

TRACE peanut study Final report: Threshold reactivity and clinical evaluation study The effect of sleep deprivation and exercise on reaction threshold in peanut-allergic adults: a randomised controlled study

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Table of Contents

Acknowledgements 2
Table of Contents
List of figures7
List of tables
Glossary of terms
Executive summary 11
Introduction and background to the study 11
The problem with Precautionary Allergen Labelling11
Establishing thresholds: The theory 13
Methods 17
Results 17
Implications
Aims and objectives of the TRACE study 19
Methods
Funding
Recruitment
Screening via website
Screening via telephone21
Study inclusion and exclusion criteria22
Inclusion criteria 22
Exclusion criteria
Screening visit
Skin Prick Testing
Blood sampling23
Other investigations
Exercise

Sleep diary	
Informed consent	24
Study design	25
Randomisation	
Challenge visits	
Baseline challenge - pre challenge assessment	
Challenge meal preparation	
Challenge Procedure	
Criteria for scoring symptoms and stopping food challenge	30
Intervention challenges	31
Single or multiple factors	31
Exercise challenge	31
Sleep challenge	32
No intervention challenge	32
Treatment of allergic reactions	32
Discharge procedures	
Data collection and management	
Ethics committee approval	35
Study outcomes	35
Analysis populations	36
Adverse events	36
Adverse event	
Serious adverse event	37
Collection and recording of adverse events	37
Grading and attribution of adverse events	38
Attribution Definitions	40
Reporting serious adverse events	40

	IDMC	41
S	Study management	41
	Independent Members	41
	Dependent (voting) members	42
	Observers	42
	Previous members	42
S	Statistical Analysis	43
	Sample size	43
	Initial Sample Size Calculation	43
	Further Simulation Work	43
	Primary outcome analysis	45
	Secondary outcome analysis	46
Pilo	ot study and alteration to initial study design	48
F	Pilot Baseline Challenges	48
A	im of the pilot baseline challenge study	48
Ν	Nethods	48
F	Results	49
C	Dutcome	49
F	Pilot Exercise Challenges	50
	Background	50
	Aims of pilot exercise study	50
	Pilot exercise method	50
	Pilot exercise results	51
	Outcome	54
F	Pilot sleep deprivation challenges	54
	Aims of pilot sleep deprivation study	54
	Pilot sleep deprivation method	. 54

Pilot sleep deprivation results	54
Outcome	56
Alteration to main study design: Placebo challenges	56
Results	58
Study populations	58
Primary outcome: Peanut thresholds and the effect of co-factors	61
Secondary outcome: Population threshold curves	69
Safety	75
Adrenaline use	75
Discussion and implications	76
Limitations	85
Appendices	88
Appendix 1: TRACE study website registration portal	88
Appendix 2: Screening Visit Form	89
Appendix 3: Modification and explanation of existing PRACTALL criteria	107
Appendix 4: TRACE study case record form	112
Appendix 5: Karolinska Sleepiness Scale	129
Appendix 6: Per protocol results	130

List of figures

Figure 1 TRACE study recruitment	24
Figure 2 Overall TRACE study design	26
Figure 3 World Allergy Organization subcutaneous immunotherapy systemic reaction grading.	39
Figure 4 Simulation scenarios to inform power calculation	45
Figure 5 Trace showing a participant's heart rate (vertical axis) during exercise bouts	51
Figure 6 Consort diagram	58
Figure 7 Dose reached (mg peanut protein) by challenge for full analysis population	62
Figure 8 Log (dose reached) by challenge for full analysis population	63
Figure 9 Dose reached in mg peanut protein by visit number (full analysis population)	64
Figure 10 Log dose reached of peanut protein by visit number	65
Figure 11 Percentage change in threshold (logged) for each covariate	66
Figure 12 Dose distribution curve for extended analysis population (n=123) with 95% confidence intervals	70
Figure 13 Threshold dose distribution model	72
Figure 14 Eliciting dose estimates (mg peanut protein) derived from threshold distribution curve	73
Figure 15 The effect of stress on mucosal mast cells and intestinal permeability	81

List of tables

Table 1 Dose regimen	30
Table 2 Treatment of severe (red) symptoms	33
Table 3 Pilot baseline (n=4) challenges and pilot exercise (n=3) challenges	53
Table 4 Pilot sleep deprivation challenges	55
Table 5 Psychomotor vigilance task results for 3 pilot healthy volunteer sleep participants	56
Table 6 Baseline characteristics for study populations	60
Table 7 Estimated effect shown in log and percentage scale, 95% confidence interval and p-value for each term in the linear mixed effects model	67
Table 8 Predicted dose (and 95% CI) that gives different probability of reactions	74

Glossary of terms

AIC	Akaike information criterion			
CTCAE	Common terminology criteria for adverse events			
CRF	Clinical research facility			
DBPCFC	Double blind placebo-controlled food challenge			
ED	Elicitation dose			
FDEIA	Food dependent exercise induced anaphylaxis			
FEV	Forced expiratory volume			
IgE	Immunoglobulin E			
IM	Intramuscular			
ICSA	Interval censored survival analysis			
IV	Intravenous			
LOAEL	Lowest observed adverse effect level			
MED	Minimum eliciting dose			
μg	Microgram			
mg	Milligram			
NOAEL	No observed adverse effect level			
NRES	National Research Ethics Service			
NSAID	Non-steroidal anti-inflammatory drug			
OFC	Oral food challenge			
PAL	Precautionary advisory labelling			

PEFR	Peak expiratory flow rate
QOL	Quality of life
SAE	Serious adverse event
SUSARS	Serious unexpected severe adverse reactions
SPT	Skin prick test
TDC	Threshold Dose distribution Curves
TMG	Trial management group
TRACE	Threshold Reactivity and Clinical Evaluation Study
TSC	Trial Steering Committee
VO2	Maximum rate of oxygen consumption
WDEIA	Wheat dependent exercise induced anaphylaxis

Executive summary

Introduction and background to the study

Presently there is no therapy available in clinical practice for the treatment of peanut allergy. Thus, the current strategy in managing this condition is careful avoidance of the allergen and rescue therapy in the event of accidental exposure. Allergens in foods present a risk to allergic individuals. To help food allergic consumers practise safe allergen avoidance, European Legislation mandated that the presence of 14 allergens, deliberately added as ingredients, must be declared on prepacked foods. In December 2014 this was extended to non-prepacked foods and foods eaten outside the home.

There were also changes to the way in which the allergenic foods were labelled. Allergens now have to be highlighted in bold and located in a single place i.e. in the ingredients list. However, an additional type of labelling exists: precautionary allergen labelling (PAL). This type of labelling is aimed at notifying allergic consumers about the risk of unintentional allergen presence which are not deliberately added as ingredients, for example by contamination during processing methods. With an increasingly complex food manufacturing process, often equipment is shared and several different types of food can be processed using the same production line. This PAL often takes the form of warnings such as 'May contain peanut', 'Not suitable for those with peanut allergy', 'Made in factory where nuts are processed' etc. Unfortunately, there is no specific legislation that governs the use of these advisory statements and furthermore, these advisory labels are voluntary, but the basis of their requirement is covered under General Food law.

As a result, these labels often send mixed messages to consumers making it difficult for them to make rational decisions about food choices.

The problem with Precautionary Allergen Labelling

PALs are present to try and convey the risk of reaction that a certain food poses to the sensitive population. Application of PAL however, is inconsistent making risk assessment difficult. Currently, due to fears over litigation with regards to accidental exposure to food allergens, advisory labels seem to be becoming increasingly common as some food manufacturers take a very risk averse approach to PAL. Patients mistakenly believe that a food with a label which reads 'May contain nuts' poses a greater risk than one which is labelled 'May contain traces'.¹

However, studies have demonstrated that there is often no relationship between the wording that is used on food labels and the amount of allergen that that food actually contains.^{2,3} This inconsistent labelling practice leads allergic consumers to become distrustful of labels leading them to act in one of two ways: either ignoring these advisory statements completely thereby placing themselves at risk or by avoiding these foods and thus narrowing their food choices considerably. The latter approach may have significant adverse consequences on their nutritional status.⁴ Food allergic consumers need to be able to trust the food label.

There is a need for specific regulation and legislation which governs when to use PAL and some standardisation of these labels. Regulation on when to use PAL could be based on, for example, reference doses for allergens such as peanut which have been derived from the distribution of individual threshold doses in the allergic population. These can be used to determine action levels below which PAL will not be required as ideally PAL should only be used when food manufacturers cannot

² Hefle SL, Furlong TJ, Niemann L, Lemon-Mule H, Sicherer S, Taylor SL. Consumer attitudes and risks associated with packaged foods having advisory labeling regarding the presence of peanuts. J Allergy Clin Immunol. 2007;120(1):171-176. doi:10.1016/j.jaci.2007.04.013

³ Remington BC, Baumert JL, Blom WM, Houben GF, Taylor SL, Kruizinga AG. Unitented allergen in precautionary labelled and unlabelled products pose a significant risk to UK allergic consumers. Allergy. 2015; 70: 813-819. doi: 10.1111/all.12625

⁴ Christie L, Hine RJ, Parker JG, Burks W. Food allergies in children affect nutrient intake and growth. J Am Diet Assoc. 2002. doi:10.1016/S0002-8223(02)90351-2

¹ Barnett J, Muncer K, Leftwich J, et al. Using "may contain" labelling to inform food choice: A qualitative study of nut allergic consumers. BMC Public Health. 2011. doi:10.1186/1471-2458-11-734

effectively manage the level of unintentional allergen presence below such defined action levels and thus pose a risk to the majority of the allergic population. The absence of the PAL should imply a clear level of agreed safety. Consumers need to be well educated about the process of allergen risk assessment to enable them to trust in food manufacturing and labelling practices. How can this problem therefore be addressed?

