




BMJ Open 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

Debra J Palmer ^{1,2} Thomas R Sullivan ^{3,4} Dianne E Campbell,^{5,6} Ralph Nanan,^{7,8} Michael S Gold,^{9,10} Peter S Hsu,^{5,6} Merryn J Netting,^{3,11} Vicki McWilliam,^{12,13} Jennifer J Koplin,^{12,14} Kirsten P Perrett ^{13,14}, Patrick Quinn,^{9,10} Michael O'Sullivan,^{15,16} Susan L Prescott,^{1,16} Rosalie Grivell,¹⁷ Maria Makrides^{3,9}

To cite: Palmer DJ, Sullivan TR, Campbell DE, *et al.* 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. *BMJ Open* 2022;**12**:e056925. doi:10.1136/bmjopen-2021-056925

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2021-056925>).

Received 30 August 2021
Accepted 25 May 2022



© Author(s) (or their employer(s)) 2022. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to

Dr Debra J Palmer;
debbie.palmer@telethonkids.org.au

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.

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.

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.

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

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 allergens⁸ as early as 22 weeks' gestation.⁹ The fetus develops regulatory immune responses to both self-antigens and to exogenous allergens¹⁰ 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 USA²⁰ 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

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 method²² 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

TIMEPOINT	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					Completion
	<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. t₁=22 weeks' gestation to birth; t₂=birth; t₃=birth to 4 months' postnatal; t₄=infant 4 months of age; t₅=infant 8 months of age; t₆=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

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

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.

Author affiliations

¹Telethon Kids Institute, The University of Western Australia, Nedlands, Western Australia, Australia

- ²UWA Medical School, The University of Western Australia, Perth, Western Australia, Australia
- ³SAHMRI Women and Kids, South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia
- ⁴School of Public Health, The University of Adelaide, Adelaide, South Australia, Australia
- ⁵Discipline of Child and Adolescent Health, The University of Sydney, Lidcombe, New South Wales, Australia
- ⁶Department of Allergy and Immunology; and Kids Research, The Children's Hospital at Westmead, Westmead, New South Wales, Australia
- ⁷Charles Perkins Center, The University of Sydney, Sydney, New South Wales, Australia
- ⁸Sydney Medical School Nepean, The University of Sydney, Sydney, New South Wales, Australia
- ⁹Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, South Australia, Australia
- ¹⁰Department of Allergy and Clinical Immunology, Women's and Children's Health Network, North Adelaide, South Australia, Australia
- ¹¹Department of Nutrition, Women's and Children's Health Network, North Adelaide, South Australia, Australia
- ¹²Murdoch Childrens Research Institute, Parkville, Victoria, Australia
- ¹³Department of Allergy and Immunology, The Royal Children's Hospital Melbourne, Parkville, Victoria, Australia
- ¹⁴Department of Paediatrics, The University of Melbourne, Melbourne, Victoria, Australia
- ¹⁵Faculty of Medicine Dentistry and Health Sciences, The University of Western Australia, Perth, Western Australia, Australia
- ¹⁶Immunology Department, Perth Children's Hospital, Nedlands, Western Australia, Australia
- ¹⁷Department of Obstetrics Gynaecology and Reproductive Medicine, Flinders University, Bedford Park, South Australia, Australia

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, M.JN and VM all contributed to refinement of the PrEggNut Study Protocol and approved the final manuscript.

Funding This trial is supported by the NHMRC project grant number 1147576. DJP is supported by the NHMRC, Medical Research Futures Fund (MRFF) Career Development Fellowship 1144544. MM is supported by an NHMRC Principal Research Fellowship 1061704. TRS is supported by an NHMRC Emerging Leadership Fellowship 1173576. MN is supported by an NHMRC Early Career (Health Practitioner) Fellowship 1156518. JK is supported by an NHMRC Career Development Fellowship 1158699. KPP is supported by a Melbourne Children's Clinician-Scientist Fellowship. SLP is supported by a fellowship from the NOVA Institute for Health.

Disclaimer The funders have no role in the study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication and have no authority over any of these activities.

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.

Provenance and peer review Not commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and

responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iDs

Debra J Palmer <http://orcid.org/0000-0003-2498-8343>
 Thomas R Sullivan <http://orcid.org/0000-0002-6930-5406>
 Kirsten P Perrett <http://orcid.org/0000-0002-5683-996X>

