (a) following the recommended intake of egg and peanuts to 4 months postnatal age, and (b) continuing breastfeeding to 4 months postnatal age. Women will be considered to have adhered to diet recommendations if they reported meeting recommendations in the last week (at least 6 eggs and 60 peanuts for the treatment group, no more than 3 eggs and 30 peanuts for the control group) across at least 75% of their scheduled pre-natal and post-natal assessments. Depending on timing of birth, pre-natal assessments are scheduled at 26, 30, 34 and 38 weeks gestation, while post-natal assessments are scheduled at 1, 2, 3 and 4 months after delivery. Women with missing data on egg and peanut intake at a scheduled appointment will be considered to have not met diet recommendations at that specific appointment. Women will be considered to have adhered to the breastfeeding recommendations if they continue any breastfeeding, including use of expressed breast milk, until at least 4 months of age. Women with missing data on breastfeeding duration will be considered to have not met breastfeeding recommendations.

For each randomised group, frequencies and percentages will be presented for:

- Overall adherence (following dietary consumption and breastfeeding recommendations);
- Adherence to breastfeeding recommendations;
- Adherence to both egg and peanut dietary consumption recommendations;
- Adherence to egg consumption recommendations;
- Adherence to peanut consumption recommendations.

The participants included in the ITT analysis dataset (see Section 4.3) will be used as the denominator in the calculation of percentages. The number of eggs and peanuts consumed in the last week at each scheduled assessment for each randomised group will also be described using medians and inter-quartile ranges.

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Frequencies and percentages (of the ITT analysis dataset) will also be presented separately by randomised group for the following protocol deviations:

- Ineligible participant randomised;
- Randomised in the wrong stratum;
- Given the wrong dietary recommendations according to randomisation;
- 12-month clinical outcome assessment not occurring between 12 and 15 months of age;
- Withdrawal from study;
- Loss to follow-up.

4.3 Analysis populations

For the primary outcome and secondary clinical outcomes (detailed in Sections 6.1 and 6.2), the planned analyses will be performed using an ITT approach. Excluding infant deaths occurring prior to outcome measurement (see Section 6.4.6 for more detail), the ITT population will include all randomised women-infant pairs, analysed as randomised, irrespective of eligibility or compliance with the protocol. For the primary outcome only, a sensitivity analysis will also be performed using a per-protocol approach. The per-protocol population will consist of all women-infant pairs that:

- Followed dietary consumption and breastfeeding recommendations (as described in Section 4.2);
- Did not report a protocol deviation (as described in Section 4.2);
- Provided data on the primary outcome at 12 months.

For exploratory laboratory outcomes (Section 6.3) collected in Perth and Sydney, the analysis population will consist of all women or infants that provided a blood sample at the relevant time point, analysed according to their randomised group.