Establishing thresholds: The theory

Food policy makers are tasked with assessing the risks posed by allergenic foods. To do this, risk assessors need information about the response characteristics of the atrisk population and the size of the population at risk. In an ideal scenario it should be possible for risk assessors to calculate the number of reactions that would occur for any given level of residual allergen in a food product if allergic individuals consumed that food. Thus, data are required on the levels of allergen which may potentially only pose a small risk to most members of the allergic population. There is cause to believe that a level of peanut allergen exists below which no member of the allergic population would react.

Indeed, in a review on existing threshold data for peanut, Taylor et al concluded that 'thresholds for common allergenic foods are finite, measurable and above zero.⁵ Indeed a 'threshold' is defined as 'a limit below which a stimulus causes no reaction'. In the field of toxicology scientists use thresholds to determine the harmful effects of chemicals and pollutants. In this area, a threshold is defined as a dose at or below which a response is not seen in an experimental setting. Techniques on modelling thresholds in toxicology studies have been transposed to modelling thresholds in food allergy. In food allergy a threshold is defined as the amount of protein which evokes an allergic reaction. An individual's elicitation threshold to an allergen is thought to lie between the No Observed Adverse Effect Level (NOAEL), the highest dose that will not produce an adverse effect in that person and the Lowest Observed Adverse Effect Level (LOAEL). Data on individuals' thresholds can be obtained

⁵ Taylor SL, Hefle SL, Bindslev-Jensen C, et al. Factors affecting the determination of threshold doses for allergenic foods: How much is too much? J Allergy Clin Immunol. 2002;109(1):24-30. doi:10.1067/mai.2002.120564

through various types of clinical study including diagnostic challenges, threshold finding trials and immunotherapy studies.

Data from these studies show that the eliciting dose or LOAEL for peanut allergic individuals can range from a tenth of a milligram to many grams.⁶ Thresholds exist at both an individual and population level. It would be logical to believe that in an allergic population the lower the dose, the milder the symptoms and the lower the proportion of reactive individuals. However, there are few data on the proportion of allergic individuals reacting to a given dose as well as limited information on how severity relates to the dose threshold for any given individual. Furthermore, it has also been reported in some very sensitive individuals that systemic reactions have resulted from exposure to microgram amounts of food.⁷

Risk managers can utilise the data on NOAELs and LOAELs gleaned from these studies to examine the distribution of clinical minimum eliciting doses. This useful statistical approach allows inferences to be made about reaction rates to doses outside the experimental range; an advantageous approach given the restriction on being able to test all individuals in the allergic population. From a public health perspective, the optimal outcome would be to define a population threshold where all members of the allergic population are protected.

However, this 'zero risk' approach unfortunately is not practicable, and it is not possible to test the reaction threshold of every member of an allergic population. A more realistic and achievable aim, therefore, is to discover an amount of protein that is unlikely to cause serious adverse effects in the majority of the population at risk. Thus, the population threshold can be redefined as the largest amount of allergenic food which will not cause a reaction when tested in a defined proportion of allergic

⁶ Taylor SL, Crevel RWR, Sheffield D, Kabourek J, Baumert J. Threshold dose for peanut: risk characterization based upon published results from challenges of peanut-allergic individuals. Food Chem Toxicol. 2009;47(6):1198-1204. doi:10.1016/j.fct.2009.02.011

⁷ Wüthrich B. Lethal or life-threatening allergic reactions to food. J Investig Allergol Clin Immunol. 2000

individuals. The Eliciting Dose_x (ED_x) approach is often used and is pragmatic. It refers to the amount of allergen that is predicted to produce a reaction in a defined proportion of the allergic population. For example, the ED₁₀, which is commonly referred to in threshold studies, is the dose which will elicit a dose in 10% of the population. Eliciting doses are used to model reference doses which are essentially an index of safe exposure. The identification of reference doses for food allergens considered safe for the majority of food allergic individuals would inform risk assessment and provide guidance on when PAL should be used.

A consensus on levels of allergens that are low risk is lacking. Studies on doses of allergen which elicit reactions in allergic individuals have been performed and attempts have been made using dose distribution modelling to define doses of allergenic protein which are likely to elicit a reaction in a proportion of the population. Recently, single dose challenges have been used to validate these doses helping to move the debate forward,⁸ but concerns remain about the general applicability of such levels and how they might be modified by everyday lifestyle factors (co-factors).⁹

Several clinical studies have examined thresholds of reaction to allergen in foodallergic participants. From these, attempts have been made to model a population threshold. Peanut, probably due to its ubiquity and also its propensity to cause severe and fatal allergic reactions, has been the most widely studied. Taylor et al showed that peanut allergen elicitation thresholds can range from 0.5mg to 10000mg of whole peanut between peanut-allergic individuals.⁶ There is clearly a wide intrinsic variation in patients' thresholds. However, a possible factor influencing this wide

⁸ Hourihane JOB, Allen KJ, Shreffler WG, Dunngalvin G, Nordlee JA, Zurzolo GA, et al. Peanut Allergen Threshold Study (PATS): Novel single-dose oral food challenge study to validate eliciting doses in children with peanut allergy. J Allergy Clin Immunol. 2017;139(5):1583–90.

⁹ Allen KJ, Remington BC, Baumert JL, Crevel RWR, Houben GF, Brooke-Taylor S, et al. Allergen reference doses for precautionary labeling (VITAL 2.0): Clinical implications. J Allergy Clin Immunol. 2014;133(1):156–64.

apparent variation is that the contributory studies used to derive these estimates have varied in dosing regime, dosing interval, study entry criteria and food matrix.

Although it is widely known that thresholds vary across individuals in a population, few data exist on the variation of thresholds within allergic individuals over time. One study by Moneret-Vautrin suggests up to a 10-fold change in threshold with successive challenges (personal communication: Professor DA Moneret Vautrin and the North American and European branches of International Life Sciences Institute), while another suggests a small negative change in threshold of 0.81 fold in a control group of participants in an immunotherapy study who undertook a peanut challenge pre and post intervention (personal communication Dr Andrew Clark, STOP 2 study). It has been suggested that intra-individual variation may occur as a result of both host factors (associated atopic or comorbid conditions, age, medications) and cofactors (exercise, sleep deprivation, alcohol, infection and non-steroidal anti-inflammatory drugs). It has also been suggested that co-factors may in some way be responsible for augmenting allergic reactions. Co-factors have been identified in up to 30% of anaphylactic reactions in adults¹⁰ with a variety of co-factors being implicated.

Indeed in peanut immunotherapy studies it has been reported that patients seemed to lose tolerance to peanut doses during both the updosing and maintenance periods when they took the doses close to periods of exercise or when they were tired.¹¹ There is good evidence that exercise may exacerbate allergic reactions to gluten although this has not been formally explored in relation to peanut.¹² In the presence

¹⁰ Wölbing F, Fischer J, Köberle M, Kaesler S, Biedermann T. About the role and underlying mechanisms of cofactors in anaphylaxis. Allergy Eur J Allergy Clin Immunol. 2013. doi:10.1111/all.12193

¹¹ Varshney P, Steele PH, Vickery BP, et al. Adverse reactions during peanut oral immunotherapy home dosing. J Allergy Clin Immunol. 2009;124(6):1351-1352. doi:10.1016/j.jaci.2009.09.042

¹² Christensen MJ, Eller E, Mortz CG, Brockow K, Bindslev-Jensen C. Exercise Lowers Threshold and Increases Severity, but Wheat-Dependent, Exercise-Induced Anaphylaxis Can Be Elicited at Rest. J Allergy Clin Immunol Pract. 2018;6:514–20.

of these factors, allergic reactions may be elicited at lower doses or may be more severe or life threatening. However, underlying mechanisms have so far yet to be elucidated.

Methods

The TRACE study was a randomised double blind placebo-controlled crossover study of peanut allergic adults recruited from the United Kingdom. The study was based at the NIHR/Welcome Trust Cambridge Clinical Research Facility (Cambridge, UK) and Royal Brompton & Harefield NHS Foundation Trust Clinical Research Facility (London, UK), and was performed in collaboration with the University of Manchester. Using population-based advertisements in the media adults aged 18-45 were recruited. Following face-to-face screening eligible participants underwent a double-blind placebo-controlled challenge to peanut (baseline challenge) in order to confirm peanut allergy. Following, these participants were randomised to receive three further peanut challenges in a random order: one with exercise following each dose, one with sleep deprivation preceding challenge, and one with no intervention. Participants were given escalating amounts of peanut protein in 8 distinct doses (dose range 3 micrograms-1000 mg) until they developed objective signs of an allergic reaction. The primary outcome was the cumulative threshold dose triggering symptoms (mg protein). Primary analysis estimated the difference between nonintervention challenge and each intervention in log threshold (as % change). As secondary outcomes, dose distributions were modelled deriving eliciting doses in the peanut-allergic population with and without cofactors applied.