REFERENCES

- Du Toit G, Roberts G, Sayre PH, *et al*. Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J Med* 2015;372:803–13.
- Ierodiakonou D, Garcia-Larsen V, Logan A, *et al*. Timing of allergenic food introduction to the infant diet and risk of allergic or autoimmune disease: a systematic review and meta-analysis. *JAMA* 2016;316:1181–92.
- Palmer DJ, Metcalfe J, Makrides M, *et al*. Early regular egg exposure in infants with eczema: A randomized controlled trial. *J Allergy Clin Immunol* 2013;132:387–92.
- Metcalfe JR, D'Vaz N, Makrides M, *et al*. Elevated IL-5 and IL-13 responses to egg proteins predate the introduction of egg in solid foods in infants with eczema. *Clin Exp Allergy* 2016;46:308–16.
- Pastor-Vargas C, Maroto AS, Diaz-Perales A, *et al*. Detection of major food allergens in amniotic fluid: initial allergenic encounter during pregnancy. *Pediatr Allergy Immunol* 2016;27:716–20.
- Szépfalusi Z, Loibichler C, Hänel-Dekan S, *et al*. Most of diaplacentally transferred allergen is retained in the placenta. *Clin Exp Allergy* 2006;36:1130–7.
- Szépfalusi Z, Loibichler C, Pichler J, *et al*. Direct evidence for transplacental allergen transfer. *Pediatr Res* 2000;48:404–7.
- Prescott SL, Macaubas C, Holt BJ, *et al*. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998;160:4730–7.
- Jones AC, Miles EA, Warner JO, *et al*. Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimuli during gestation. *Pediatr Allergy Immunol* 1996;7:109–16.
- Thornton CA, Upham JW, Wikström ME, *et al*. Functional maturation of CD4+CD25+CTLA4+CD45RA+ T regulatory cells in human neonatal T cell responses to environmental antigens/allergens. *J Immunol* 2004;173:3084–92.
- Mold JE, McCune JM. At the crossroads between tolerance and aggression: Revisiting the "layered immune system" hypothesis. *Chimerism* 2011;2:35–41.
- Mold JE, McCune JM. Immunological tolerance during fetal development: from mouse to man. *Adv Immunol* 2012;115:73–111.
- Bernard H, Ah-Leung S, Drumare M-F, *et al*. Peanut allergens are rapidly transferred in human breast milk and can prevent sensitization in mice. *Allergy* 2014;69:888–97.
- Mosconi E, Rekima A, Seitz-Polski B, *et al*. Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development. *Mucosal Immunol* 2010;3:461–74.
- Macchiaverni P, Arslanian C, Frazão JB, *et al*. Mother to child transfer of IgG and IgA antibodies against Dermatophagoides pteronyssinus. *Scand J Immunol* 2011;74:619–27.
- Metcalfe JR, Marsh JA, D'Vaz N, D'Vaz N, *et al*. Effects of maternal dietary egg intake during early lactation on human milk ovalbumin concentration: a randomized controlled trial. *Clin Exp Allergy* 2016;46:1605–13.
- Palmer DJ, Gold MS, Makrides M. Effect of cooked and raw egg consumption on ovalbumin content of human milk. *Clin Exp Allergy* 2005;35:173–8.
- Palmer DJ, Gold MS, Makrides M. Effect of maternal egg consumption on breast milk ovalbumin concentration. *Clin Exp Allergy* 2008;38:1186–91.



- 19 Perkin MR, Logan K, Tseng A, *et al.* Randomized trial of introduction of allergenic foods in breast-fed infants. *N Engl J Med* 2016;374:1733–43.
- 20 Frazier AL, Camargo CA, Malspeis S, *et al.* Prospective study of peripregnancy consumption of peanuts or tree nuts by mothers and the risk of peanut or tree nut allergy in their offspring. *JAMA Pediatr* 2014;168:156–62.
- 21 Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, *et al.* Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report. *J Allergy Clin Immunol* 2012;130:1260–74.
- 22 Kunz B, Oranje AP, Labrèze L, *et al.* Clinical validation and guidelines for the SCORAD index: consensus report of the European Task force on atopic dermatitis. *Dermatology* 1997;195:10–19.
- 23 Joshi PA, Smith J, Vale S, *et al.* The Australasian Society of clinical immunology and allergy infant feeding for allergy prevention guidelines. *Med J Aust* 2019;210:89–93.
- 24 Koplin JJ, Allen KJ, Gurrin LC, *et al.* The impact of family history of allergy on risk of food allergy: a population-based study of infants. *Int J Environ Res Public Health* 2013;10:5364–77.
- 25 Hill DJ, Hosking CS, Zhie CY, *et al.* The frequency of food allergy in Australia and Asia. *Environ Toxicol Pharmacol* 1997;4:101–10.
- 26 Palmer DJ, Sullivan TR, Gold MS, *et al.* Randomized controlled trial of early regular egg intake to prevent egg allergy. *J Allergy Clin Immunol* 2017;139:1600–7.
- 27 O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics* 1979;35:549–56.
- 28 Sullivan TR, White IR, Salter AB, *et al.* Should multiple imputation be the method of choice for handling missing data in randomized trials? *Stat Methods Med Res* 2018;27:2610–26.