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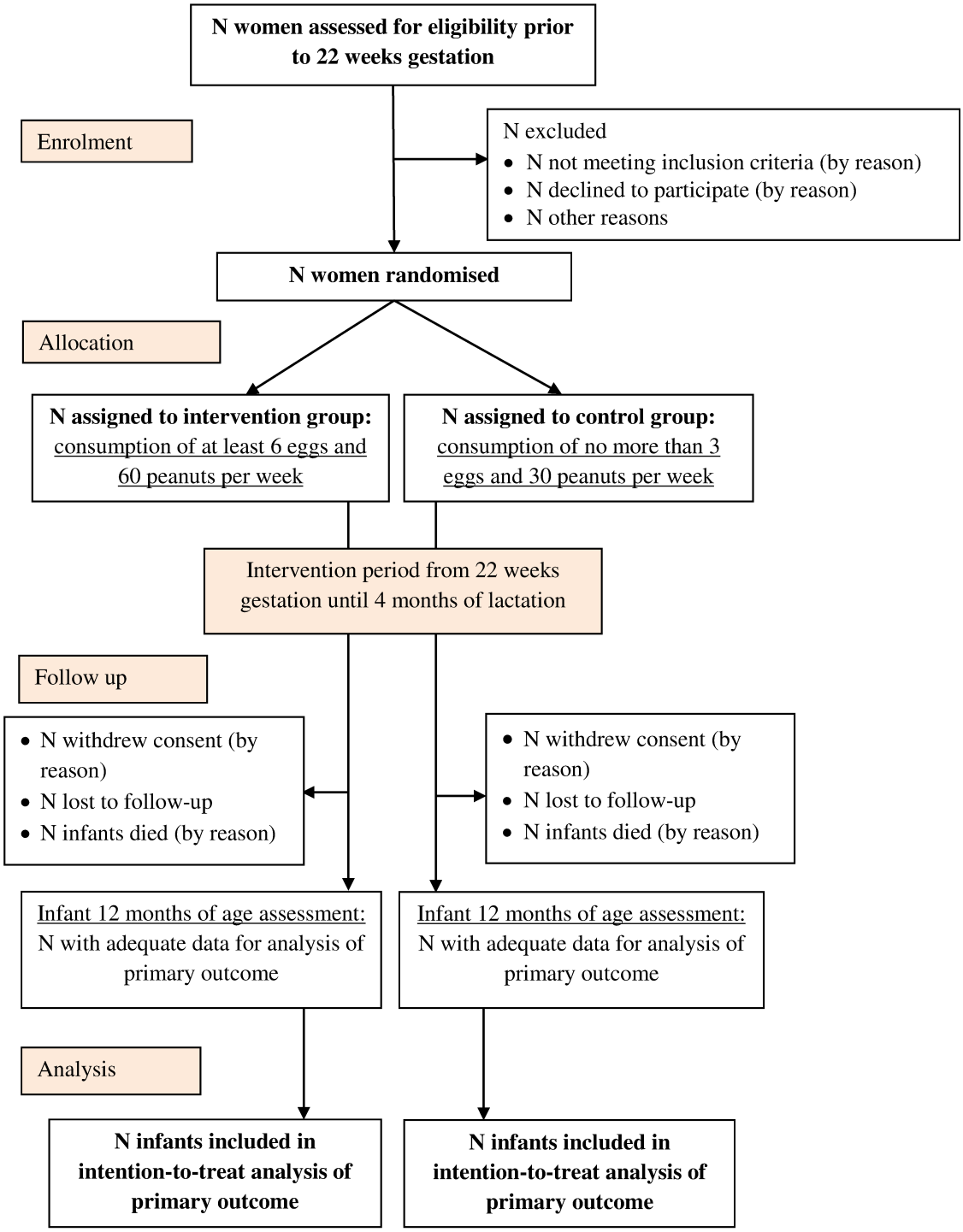
5 TRIAL POPULATION

5.1 Screening data, eligibility, recruitment and withdrawal/follow-up

The following CONSORT flow diagram will be completed to document numbers screened and randomised and the flow of participants through the trial.



The number of screened women and exclusions from the trial (not meeting the protocol-defined inclusion criteria, declined to participate, withdrew consent and loss to follow-up) will also be reported separately for each city.

5.2 Baseline characteristics

A descriptive comparison of the randomised groups will be conducted on the baseline characteristics presented in the following table.

Baseline characteristic	**Categories**
City	Adelaide Melbourne Perth Sydney
Maternal age in years	-
Maternal ethnicity	Caucasian Aboriginal/Torres Strait Islander Maori/Pacific Islander African Middle Eastern Mediterranean East Asian South/South East Asian Other
Maternal school education - completion of secondary school	Yes/No
Maternal further education	No further study Certificate/diploma Degree Higher degree
Maternal smoking	Yes/No
Other smoking in the household	Yes/No
Pet dog(s)	Yes/No
Pet cat(s)	Yes/No

Pet chicken(s)	Yes/No
Parity	First born child Subsequent born child
Gestation at randomisation in weeks	-
Pre-pregnancy weight in kg	-
Current weight in kg	-
Maternal BMI in kg/m^2	-
Maternal history of allergic disease	Yes/No
Paternal history of allergic disease	Yes/No
Sibling history of allergic disease	Yes No Not applicable
Maternal history of food allergy	Yes/No
Paternal history of food allergy	Yes/No
Sibling history of food allergy	Yes No Not applicable
Maternal peanut intake per week	-
Total household peanut intake per week	-
Maternal egg intake per week	-
Total household egg intake per week	-
Maternal egg specific-IgG4 levels (Perth and Sydney women only)	-
Maternal peanut specific-IgG4 levels (Perth and Sydney women only)	-
Infant sex	Female Male

Means and standard deviations, or medians and interquartile ranges will be reported for continuous variables. Frequencies and percentages will be reported for categorical variables. The clinical importance of any observed imbalances will be noted.