Results

There were 1043 registrants on the study website and 222 participants attended face to face screening visits. Baseline challenges were performed in 123 participants, 100 were randomized and 81 (mean age 25y) completed at least one further challenge (full analysis population). Sixty-four participants completed the study (per-protocol population). For the primary outcome, the mean (SD) threshold was 214 mg (330mg) for non-intervention challenges and this was reduced by 45% (95% confidence interval 21,61 p=0.001) and 45% (22,62 p=0.001) for exercise and sleep deprivation, respectively. As a secondary outcome, mean (95% confidence interval) estimated eliciting doses for 1% of the population were 1.5mg (0.8,2.5) during non-intervention

challenge (n=81), 0.5mg (0.2,0.8) following sleep and 0.3mg (0.1,0.6) following exercise.

Implications

We showed that exercise and sleep deprivation each significantly and independently reduce the threshold of reactivity in people with peanut allergy, putting them at greater risk of an allergic reaction. Everyday co-factors such as exercise and sleep deprivation have the ability to lower reaction threshold by approximately half. This needs to be accounted for when defining allergen reference doses for allergen food labelling. Adjusting reference doses using these data will improve allergen risk-management and labelling to optimize protection of peanut-allergic consumers.

Aims and objectives of the TRACE study

Food challenges from which threshold data are derived are usually performed under 'ideal' test conditions that do not reflect everyday exposure conditions. The effects of co-factors have not been investigated in a prospective study. If co-factors can affect the threshold dose at which allergic reactions are elicited, then there is a need to account for this in population threshold modelling.

Our aims were to conduct a robust, prospective examination of the threshold of peanut reactivity in allergic adults and examine the influence of each of two important, every day co-factors, exercise and sleep deprivation.

Methods

The TRACE study was a population-based multicentre randomised 52 week crossover study which enrolled peanut allergic adults from the United Kingdom. The study was performed between 2013 and June 2016 at the NIHR/Welcome Trust Cambridge Clinical Research Facility (Cambridge, UK) and Royal Brompton & Harefield NHS Foundation Trust Clinical Research Facility (London, UK), in collaboration with the University of Manchester.

Funding

The UK Food Standards Agency funded the study.

Recruitment

Participants were recruited from the general adult population. A media agency (MWI) was employed to generate a study identity for a website and advertising materials. Participants were recruited through advertisements on London and Cambridge-based Newspapers (Metro, Evening Standard and Cambridge News), Facebook, Google Words and Twitter advertising campaigns. Participants were also recruited through national patient support groups such as the Anaphylaxis Campaign and through Allergy UK. Information was also given to peanut allergic patients attending general allergy clinics in both Cambridge and London.

Screening via website

Interested participants were directed to a dedicated study website (Appendix 1) where further information about the trial was available. Through this website, potential participants registered their interest and answered some simple screening questions including: 'Has your worst reaction to peanut only been mouth or lip swelling?', 'Has your allergy been diagnosed by a doctor?', 'Are you able to run on a treadmill for 10 minutes?' and 'Are you available to take part in the study for the next 12 months?'. Registered participants were added automatically to a database including the responses to screening questions. Following this, registered eligible participants underwent a brief telephone consultation as a further screening stage.

Screening via telephone

Further detailed questions were asked of website registrants to elicit more information about their allergy and general health to ensure that appropriate individuals were called for face-face screening visits. The telephone screening questions were as follows.

- Tell me about your reaction to peanut. (Exclude if oral allergy to peanut or if history of severe anaphylaxis to peanut involving hypotension, hypoxia, neurological compromise.)
- 2. **Do you have any other food allergies?** (i.e. to matrix components of the food challenge vehicle)
- 3. Do you have asthma? (If no jump to Question 9)
- 4. What triggers your asthma? (Significant exercise induced component?)
- 5. Does your asthma keep you awake at night or wake you up in the morning earlier than usual? (Suggesting poor control)
- What treatment are you on at the moment? (Are they on systemic therapy? How often are you using a short acting B2 agonist?)
- How many courses of oral steroids have you needed in the last 2 years for your asthma? (Poor control?)
- 8. Have you had any hospital admissions for your asthma if so how many? Any admissions to ITU/HDU?
- 9. Are you pregnant or planning to become pregnant in the next year?
- 10. **Do you have any musculoskeletal problems?** (that would cause problems with the exercise challenge)
- 11. Are you on any regular medication? (Exclude if on immunosuppressants, beta blockers or ACE inhibitors, regular corticosteroids, sedatives or tricyclic antidepressants.
- 12. Do you have any other major health problems? (Exclude if mastocytosis, coronary artery disease, eosinophilic oesophagitis, gastric or duodenal ulcer, clinically significant ECG abnormalities, arrhythmias etc, history of recurrent autonomic dysfunction, significant sleep or psychiatric disorder)

Study inclusion and exclusion criteria

Inclusion criteria

- Male and female subjects who are 18-45 years of age at the time of study entry
- A diagnosis of peanut allergy as manifested by urticaria, angioedema or respiratory/gastrointestinal tract symptoms, with acute onset of symptoms after ingestion (up to 2h).
- A positive peanut DBPCFC at baseline (Visit 1). This outcome was defined as the onset of objective or significant subjective allergic events after ingestion of peanut protein but not to the placebo.
- Sensitisation to peanut demonstrated by skin prick test, or serum specific IgE

Exclusion criteria

- Oral allergy syndrome to peanut (defined as a clinical history of only oral allergy symptoms on exposure to peanut and principal sensitization to only PR10 homologues of peanut (Ara h 8)
- Monosensitisation to Ara h 9
- History of hypersensitivity to the matrix components used within the challenge material.
- Poorly controlled asthma.
- History of significant and repeated exercise –induced asthma attacks requiring treatment, independent of food ingestion or a drop in FEV1 of >15% during screening Vo2max exercise session
- History of any of the following:
 - Severe anaphylaxis to peanut as defined by hypoxia (SpO2 < 92%) or hypotension (>30% drop in systolic blood pressure), with or without neurological compromise
 - A previous reaction to peanut that in the opinion of the investigator was life-threatening
 - o Mastocytosis

Other exclusion criteria include conditions which would directly impair the participant's ability to undertake the study protocol such as musculoskeletal disorders impairing exercise and shift working impairing the sleep deprivation challenge.

Screening visit

Suitable participants were invited for a screening visit which involved a detailed history, skin prick and blood tests to determine their allergic status. A copy of the Screening visit form is included in Appendix 2. Participants were included in the study if they were aged 18-45 years with a history of an immediate systemic allergic reaction after peanut ingestion with evidence of sensitisation to peanut and the diagnosis confirmed by positive DBPC peanut challenge.

Skin Prick Testing

Skin prick testing was performed on all individuals at the face to face screening visit. Skin prick tests were performed to the peanut and tree nut panels, aeroallergens and to common foods including egg, milk, wheat, sesame, soya, lupin, peach, cod and shrimp. Sensitisation was defined as a positive skin prick test to peanut (extract ALK-Abello, Hørsholm, Denmark), skin weal of \geq 3mm greater than the negative control or serum specific IgE to peanut >0.35 kUA/L (ImmunoCAP).

Blood sampling

Venous blood was obtained at the screening visit. Participants were screened for the presence of specific IgE to peanut and Arah1, 2, 3, 8 and 9. As part of a general health screen, blood was also analysed for full blood count and renal function.

Other investigations

A baseline ECG was performed. A pregnancy test was performed to exclude pregnancy where appropriate.

Exercise

Each participant underwent a VO₂ max test to ascertain their maximum exercise capacity which was used to determine the exercise intensity for their exercise challenge. Lung function was assessed through spirometry and participants with asthma undertook the Asthma Control Test to determine their asthma control. Pre and post exercise lung function was used to exclude any participants with exercise induced asthma. For safety, a fall in FEV1 of greater than 15% following exercise excluded participants from the study.

Sleep diary

In preparation for the sleep deprivation challenge and to gain an understanding of participants' normal sleep patterns, participants were asked to complete a sleep diary for the two weeks preceding the sleep deprivation challenge.

Informed consent

Following telephone screening, each potentially eligible participant received a Participant Information Leaflet and a detailed verbal explanation of the study protocol including the risks and benefits of participation. Potential participants were given the opportunity to ask questions and were provided with sufficient time to make a decision. No clinical procedures were undertaken until informed consent had been obtained. Participants could withdraw at any time without the need for explanation.

Figure 1: TRACE study recruitment



Study design

Each participant underwent a baseline challenge (to determine their allergic status and initial threshold) followed by a further 3 interventional challenges (exercise, sleep and no intervention) spaced 3 months apart to reduce the possibility of a desensitisation effect produced by repeated peanut challenges (Figure 2). The initial baseline challenge was double blind and placebo-controlled and took place on 2 separate days: one day active and one day placebo. The order of the two days was randomly assigned and determined by randomisation lists produced by the study statisticians. Both participants and investigators were blinded to the ordering of the challenge days. Following this there was a further randomisation step. There were six allocation arms which varied by the order of the final 3 challenges (exercise/sleep/no intervention). The order of challenges between the six groups was balanced by employing a Latin square design. Participants were randomly assigned to one of the six arms using a secure online tool with audit trail. The six possible sequences were, ABC/BCA/CAB/ACB/BAC/CBA with each letter representing a different co-factor: A for exercise, B for sleep deprivation and C for no intervention. The strong degree of balance allowed for natural variations in intra-individual threshold over time. Randomisation was stratified by age, centre and the presence of asthma. (Figure 2). The final three challenges were not placebo controlled and participants received the active arm only.

Figure 2: Overall TRACE study design



Randomisation

There was no blinding regarding treatment allocation, but initially each DBPCFC consisted of two days, one active and one placebo. Both participants and the investigators were blinded to the ordering of challenge days. The protocol was amended so that only the baseline challenge consisted of two days (active and placebo). Thereafter for each participant, the remaining three interventional challenges (repeat baseline, sleep and exercise) consisted of only a single active day. Both participants and the investigators were blinded to the ordering of challenge days.

Within each combination of stratification levels, the sequence of treatments within participants were randomised using blocked randomisation utilising blocks of size 6. In parallel with the choice of challenge sequence, the order of the DBPCFC days (day 1: peanut, day 2: placebo or vice versa) were chosen. Within each block of challenge sequences, the ordering was balanced across participants within period and treatment. This was achieved by finding the pairs of identical treatments within each block and period and randomly assigning with equal probability one of the two possible sequences of orderings within the pair.

Challenge visits

Baseline challenge - pre challenge assessment

A copy of the case record form is shown in Appendix 3. A pre-challenge history was performed immediately prior to the challenge. This was to ascertain the participant's health on the day of the challenge and to ensure that other conditions such as asthma (using the Asthma Control Test and spirometry), rhinitis (using a Total Nasal Symptom Score) and eczema (using Patient Oriented Eczema Measure) were well controlled. Challenges were postponed if these conditions were inadequately controlled or if the participant was unwell with an infective illness. If the participant had had an allergic reaction to peanut in the last 3 months challenges were postponed. Participants were also assessed for the presence of any other co-factors (for example infection, alcohol or the use of non-steroidal anti-inflammatory drugs) which may interfere with the challenge outcome. The participant was examined, and skin was assessed to provide a baseline in anticipation of cutaneous features developing later. For safety an 18 or 20 gauge cannula was inserted prior to challenge and emergency medication was checked. Baseline observations including

blood pressure, pulse, peak expiratory flow rate (PEFR) reading and oxygen saturations were performed. Providing those were satisfactory the challenge dose was administered 5 minutes later.

Challenge meal preparation

A series of eight doses of peanut in the form of peanut flour were prepared. This was incorporated into a masked dessert food matrix developed for a European epidemiological project (Europrevall)¹³ and manufactured at the University of Manchester and then distributed to the study centres for reconstitution at the point of use. Participant either ingested the matrix either alone (placebo) or containing peanut allergen (active, 12.5% fat, light roast peanut flour from the Golden Peanut Company, Alphretta, GA, USA) until they developed an objective allergic reaction (definition below). Microbiological safety and allergen content were confirmed before materials sent out to the clinical centres. An unblinded scientist with no interaction with the participant or the study team was responsible for the randomisation of subjects and preparation of the challenge material.

Challenge Procedure

The challenges were undertaken using a harmonised protocol in accordance with best practice the dosing regimen is shown in Table 1. Numerous dosing schedules are currently in use for performing food challenges. Incremental scales vary from 10-fold increases, semi-logarithmic, doubling dose or even smaller increases with the latter associated with schedules aiming to deliver cumulative doses with shorter time intervals between doses (15 minutes). With schedules aimed at delivering discrete doses, intervals are typically longer (30 minutes). Using lower starting doses and prolonged intervals can increase the likelihood or partial desensitisation and falsenegative results.¹⁴ Data from the literature suggests that a starting dose of 3

¹³ Cochrane SA, Salt LJ, Wantling E, et al. Development of a standardized low-dose double-blind placebo-controlled challenge vehicle for the EuroPrevall project. 2012;67(4):107-113. doi:10.1111/j.1398-9995.2011.02715.x

¹⁴ Niggemann B, Lange L, Finger A, Ziegert M, Müller V, Beyer K. Accurate oral food challenge requires a cumulative dose on a subsequent day. J Allergy Clin Immunol. 2012;130(1):261-263. doi:10.1016/j.jaci.2012.03.021

micrograms should be low enough to provide No Observed Adverse Effect levels (NOAELS, the highest dose known to not induce an objective allergic reaction). However, starting at very low doses can make it more difficult to achieve meaningful top doses with acceptable increments in an acceptable period of time. Thus, a combination of logarithmic increments (from 3 micrograms to 30mg) followed by semi-logarithmic increments thereafter was used. Usually dosing regimens escalate to high cumulative dose of allergen protein to reduce the risk of a false negative challenge. Sicherer et al reported approximately 5% false-negative challenge results with a top dose of 876mg of protein.¹⁵ In this study, doses were delivered at 30 minute intervals which has been proposed as a suitable interval for the investigation of IgE associated reactions.¹⁶ However if significant symptoms evolved during the interval, the clinical investigator could increase the interval to 60 minutes. Longer time intervals however lengthen the challenge procedure and decrease the chance of accumulating high doses which may result in more severe reactions.¹⁷ The doses were given until the participant was judged to have developed objective signs of an allergic reaction and thus have reached their clinical threshold. Allergic reactions were treated appropriately, and all treatments and their effect were recorded. On a separate day the participant underwent a further challenge where all doses administered were placebo. The placebo dessert matrix matched the active dessert having previously been subjected to blinding tests at the University of Manchester. Placebo challenges were carried out under similar conditions. Both active and

¹⁵ Sicherer SH, Morrow EH, Sampson HA. Dose-response in double-blind, placebocontrolled oral food challenges in children with atopic dermatitis. J Allergy Clin Immunol. 2000;105(3):582-586. doi:10.1067/mai.2000.104941

¹⁶ Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, et al. Standardizing doubleblind, 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(6):1260-1274. doi:10.1016/j.jaci.2012.10.017

¹⁷ Niggemann B, Beyer K. Pitfalls in double-blind, placebo-controlled oral food challenges. Allergy Eur J Allergy Clin Immunol. 2007. doi:10.1111/j.1398-9995.2007.01396.x

placebo challenges occurred in a random order for each participant and were spaced a week apart.

Table	1:	Dose	regimen
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Dose Number	1	2	3	4	5	6	7	8
Amount of	Зµg	30µg	300µg	3mg	30mg	100mg	300mg	1g
peanut								
protein								

Criteria for scoring symptoms and stopping food challenge

In this study the PRACTALL criteria proposed by Sampson et al¹⁸ were adapted following our pilot challenge experience (described in 'Pilot Work') (Appendix 4). Participants were deemed to have reached their threshold, and the challenge stopped if they developed three concurrent yellow symptoms within one organ system or across different organ systems or 1 red symptom in any organ system. If the participant was almost at their threshold (e.g. 2 yellow symptoms) and the investigator was concerned about escalating to the next dose level for fear of inducing severe symptoms, then the dose could be repeated.

The colour coded symptom grading system was used as follows:

Green (mild) symptoms were not an indication to alter dosing.

Yellow (moderate) symptoms if present singly would be an indication for the investigator to proceed with caution. If three yellow symptoms were present

¹⁸ Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, et al. Standardizing doubleblind, 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(6):1260-1274. doi:10.1016/j.jaci.2012.10.017

concurrently within the same organ or across different organ systems, then this was an indication to stop.

Red (severe) symptoms if present singly was an immediate indication to stop.

The challenge could also be stopped at the investigator's discretion if they believed that continuing the challenge would place the participant at risk or also if the participant did not wish to continue.

Any extra symptoms which did not form part of the stopping criteria were recorded on the case record form as 'Free text symptoms'.

Intervention challenges

Unlike the baseline challenge, the interventional challenges were open, with only one 'active' challenge taking place. Reasons for this difference are explained in the **Alteration to main study design section.**

Single or multiple factors

Consideration was given to studying multiple extrinsic factors within a single challenge (i.e. sleep restriction + exercise). This would maximize the chances of detecting any effect of extrinsic factors, but we would be unable to define which individual factor was responsible for the change in threshold. As there was a reasonable degree of confidence that the single factors applied would create an effect, each factor was examined in isolation.

Exercise challenge

On the challenge day the participant was admitted to the ward on the day of the exercise challenge. Participants were given each dose followed 5 minutes later by a 10-minute bout of exercise at 85% VO2 max on a static bike. Heart rate was measured throughout the challenge using an Actiheart monitor to ensure that they achieved their target heart rate. The participant was allowed to drink water but not able to eat any food apart from the challenge meal. Providing the participant had not met the stopping criteria for the challenge the second challenge dose was administered and followed by an identical exercise bout. This sequence of incremental dose followed by identical exercise will be repeated until all the doses have been consumed, or the challenge has been terminated because of an apparent reaction.

Sleep challenge

Participants received a peanut challenge after being sleep deprived. Participants were admitted to the research ward on the night before the food challenge. They were allowed to sleep for a maximum of 2 hours during the night. All participants were woken by 3am regardless of whether they have slept and were kept awake by nursing staff who kept a log of the participant's activities every 15 minutes until the morning peanut challenge. Dosing was conducted in the same manner as the baseline challenge. Tiredness was assessed objectively using the Psychomotor Vigilance Task¹⁹ and subjectively using the Karolinska Sleepiness Scale.²⁰ (Appendix 5)

No intervention challenge

This was conducted under the same conditions as the baseline challenge.

Treatment of allergic reactions

For safety and to harmonise practice across both centres, guidance was created for treating allergic reactions.

Treatment of less severe symptoms (yellow and green symptoms) was based on the investigator's clinical judgement. Oral or intravenous antihistamines and steroids could be used along with inhaled or nebulized β 2 agonists if required.