5.3 Post-randomisation characteristics

A descriptive comparison of the randomised groups will be conducted on the post randomisation characteristics presented in the following table:

Post-randomisation characteristic	**Categories**
Infant birth weight in kg	-
Infant gestational age at birth in weeks	-
Preterm birth < 37 weeks	Yes/No
Early preterm birth < 34 weeks	Yes/No
Infant birth mode of delivery	Vaginal Caesarean section
Ever breastfed	Yes/No
Breastfeeding during the whole intervention period until infant is 4 months of age	Yes/No
Breastfeeding up to 8 months of age	Yes/No
Breastfeeding up to 12 months of age	Yes/No
Breastfeeding duration in months*	-
Maternal mastitis during the intervention period	Yes/No
Maternal weight change during intervention period in kg	-
Infant given any infant formula during intervention period	Yes/No
Age at introduction to infant formula in months in infants given formula	-
Age at introduction to solid foods in months*	-
Age of introduction to egg in months*	-
Age of introduction to peanut in months*	-
Infant weight at 4 months of age in kg	-

* Time to event variable

Means and standard deviations, or medians and interquartile ranges will be reported for continuous and time to event variables. Frequencies and percentages will be reported for categorical variables.

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Differences between study groups will be tested using t-tests for continuous variables, Fisher exact tests for categorical variables and log-rank tests for time to event variables.

6 ANALYSIS

6.1 Primary outcome

6.1.1 IgE-mediated egg and/or peanut allergy at 12 months of age

Binary outcome based on measurements on the infant at 12 months of age. IgE-mediated egg and/or peanut allergy is defined as an allergic reaction to egg with sensitisation to egg and/or an allergic reaction to peanut with sensitisation to peanut. Sensitisation is defined as a 3mm or greater weal size based on a skin prick test (SPT). Infants with a positive SPT who do not proceed with the corresponding food challenge due to previous anaphylaxis or allergic reaction will be considered positive for IgE-mediated egg and/or peanut allergy; otherwise, the allergic reaction will be established according to an oral food challenge.

Note: Sensitisation is defined the same way throughout Section 6.2.

6.2 Secondary clinical outcomes

6.2.1 Sensitisation to egg

Binary outcome based on a positive SPT to egg.

6.2.2 Sensitisation to peanut

Binary outcome based on a positive SPT to peanut.

6.2.3 IgE-mediated egg allergy

Binary outcome based on an allergic reaction to egg with sensitisation to egg. Infants with a positive SPT for egg who do not proceed with the egg food challenge due to previous anaphylaxis or allergic reaction to egg will be considered positive for IgE-mediated egg allergy; otherwise, the allergic reaction will be established according to an oral egg challenge.

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6.2.4 IgE-mediated peanut allergy

Binary outcome based on an allergic reaction to peanut with sensitisation to peanut. Infants with a positive SPT for peanut who do not proceed with the peanut food challenge due to previous anaphylaxis or allergic reaction to peanut will be considered positive for IgE-mediated peanut allergy; otherwise, the allergic reaction will be established according to an oral peanut challenge.

6.2.5 Infant eczema

Binary outcomes based on a medical diagnosis of infant eczema by 4 and 12 months of age.

6.3 Exploratory laboratory outcomes

6.3.1 Maternal egg specific IgG4 concentrations

Continuous outcomes based on egg specific IgG4 levels (mgA/L) in maternal blood samples at 34-38 weeks gestation and 4 months postnatal age (Perth and Sydney women only). Egg-specific IgG4 serum antibody concentrations will be measured using the ImmunoCAP 250 system, which has a lower limit of detection of 0.07 mgA/L.

6.3.2 Maternal peanut specific IgG4 concentrations

Continuous outcomes based on peanut specific IgG4 levels (mgA/L) in maternal blood samples at 34-38 weeks gestation and 4 months postnatal age (Perth and Sydney women only). Peanut-specific IgG4 serum antibody concentrations will be measured using the ImmunoCAP 250 system, which has a lower limit of detection of 0.07 mgA/L.