¹⁹ Dinges DF, Powell JW. Microcomputer analyses of performance on a portable, simple visual RT task during sustained operations. Behav Res Methods, Instruments, Comput. 1985. doi:10.3758/BF03200977

²⁰ Åkerstedt T, Anund A, Axelsson J, Kecklund G. Subjective sleepiness is a sensitive indicator of insufficient sleep and impaired waking function. J Sleep Res. 2014. doi:10.1111/jsr.12158

Signs and symptoms	Stopping criteria	Recommended treatment
Skin		
Urticaria/Angioede ma	< 10 hives but ≥3, or significant lip or face oedema	In isolation: follow local procedures, consider fast acting anti-histamines (eg. cetirizine) first In combination with any symptom from a different system, consider: 0.5 mL adrenaline (1:1000) IM
	Generalized involvement	0.5 mL adrenaline (1:1000) IM
Rash	Generalized marked erythema (>50%)	0.5 mL adrenaline (1:1000) IM
Lower respiratory		
Wheezing	Expiratory wheezing on auscultation	0.5 mL adrenaline (1:1000) IM +SABA
	Mild audible (inspiratory and) expiratory wheezing	0.5 mL adrenaline (1:1000) IM +SABA
	Use of accessory muscles and/or audible wheezing (or silent lung)	0.5 mL adrenaline (1:1000) IM +SABA
Laryngeal	Hoarseness, frequent dry cough Stridor	 0.5 mL adrenaline (1:1000) IM, consider nebulised adrenaline (1mg in 5ml saline). 0.5 mL adrenaline (1:1000) IM, consider nebulised adrenaline (1mg in 5ml saline). Notify anaesthetist / ICU.
Gastrointestinal		
Emesis/diarrhoea	2-3 episodes of emesis or diarrhoea or 1 of each	0.5 mL adrenaline (1:1000) IM)+ 1000 mL, consider 1 litre 0.9% saline bolus over 1-3 minutes
	>3 episodes of emesis or diarrhoea or 2 of each	saline bolus over 1-3 minutes

Table 2: Treatment of severe (red) symptoms

Signs and symptoms	Stopping criteria	Recommended treatment
Cardiovascular/ne	urologic	
	Drop in blood pressure and/or >20% from baseline, or significant change in mental status. Cardiovascular collapse, signs of impaired circulation (unconscious)	0.5 mL adrenaline (1:1000) IM. Inform ICU/ anaesthetist +1000 mL 0.9% saline bolus over 1-3 minutes (repeat as required) Consider IV adrenaline; diluted to at least 1:10,000, Start infusion at 5-15 μg/min. ECG /P/BP monitoring essential. Contact ICU / anaesthetist.

Discharge procedures

Following a positive reaction, participants were observed for two hours following the ingestion of the last dose and until they had completely recovered from the reaction. Upon discharge participants were provided with a treatment plan for managing any late or future allergic reactions. Their adrenaline autoinjector was checked to make sure it was in date and participants were retrained on its usage. Participants were counselled not to undertake vigorous or unaccustomed physical exercise, ingest alcohol or take NSAIDs for 4 hours after the last challenge dose. Participants using adrenaline due to a suspected reaction after discharge were advised to immediately return to the investigative site (if before 17:00) or go to the closest emergency department for additional assessment.

Data collection and management

Participant demographic data, details of symptoms during challenge including timing of onset and resolution, threshold reached, and treatment administered during challenge was collected on a paper case record form.

An adaptation of an existing Allerg-e-lab concept was developed for the TRACE Study by the University of Manchester. The Open-CDMS platform, an open-source clinical data management system was used. A database template was developed to build the database from the paper clinical case record forms. Field limits were decided for the numerical values in the case record forms (for example heart rate etc). Through consultation with the study team the OpenCDMS team in Manchester produced electronic challenge documents and uploaded these onto a live test system. These were then tested by the investigators at each site and any amendments were included. The live system was then launched which could be accessed by investigators at both sites. Data from the CRF were then entered by the study team onto a centralised electronic database.

Quality control of the data and data checking was carried out by an independent person at each site who checked 100% of the primary outcome data (cumulative peanut dose reached) and symptoms at the time of onset of reaction.

Data could be exported from the database into csv files for analysis.

Ethics committee approval

This study was approved by the NRES committee East of England (12 EE02/89). Informed, written consent was obtained from all participants prior to participation in the study. An Independent Data Monitoring Committee consisting of a team of experienced allergists oversaw safety data and assessed severe reactions. RD approval at each site and CRF permissions were obtained.

Study outcomes

The primary outcome was the peanut threshold in each individual (or dose triggering symptoms) and defined as the Lowest Observed Adverse Effect Level (LOAEL), the lowest cumulative dose that causes an objective allergic reaction (defined below). This was measured in mg peanut protein (mean, SD, minimum and maximum) and summarised by challenge type and timing of challenge.

As secondary outcomes, threshold dose distribution curves were derived for the different challenge types and probability distribution modelling was used to determine population thresholds, the cumulative dose of peanut protein predicted to provoke reactions in different percentages of the peanut-allergic population (Eliciting Dose-ED_{x%}).

Reaction severity was not measured as a pre-planned main outcome in this study. However, a detailed post-hoc analysis of reaction severity and symptom pattern and discussion of development of a severity score will be reported separately.

Analysis populations

The primary analysis population was the full-analysis set, which was defined as all participants who had completed at least one post-baseline challenge. Analyses on the per-protocol population, defined as participants who completed all three postbaseline challenges were also performed. The extended analysis set consisted of all participants who received a baseline challenge. The safety population consisted of all participants who underwent at least one challenge.

Adverse events

Safety data were recorded on a specifically designed case report form (CRF). All serious adverse events (SAEs) were reported on an SAE report in addition to CRFs. Safety data were reviewed three monthly by the IDMC. The IDMC reserved the authority to recommend termination of the trial because of safety findings.

Adverse events that were classified as serious were reported promptly and appropriately to the NIHR, Cambridge University (sponsor), principal investigators in the trial, TSC chair and deputy chair, IDMC chair, the Food Standards Agency Food Allergy Branch, and the Ethics Committee.

The types of adverse events were as follows compliant with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0 (December 12, 2003).

Adverse event

An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding or disease that occurs during participation in the study. An adverse event will be followed until it resolves or until 30 days after a participant terminates from the study, whichever comes first.

An adverse event is considered as 'unexpected' when its nature or severity is not consistent with the investigator's protocol.

Adverse event were described as 'expected' they caused symptoms and/or signs that could be reasonably described as a consequence of an allergic reaction, exercise or
sleep deprivation. Symptoms of an allergic reaction were defined as any described within this protocol or the standard operating procedures, or those in the view of the investigator that were an expected consequence of a food challenge, exercise or sleep deprivation.

Serious adverse event

A Serious Adverse Event (SAE) is any untoward and unexpected medical occurrence or effect that:

- Results in death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow up after the completion of therapy must be reported whether is considered treatment related or not.
- Is life threatening- refers to an event in which in the view of the investigator the subject was at risk of death at the time of the event.
- Requires inpatient hospitalisation or prolongation of existing hospitalisation.
- Results in persistent of significant disability of incapacity.
- An event that requires intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalisation may be considered an SAE when based on appropriate medical judgement it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Collection and recording of adverse events

Adverse events were collected from the time the participant provided consent until the time the event resolved or until 30 days after the participant completed study treatment. Adverse events were discovered through observing and questioning the participant or receiving an unsolicited complaint and questioning the participant in an objective manner. Throughout the study, the investigator recorded all adverse events on the appropriate adverse event CRF regardless of their severity or relation to study medication or study procedure. The investigator treated participants experiencing adverse events appropriately and observed them at suitable intervals until their symptoms resolved or their status stabilised. SAEs were recorded on the adverse event CRF and health authorities notified.

Grading and attribution of adverse events

Grading Criteria

The study site graded the severity of adverse events experienced by study participants according to the criteria set forth in the NCI-CTCAE Version 3.0. This document provided a common language to describe levels of severity, to analyse and interpret data, and to articulate the clinical significance of all adverse events. Adverse events were graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

- Grade 1 mild adverse event
- Grade 2 moderate adverse event
- Grade 3 severe and undesirable adverse event
- Grade 4 life-threatening or disabling adverse event

Grade 5 - death

All adverse events were recorded and graded whether they were or were not related to disease progression or treatment. The NCI-CTCAE grades were used as the primary source for scoring. If uncertainty arose then the WAO criteria were mapped to these grades and could be used to help interpret which grade to use (see Figure 3 -adapted from J Allergy Clin Immunol 2010;125:569-74, 74 e1-74 e7).

Figure 3. Adapted from World Allergy Organization Subcutaneous Immunotherapy Systemic Reactions Grading

Grade	Symptoms
Grade 1	Symptoms/signs of one organ system present
	Cutaneous
	Generalized pruritus, urticarial, flushing or sensation of heat or warmth or Angieodema
	Upper respiratory
	Rihinitis or Throat clearing, itchy throat or Cough perceived to come from the upper airway, not lungs, larynx or trachea
	Conjunctivitis
	Other
	Nausea, abnormal taste or headache
Grade 2	Symptoms/signs of more than one organ system present
	Lower respiratory
	Asthma: cough, wheezing, shortness of breath (e.g. less than 40% PEF/FEV1 drop, responding to an inhaled bronchodilator)
	Gastrointestinal
	Abdominal cramps, vomiting or diarrhoea
	Other
	Uterine cramps

Grade	Symptoms
Grade 3	Lower respiratory
	Asthma (e.g. 40% drop in PEF/FEV1) not responding to inhaled bronchodilator
	Upper respiratory
	Laryngeal, uvula or tongue oedema with respiratory distress
Grade 4	Lower or upper respiratory
	Respiratory failure with or without loss of consciousness
	Cardiovascular
	Hypertension with or without loss of consciousness
Grade 5	Death

Patients may also have a sense of impending doom, especially in grades 2, 3 or 4.

Attribution Definitions

The relation, or attribution, of an adverse event to study participation was determined by the investigator and recorded on CRF and/or SAE reporting form.

Reporting serious adverse events

Timeline

Serious adverse events were reported to the PI within 24 hours

Options for Reporting of Serious Adverse Events. All SAEs were reported to the IDMC Chair and Deputy chair of the Trial Steering Group, Cambridge Central Ethics Committee and IDMC and trial Food Standards Agency and sponsor (Cambridge University Hospitals Trust Research and Development Department) where in the opinion of the Investigator the event was related (resulted from the administration of any of the research procedures) and expected (e.g. an event not listed in the protocol

as an expected occurrence). Reports were submitted within 30 days of the CI being made aware of the event. All serious unexpected severe adverse reactions (SUSARS) were reported to the above bodies and individuals within seven days of the CI being made aware of the event

Regardless of the relation of the adverse event to study participation the event must be reported as a resinous adverse event if it meets any of the above definition.

IDMC

All safety data were reviewed periodically by the Independent Data Monitoring Committee (IDMC) which consisted of clinicians and statisticians not directly involved in the study. The IDMC reviewed safety data after recruitment of 5, 25, 50, 75 participants. The IMDC provided advice to the Trial Management Group (TMG). They also reviewed severe events reported by the Principal Investigators and had the authority to withdraw any participants and/or terminate the study because of safety findings.

Study management

The role of the TRACE study Trial Steering Committee (TSC) was the main decisionmaking body. It had overall responsibility for scientific strategy and direction and had ultimate responsibility for ensuring the project's aims were delivered on time and within budget.

The members of the TSC at completion of the project were:

Independent Members

- Prof Graham Roberts, consultant in paediatric allergy & respiratory medicine, Southampton University (Chair)
- 2. Ms Moira Austin OR Ms Hazel Gowland, Anaphylaxis Campaign
- Dr Steve Till, consultant allergist, King's College London/Guy's & St Thomas' NHS Foundation Trust, Vice -Chair
- 4. Dr Phillipa Caudwell (exercise physiologist) Research Fellow, Biopsychology Group, Institute of Psychological Sciences, University of Leeds
- Dr Victoria Cornelius (medical statistician) Senior Lecturer in Medical Statistics, Department of Primary Care and Public Health Sciences, King's College London

 Dr Pina Guissepina (Consultant allergist), The Royal Free Hospital, Deputy Vice-Chair

Dependent (voting) members

- 1) Dr Andrew Clark, chief investigator
- 2) Dr Pamela Ewan, principal investigator, Addenbrooke's Hospital.
- 3) Prof Clare Mills, principal investigator, University of Manchester
- Dr Robert Boyle, principal investigator, NIHR Clinical Lecturer in Paediatric Allergy at Imperial College London (Principle Investigator at St, Mary's Hospital)
- 5) Dr Shelley Dua, investigator, University of Cambridge
- 6) Dr Monica Ruiz-Garcia, investigator, Imperial College, London
- Dr James Wason, trial statistician Institute of Health and Society, Newcastle University
- Dr Simon Bond, trial statistician MRC Biostatistics Unit, Cambridge Institute of Public Health
- 9) Prof Ian Kimber, Professor of Toxicology, University of Manchester

Observers

- 1) Professor Stephen Durham, Head of the Allergy & Clinical Immunology Section within the National Heart and Lung Institute, Imperial College London
- 2) Dr Isabel Skypala, Royal Brompton and Harefield NHS Trust
- Mr Ross Yarham, Food Allergy & Intolerance Research Programme Manager, Food Standards Agency
- 4) Dr Chun-Han Chan, Senior Allergy Policy Advisor Food Standards Agency

Previous members

- 1) Miss Sarah Hardy, Food Standards Agency
- 2) Mrs Ruth Willis, Food Standards Agency

Statistical Analysis

Sample size

As there were no published data on intra-individual variation in thresholds over time from repeat challenges, we considered different scenarios with power assessed by simulation.

Initial Sample Size Calculation

It was difficult to provide a power calculation for a cross-over study so sample size justification was in part based on parallel group design along with the general argument that crossovers required fewer participants while precision of results depended on obtaining large sample sizes. As a guide, numbers required could be derived from considering the ratio of decreased to increased thresholds following challenge, with or without the modulating factor. There were no published data on intra-individual variation in thresholds over time from repeat challenges, but some pilot data was available through collaboration with Prof DA Moneret Vautrin and the North American and European branches of ISLI (International Life Sciences Institute) to inform a power calculation. These data were based on n=118 peanut allergic subjects with complete threshold data for two sequential challenges.

These data demonstrated a decreased threshold in 34%, an increased threshold in 47% and 20% showed no change. Based on this, with 120 subjects, we would have at least 80% power to detect 40% showing decreased threshold (of up to 10 fold), 20% showing an increased threshold (the balance of 40% showing no change)- i.e. approximately the reverse of the observed changed in the pilot data. Although threshold levels have been used for the power calculation, changes in severity scores will also be considered during the final analysis.

For the cross-over study to remain well-balanced it was preferable to target a sample size that was a multiple of 3, and consideration of feasibility led to the choice of 72 subjects (adults) as being the minimum number required. A smaller number of participants than 120 per cohort is allowable in this context as the cross over design is more powerful than a parallel design.

Further Simulation Work

Further to the above calculation – simulations were performed to ensure the study would be suitably powered to detect clinically relevant changes in the continuous

threshold variable as opposed to the categorical "change vs. no change". Simulations were required since there is no previous data or publications to provide an estimate of within participant variability in threshold as a result of repeat trials with or without extrinsic factors. Therefore, a wide range of hypothetical scenarios were investigated.

A sample size of 72 was simulated with each participant's changes in log transformed threshold from baseline to challenges (no intervention, exercise and sleep deprivation) generated from a multivariate normal distribution. The different scenarios incorporated different values of the parameters in the distribution (mean, variance, correlation). Informed by clinician advice and a small amount of pilot data, 48 scenarios were created in which the mean change in log threshold from baseline to no-intervention challenge was held fixed at 0, the mean change in log threshold from baseline to both interventions ranged from -0.3 to -1.3, within participant correlation took values of 0.5 and 0.7, and within participant variance took values of 1, 2, 3 and 4.

Each scenario was simulated 500 times and the power to detect a significant difference and a CI width <1 log was estimated as well as the power to detect a significant difference alone. The results are shown in Figure 4 below.





In the most conservative scenario investigated (within-person correlation=0.5 and variance=4) 72 completed participants would mean 80% power (5% two-sided significance level) to detect a minimum change in natural log threshold of approx. - 0.9 (60% reduction in mg threshold from baseline to intervention challenge).

In the scenario most similar to the pilot data (repeat challenges in children) (correlation=0.7 and variance=1) with 72 participants there was 80% power to detect a minimum change in natural log threshold of approx. -0.3 (25% reduction in mg threshold from baseline to intervention challenge).

Taking the secondary aim of having narrow CI limits into account only impacts power negatively in scenarios with low correlation and high variance.

Primary outcome analysis

A linear mixed-effects model on the change in log-threshold from baseline to each challenge was fitted to the data. Random effects for each participant were included.

Fixed effects included the challenge type (exercise, sleep-deprivation), age, sex, timing of the challenge, baseline log-threshold, presence of asthma, centre, and baseline Ara h 2. Each datapoint was assumed to have an error term that was identically and independently normally distributed with constant variance. Random effects for participant were also assumed to be normally distributed with constant variance variance.

From this model, assumptions such as normally distributed error with constant variance were checked. The individual error variance and intraclass correlation coefficient parameters were estimated.

The primary analysis estimated the effect of type of challenge (i.e. the difference between non-intervention challenge and each intervention challenge in log LOAEL) from the model along with confidence interval and p-value for whether the difference was significant.

The raw output from the mixed model was recorded in R, including parameter estimates and fixed effects correlation matrix allowing inferences on the difference between any two challenges to be found.

As an additional analysis of the LOAEL outcome, binary outcomes for each challenge were formed that measured whether the LOAEL is higher than baseline for that challenge. These were analysed using a suitable paired binary test.

Secondary outcome analysis

Population threshold curve

A secondary objective was to derive the population threshold curve for the different challenge types. To do this parametric interval-censored survival analysis methods were used, similarly to Taylor et al.⁶ The LOAEL values were included as interval censored data between the dose one below the one which caused the first reaction and the dose at which the reaction occurred. If the first dose caused a reaction the data was left censored at the first dose. If no reaction took place for any dose, it was right censored at the final dose.

The survreg function in the Survival package in R was fitted to normal, log-logistic and Weibull models and the model which fitted the data best according to AIC was chosen. This model was used to find the dose predicted to provoke reactions in different proportions of the peanut-allergic population (ED1, ED5, ED10, ED50, ED80, ED95). For each type of challenge a different curve was derived.

We noted that the interpretation of the curve was dependent on the analysis population used. For the baseline challenge data from all individuals completing a both baseline challenges was available, but for other challenges only individuals who were randomised (meeting eligibility criteria) were included.

For the baseline challenge curve, the analysis in the extended analysis set was repeated.

Pilot study and alteration to initial study design

The pilot process which led to the finalisation of the above study protocols is detailed below.