6.3.3 Infant egg specific IgG4 concentrations

Continuous outcomes based on egg specific IgG4 levels (mgA/L) in infant blood samples at 4 and 12 months of age (Perth and Sydney infants only). Egg-specific IgG4 serum antibody concentrations will be measured using the ImmunoCAP 250 system, which has a lower limit of detection of 0.07 mgA/L.

6.3.4 Infant peanut specific IgG4 concentrations

Continuous outcomes based on peanut specific IgG4 levels (mgA/L) in infant blood samples at 4 and 12 months of age (Perth and Sydney infants only). Peanut-specific IgG4 serum antibody concentrations will be measured using the ImmunoCAP 250 system, which has a lower limit of detection of 0.07 mgA/L.

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6.3.5 Infant egg specific IgE concentrations

Continuous outcomes based on egg specific IgE levels (kUA/L) in infant blood samples at 4 and 12 months of age (Perth and Sydney infants only). Egg-specific IgE serum antibody concentrations will be measured using the ImmunoCAP 250 system, which has a lower limit of detection of 0.1 kUA/L.

6.3.6 Infant peanut specific IgE concentrations

Continuous outcomes based on peanut specific IgE levels (kUA/L) in infant blood samples at 4 and 12 months of age (Perth and Sydney infants only). Peanut-specific IgE serum antibody concentrations will be measured using the ImmunoCAP 250 system, which has a lower limit of detection of 0.1 kUA/L.

6.4 Analysis methods

6.4.1 Overall analysis approach

The primary outcome and all secondary clinical outcomes (see Sections 6.1 and 6.2) will be analysed using log binomial regression models, with the effect of treatment described as a relative risk with a 95% confidence interval. Should any of the models fail to converge, a known problem with log binomial regression, a log Poisson model using generalised estimating equations (independence working correlation structure assumed) will be used for analysis (2). If the number of infants experiencing an outcome is considered too small for a regression model to be sensible (less than 5 events in either randomised group), then, regardless of convergence, a Fisher exact test will be performed instead.

To account for censoring due to the lower detection limits of the measuring equipment, the exploratory laboratory outcomes (Section 6.3) will be analysed using tobit regression models. Based on previous experience with these measures (3), the IgG4 and IgE concentrations will be log transformed prior to analysis to satisfy an assumption of homogeneous error variance. The effect of treatment on the latent (uncensored) IgG4 and IgE concentrations will be described as a ratio of means with a 95% confidence interval. Should the assumption of homogeneous error variance on the log scale be deemed unreasonable, based on a scatterplot of model residuals versus fitted values, other transformations will be explored and justified as appropriate.

6.4.2 Covariate adjustment

Given recommendations to adjust for variables used to stratify the randomisation when estimating treatment effects (4), analyses will be adjusted for city (Adelaide, Melbourne, Perth, Sydney) and

birth order (first or subsequent born child to the mother). Adjustment will also be made for infant sex and maternal history of allergic disease (defined as a medical diagnosis of asthma, eczema, hayfever or food allergy), both considered important prognostic variables for infant allergic disease outcomes (5, 6). All adjustment variables will be treated as fixed effects in the analysis models. For each outcome, both unadjusted and adjusted analyses will be performed, with the adjusted analyses used to draw final conclusions about the effect of treatment.

If adjusted models fail to converge for any of the outcomes, adjustment variables will be removed sequentially (infant sex, then maternal history of allergic disease, then city, then birth order) until convergence is achieved. Adjusted analyses will not be considered for binary outcomes analysed using a Fisher exact test (see Section 6.4.1).

6.4.3 Planned subgroup analyses

For the primary outcome only, analyses will be performed to test for evidence of effect modification by (1) birth order (first or subsequent born child to the mother), (2) baseline total household egg intake, (3) baseline total household peanut intake, and (4) socio-economic status, determined using the home post-code of the mother and the index of relative socio-economic advantage and disadvantage (www.abs.gov.au/websitedbs/censushome.nsf/ home/seifa). Effect modification by birth order will be assessed by including this subgroup variable as well as its interaction with treatment group into the log binomial regression model for the primary outcome (with the covariates detailed in Section 6.4.2 also included in the model). Effect modification by total household egg intake, total household peanut intake and socio-economic status will be assessed in a similar fashion in separate log binomial models, but with these variables treated as continuous rather than categorical in the analysis. To account for potential non-linear effects, two-term fractional polynomials will be fitted using the "mfpi" command in Stata v16 (or later) using default settings (7). For each potential effect modifier, the p-value for the interaction term with treatment group will be reported. Independent of the statistical significance of the interaction p-value, estimates of the treatment effect with 95% confidence intervals will be reported for each birth order subgroup or in treatment effect plots for total household egg intake, total household peanut intake and socio-economic status.