Pilot Baseline Challenges

Double blind placebo-controlled food challenge (DBPCFC) is the gold standard investigation for diagnosing food allergy and for establishing the allergen threshold of reactivity. Assessing symptoms elicited during challenge and deciding whether or not they are objective is a critical part of a threshold finding study and variability in interpretation can adversely affect threshold estimation. Adopting a standard way of assessing symptoms including how to classify subjective or objective symptoms, allows comparison of outcome during DBPCFC. Moreover, the scoring and stopping criteria designed for conducting food challenges can affect the threshold estimate of a study. Therefore, the parameters for stopping and declaring a challenge should be prespecified in challenge protocols. The only published practice parameter on oral food challenge in existence at the commencement of the study was the PRACTALL consensus report for food challenge which had received broad consensus from US and European allergists hence the decision to follow these criteria. (Appendix 4)

Aim of the pilot baseline challenge study

To determine whether the PRACTALL challenge stopping criteria which had been designed for use in other studies could be safely applied to the TRACE study

Methods

4 peanut-allergic participants underwent a pilot baseline challenge to peanut (active arm only) using the method described above (Main Study Method: Baseline Challenge). Challenges were scored using the PRACTALL consensus criteria. This scoring system indicates symptoms and signs that may merit caution and aims to inform the investigator whether a dose should be delayed, repeated or that the challenge should be stopped. Symptoms and signs are scored using a traffic light warning system.

Results

Four peanut allergic participants, three female and one male, age range 18-41 years underwent challenges. Three participants developed objective symptoms based on the challenge stopping criteria which allowed their reaction threshold to be defined. One participant developed subjective symptoms only, but we took the decision to stop the challenge as this was the first one we had conducted. It was decided for safety reasons that further refinement of the criteria was needed. We felt that many of the original criteria were based on symptoms experienced by children during oral food challenge and required alteration to make them applicable to adults. Furthermore, some participants, during the pilot baseline challenges were experiencing a rapid evolution of respiratory symptoms. By adding further refinement to airway symptoms, we felt that we could detect warning signs, prevent rapid progression to severe symptoms and thus enhance safety. In addition, we incorporated the peak expiratory flow rate as a functional measurement. Gastrointestinal symptoms were also further defined in terms of their persistence. Based on the pilot challenge reactions the weighting of various symptoms was changed. In the original PRACTALL consensus criteria it was suggested that challenges could be stopped on the basis of green (mild) symptoms present for greater than 120 minutes. We regarded these as subjective symptoms and stopping a challenge for subjective symptoms increased the risk of a false positive test. Therefore, we decided to base a threshold estimate on objective symptoms (yellow or red symptoms as previously described) only.

Outcome

The PRACTALL consensus criteria have been modified for use in this study.

Pilot Exercise Challenges

Background

Anaphylaxis during exercise has been reported to occur during bouts of physical activity of varying intensities. This ranges from high intensity activity such as running or jogging to even ordinary physical activity such as gardening.²¹ In a study by Pals et al it was shown that small intestinal permeability, assessed by sugar excretion, was increased after exercising at 80% VO2 max for 60 minutes and not at lower intensities (40 and 60% maximal oxygen uptake).²² However it would be impractical to exercise our participants at this intensity for the same duration on repeated occasions during a single challenge day.

Aims of pilot exercise study

- To determine an acceptable and tolerable intensity and duration of exercise for participants during the study
- Aim to imitate real life exercise
- Detect any ingestion of food ingestion on exercise capacity (healthy volunteers will undergo the exercise protocol with the placebo dessert matrix).

Pilot exercise method

Eight healthy volunteers of varying levels of fitness undertook a V0₂ max test to determine their maximum exercise capacity as measured by maximum oxygen uptake. ECGs were performed on all volunteers prior to exercise. Participants performed varying durations of exercise at 85% VO₂ max to determine tolerability. Exercise was performed initially on a treadmill but then latterly on a static bike for reasons outlined in results. In five out of seven healthy volunteers, placebo dessert matrix was given five minutes prior to each bout of exercise. Following finalisation of the protocol, three peanut allergic participants underwent a pilot exercise challenge

²¹ Robson-Ansley P, Toit G Du. Pathophysiology, diagnosis and management of exercise-induced anaphylaxis. Curr Opin Allergy Clin Immunol. 2010. doi:10.1097/ACI.0b013e32833b9bb0

²² Pals KL, Chang R, Ryan AJ, et al. Effect of running intensity on intestinal permeability Effect of running intensity on intestinal permeability. 2013:571-576.

to determine safety. Blood lactate was measured during exercise, an elevated lactate is indicative of a normal physiological response to exertion. Dramatic increases in lactate characterise a normal response to exercise if a participant exceeds the work rate at which lactate can be removed from the blood as quickly as it enters the blood. Serum lactate was measured before and after each exercise bout.

Pilot exercise results

Two healthy volunteers undertook 8 x 15 minute bouts of exercise at 85% VO₂ max on a treadmill but this protocol was deemed to be too intense and unacceptable. One healthy volunteer undertook 8 x 5 minute bouts of exercise at 85% VO₂ max (treadmill) but this was deemed to be too easy and subjectively did not tire the participant. Four healthy volunteers undertook 8 x 10 minute bouts of exercise at 85% VO₂ max (treadmill). One participant had to withdraw during the exercise due to a pre-existing knee injury. Otherwise this protocol was well tolerated and caused sufficient cardiovascular exertion. Ingestion of the challenge matrix did not affect exercise capacity. Due to logistical issues in obtaining a treadmill in the other study centre the exercise mode was switched to a static bike. The protocol of 8 x 10 minute bouts of 85% VO₂ max was piloted on three further healthy participants on a static bike. The exercise protocol was replicated with good results and a high heart rate was achieved during the bouts (Figure 5). Serum lactate was measured before and after each exercise bout and demonstrated an increase post exercise compared to pre-exercise levels (data not shown).

Figure 5: Trace showing an individual participant's heart rate (vertical axis) during exercise bouts



Following an initial open baseline challenge with active peanut doses, three peanut allergic pilot volunteers undertook exercise challenges also with active doses. Two performed the exercise challenge on a treadmill (prior to the change) and one performed the challenge on a static bike. Two participants developed objective symptoms and one participant completed all 8 doses without reaction. For the two reactive participants there appeared to be an increase in reaction severity compared to their baseline challenge (Table 3). Both required treatment with intramuscular adrenaline for severe symptoms which was not needed in their baseline challenges.

Table 3: Pilot baseline (n=4|) challenges and pilot exercise (n=3) challenges

Mild reaction

OP Oropharyngeal pruritus/tingling, PruG Generalised pruritus, ECP Ear canal pruritus, Ch subjective chest tightness

Moderate reaction

Na Nausea persistent, AP Abdominal pain persistent, Rh Rhinorrhoea (persistent), U Urticaria localised, EryL Erythema Localised, ThT throat tightness, E Emesis

Severe reaction

ERep Repeated emesis EryG Generalised erythema, Co Dry cough persistent, Wh Wheeze audible AI C altered consciousness

Treatment

OAH Oral antihistamines **OP** Oral prednisolone **IVAH** IV antihistamines **IVHy** IV hydrocortisone **Adr** Adrenaline **Salb** salbutamol

Participant number	Baseline threshold dose	Baseline challenge reaction symptoms	Treatment given	Exercise threshold dose	Exercise challenge reaction symptoms	Treatment administered
0115	5 (30mg)	OP, ECP, Na, AP,E (1)	IVAH, IVHy	5	OP, ECP, AP, Rh, Na, ERep (10)	IVAH, IVHy, Adr, IV fluids
0110	6 (100mg)	OP, ECP AP, Rh, U	OAH, OPr	6	OP, Na, PruG, Rh, EryG, Co, Wh	IVAH, IVHy, Adr, Salb
0109	6 (100mg)	OP, Ch,	OAH	Completed	OP	None
0108	5 (30mg)	OP, Rh, Na, E (1), EryL	IVAH, IVHy			

NB: Participant 0108 completed baseline challenge only and did not undergo exercise challenge.

Outcome

It was decided that the optimal regime was for participants to undertake 8 x 10minute bouts of exercise at an intensity of 85% VO₂ max during the exercise challenge days.

Pilot sleep deprivation challenges Aims of pilot sleep deprivation study

- To determine an acceptable and tolerable amount of sleep restriction for participants.
- To aim to imitate sleep restriction in the community.
- To pilot the use of objective and subjective measures of tiredness namely the Psychomotor Vigilance Task and the Karolinska Sleepiness Scale.

Pilot sleep deprivation method

Three peanut allergic participants underwent a peanut challenge with active doses following restricted sleep of 3 hours.

Pilot sleep deprivation results

Three peanut allergic participants, one male and two female, age range 18-28 completed the pilot sleep deprivation challenges. All participants developed objective symptoms during challenge and reaction thresholds could be established (Table 4).