6.4.4 Methods for addressing outlying values

Outliers will be queried during data collection and the statistical analysis. Unless confirmed as a data entry error, outliers will not be excluded from any analyses.

6.4.5 Methods for handling missing data

Missing data will be summarised descriptively by treatment group for all baseline characteristics (Section 5.2), post-randomisation characteristics (Section 5.3), outcome variables (Sections 6.1 to 6.3), covariates for adjustment (Section 6.4.2) and covariates for subgroup analyses (Section 6.4.3). Composite binary outcomes (6.1.1, 6.2.3 and 6.2.4) will be treated as missing if any of the individual components of the outcome are missing, even if the composite can be derived from the observed components. Variables not ascertained due to infant death will not be treated as missing, as such data are not meaningful for analysis. Instead, these data will be treated as undefined and excluded from analyses (see Section 6.4.6).

To address missing data on the primary outcome and secondary clinical outcomes, multiple imputation performed under a missing at random assumption will be used to create 100 complete datasets for analysis, even if only a small percentage of data are missing. Use of 100 imputations ensures that the loss of power compared to full information maximum likelihood methods is minimal (8), which is important in the context of a confirmatory clinical trial. Imputation will be performed separately by treatment group using fully conditional specification, also known as chained equations. The conditional logistic imputation models for the incomplete outcomes will include covariates pre-specified for adjustment (Section 6.4.2) and for conducting subgroup analyses (Section 6.4.3). Additional auxiliary variables associated with the incomplete outcomes will also be added to the imputation model as appropriate to improve the prediction of missing values and the plausibility of the missing at random assumption. For composite binary outcomes (6.1.1, 6.2.3 and 6.2.4), conditional logistic imputation models will be defined for each component and the composite outcome calculated following imputation.

For the primary outcome, the sensitivity of results to the missing at random assumption will be explored by considering missing not at random mechanisms. Using pattern mixture models, the odds of IgE mediated egg and/or peanut allergy will be assumed to be between half and twice as high in children with missing data compared to children with observed data. These differences will be applied to control group children only, treatment group children only and children in both treatment groups.

For the primary outcome and secondary clinical outcomes, a complete case analysis will also be performed on the unimputed data for comparison with imputed results. However, these analyses will not be used to inform conclusions about the effect of treatment.

Considering the exploratory nature of the laboratory outcomes and the expectation of little missing data among the analysis population of women and infants providing a blood sample at each time point, analyses of these outcomes will be restricted to participants with complete outcome data.

6.4.6 Methods for addressing infant deaths

It is anticipated that a small proportion of infants will die during the study, either during pregnancy or following birth, preventing the later measurement of outcome variables. In these cases, the outcomes will be treated as undefined and excluded from analyses. Such a "survivors analysis" has been recommended in settings where the intervention is considered biologically unlikely to impact on the risk of mortality (9), as is the case here with peanut and egg consumption during pregnancy and lactation. If there is any evidence to suggest that the risk of infant death is influenced by the intervention ($p<0.20$ according to a Fisher exact test), we will also consider the effect of the intervention on the composite of death and IgE-mediated egg and/or peanut allergy at 12 months of age (i.e., the primary outcome) in a post-hoc analysis.

6.5 Harms

The number and percentage of mother-infant pairs experiencing an adverse event (AE) will be reported for each treatment group (irrespective of eligibility or compliance with the protocol) and compared across groups using Fisher exact tests. The following AEs will be evaluated:

- Maternal admission to intensive care unit (serious adverse event, SAE)
- Maternal death (SAE)
- Infant admission to intensive care unit (SAE)
- Infant death, including stillbirths (SAE)
- Any SAE
- Maternal hospitalisation > 24 hours, excluding admission for baby's birth (AE)
- Infant hospitalisation > 24 hours, excluding admission for baby's birth (AE)
- Infant anaphylaxis to egg or peanut (AE)

6.6 Analysis Software

All analyses will be performed using Stata v16 or later (College Station, TX: StataCorp LP).

7 REFERENCES

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