Table 4: Pilot sleep deprivation challenges

Mild reaction

OP Oropharyngeal pruritus/tingling, PruG Generalised pruritus, ECP Ear canal pruritus, Ch subjective chest tightness

Moderate reaction

Na Nausea persistent, AP Abdominal pain persistent, Rh Rhinorrhoea (persistent), U Urticaria localised, EryL Erythema Localised, ThT throat tightness, E Emesis

Severe reaction

ERep Repeated emesis EryG Generalised erythema, Co Dry cough persistent, Wh Wheeze audible AI C altered consciousness

Treatment

OAH Oral antihistamines **OP** Oral prednisolone **IVAH** IV antihistamines **IVHy** IV hydrocortisone **Adr** Adrenaline **Salb** salbutamol

Participant number	Sleep dep threshold dose	Sleep deprivation challenge reaction symptoms	Treatment given
0115	5 (30mg)	ECP, OP, Na, AP, E (1)	IVAH, IVHy
0121	5 (30mg)	OP, Co, AI C	IV AH, IV Hy, Salb, Adr
0126	6 (100mg)	OP, ThT, EryL, U	IV AH, OPr

However, two of three peanut allergic participants subjectively reported that they did not feel tired following the sleep restriction of 3 hours. One participant did subjectively report tiredness. Based on these pilot findings it was decided to further restrict the amount of sleep in the protocol to ensure adequate fatigue and to use formal objective and subjective measurements of tiredness (the Karolinska Sleepiness Scale (Appendix 5) and Psychomotor Vigilance Task). Therefore three further healthy volunteers were asked to pilot the protocol with 2 hours of sleep restriction. This amount achieved adequate levels of tiredness (Table 5) and was tolerated well. Table 5: Psychomotor vigilance task results for 3 pilot healthy volunteer sleep participants. A reaction time of greater than 300 milliseconds is classed as an impaired response time.

	Average reaction time (milliseconds)	Average false starts	Average missed signals
PRE sleep deprivation	278	1	1
POST sleep deprivation	357	0	2

Outcome

Sleep restriction to 2 hours was decided upon for the final protocol.

Alteration to main study design: Placebo challenges

The intention of the original study design was to have a placebo arm for every active arm. The rationale of including placebos was to eliminate investigator or participant influence on the challenge outcome at every stage and also to validate the symptoms and signs which occur on active days.

However, there were delays in starting the baseline challenges due to appointment of staff, obtaining local site permissions and additional piloting work, described above, for protocol refinement. It became clear that keeping to the original study design would result in challenge burden exceeding site capacity and the study not being completed by the funder deadline. Therefore, as a study team we had to propose a study redesign to reduce the challenge burden whilst maintaining scientific validity and participant safety. Various proposals were explored including eliminating an intervention (either sleep or exercise), removing placebos from some challenges, interspersing placebo doses within the active doses, removing 'predictable' placebos i.e. ones occurring on day 2 of the challenge pair or removing placebos from interventional challenges. We performed a sensitivity analysis looking at data from 29 baseline challenge pairs (placebo and active) and found that there was no difference in threshold with the challenge method applied. The prediction of the researcher as to whether each

day was placebo or active, based on the symptoms encountered was correct in every case. We also examined the symptoms and signs that occurred on placebo days. In every case these were minor and would not have triggered the challenge to be stopped or scored positive. Furthermore, we observed that participants who received the active challenge on Day 1 of the challenge pair were essentially unblinded and were expecting to have an allergic reaction on Day 2 reducing the validity of the placebo arm.

It was decided that a placebo must be retained for the initial baseline challenge as this is essential for correct diagnosis in peanut allergy. However, the decision was taken to remove placebos from the intervention arms. This also meant that each participant only had to attend 5 instead of 8 challenge appointments which also helped to improve booking challenges, study retention and reducing drop outs.

With no placebos, the participants would attend each interventional challenge knowing they would receive peanut. It could be argued that this would change their expectation and somehow alter their reporting of symptoms, compared to the original design. This might be true if subjective symptoms were being used to determine challenge outcome (e.g. itching or discomfort), however the stopping criteria used on active days required three concurrent 'yellow' symptoms or signs to be present. It is unrealistic to believe that such objective symptoms could be induced by a placebo effect, and our analysis indicated it is very unlikely to occur. A further criticism might be that comparing the original DBPCFC with later non-placebo interventional challenges would not be comparing 'like with like'. However, this is controlled for in the study design as the main outcome is the difference between (baseline-repeat baseline) and (baseline-intervention). Both the repeat baseline and intervention challenges in this scenario will be internally controlled (neither have placebo).

Results

Study recruitment took place between May 2013 and September 2016. A consort diagram for the study is shown in Figure 6. Two hundred and twenty-two participants were screened. Out of the 222 screened participants, 123 (55%) were eligible to attend for baseline challenge. One hundred and twenty-three participants undertook baseline challenges. Positive DBPCFC confirmed the diagnosis of peanut allergy in 109 subjects (89%). In total 100 participants were randomised. Of the remaining 23, fourteen participants tolerated all challenge doses with no or only subjective symptoms and thus a threshold could not be identified. Two participants suffered severe reactions at baseline and were therefore excluded on safety grounds and one participant was unable to tolerate the taste of the dessert matrix. Six baselines challenges were completed after the randomised quota had been met.

Study populations

In total 64 participants completed the full study (i.e. all five challenges) and are referred to as the per-protocol study population. Eighty-one participants attended for at least one intervention and this group is referred to as the full analysis population. Data from the full analysis population are shown and the per-protocol results which are reflective of the full analysis results are included in Appendix 6. The baseline characteristics of the randomised participants are listed in Table 6.

Figure 6: Consort diagram one was excluded after review on the grounds that it had been stopped prematurely*, resulting in a full analysis population of 81 participants



Table 6: Baseline characteristics for study populations

For binary variables, number and percentage (in parentheses) are shown; for continuous variables the mean and standard variation (in parentheses) are shown.

Characteristic	All randomised (n=100)	Full analysis set (n=81)
Age (years)	24.7 (6.6)	25.2 (7)
Gender: Female	53 (53 %)	43 (53 %)
Site: Cambridge	53 (53 %)	46 (57 %)
Index reaction Adrenaline use	34 (34 %)	30 (37 %)
Index reaction wheeze	45 (45%)	38 (47%)
Presence of Asthma	55 (55%)	45 (56%)
Rhinitis	80 (80%)	65 (80%)
Eczema	53 (53%)	46 (57%)
Peanut SPT wheal (mm)	11.5 (4.2)	11.2 (3.8)
V02 max (ml/min/kg)	34.5 (11)	34 (10)
Peanut specific IgE (kU₄/L)	30 (34)	31.6 (35)
Ara h 2 specific IgE (kU₄/L)	20.6 (28)	21.3 (29)

Characteristic	All randomised (n=100)	Full analysis set (n=81)
FEV1 (I)	3.9 (0.8)	3.9 (0.78)
FEV1 (I, % predicted)	105.8 (12)	106 (13)
Number of historical reactions	8.6 (3.4)	8.7 (3.5)
Baseline LOAEL (mg protein)	304 (410)	330.1 (420)
PEFR (I/min)	511.8 (110)	506.7 (110)

Primary outcome: Peanut thresholds and the effect of co-factors

The mean (SD) cumulative threshold for baseline challenges was 330mg (424mg) peanut protein for the full analysis population, 191mg (358mg) for exercise challenges, 157mg (300mg) for sleep deprivation challenges and 214mg (330mg) for non-intervention challenges. When assessing the impact of each intervention on threshold, the estimated change in (natural) log threshold for the sleep deprivation challenge compared to the non-intervention challenge was -0.61 (-0.97, -0.25; p=0.0011) and for the exercise challenge was -0.60 (-0.95, -0.24; p=0.0013). Both changes equate to a reduction in threshold of 45% shown in Figure 7, 8 and 11 and Table 7. No participant reacted on the first dose (3μ g protein), therefore there were no left-censored participants.

There was a trend towards reduction in threshold for each successive intervention visit (Figures 9, 10 and 11) which became significant only for the third post-baseline challenge versus the first post-baseline challenge: threshold (logged) = -0.47 (95% CI -0.83, -0.11); p=0.011.

Figure 7: Dose reached (mg peanut protein) by challenge for full analysis population.



Figure 8: Log(dose reached) by challenge for full analysis population.



Figure 9: Dose reached in mg peanut protein by visit number (full analysis population)







Figure 11: Percentage change in threshold (logged) for each covariate. Fullanalysis population n=81. Visits 1-3 refer to the chronological order of postbaseline challenge days. LOAEL = lowest observed adverse effect level is the reactive threshold in mg peanut protein during baseline challenge.



Table 7: Estimated effect shown in log and percentage scale, 95% confidence interval and p-value for each term in the linear mixed effects model. Fullanalysis population, n=81. Visits 1-3 refer to the chronological order of postbaseline challenge days. LOAEL = lowest observed adverse effect level is the reactive threshold in mg peanut protein during baseline challenge. The estimates for binary variables indicate the modelled difference from reference category in log LOAEL (and absolute percentage change). The estimates for continuous variables (Arah2, Age and baseline LOAEL) indicate the modelled change in log LOAEL per one-unit increase.

Variables	Estimate (log-scale)	СІ	Estimate (absolute change in %)	СІ	p-value
Baseline LOAEL (log-scale)	-0.244	(-0.436,- 0.052)	-22	(-35,-5)	0.014
Non- intervention	Reference				
Exercise	-0.596	(-0.953,- 0.239)	-45	(-61,- 21)	0.0013
Sleep	-0.599	(-0.959,- 0.239)	-45	(-62,- 21)	0.0013
Post baseline visit 1	Reference				
Post baseline visit 2	-0.148	(-0.497,0.2)	-14	(- 39,+22)	0.40
Post baseline visit 3	-0.469	(-0.83,- 0.107)	-37	(-56,- 10)	0.011

Variables	Estimate (log-scale)	CI	Estimate (absolute change in %)	СІ	p-value
Cambridge	Reference				
London	-0.820	(-1.33,- 0.309)	-56	(-74,- 27)	0.002
No asthma at baseline	Reference				
Asthma at baseline	-0.456	(- 0.963,0.051)	-37	(-62,+5)	0.077
Arah2 (per 10 units)	-0.039	(- 0.133,0.055)	-4	(-12,+6)	0.41
Female	Reference				
Male	0.332	(- 0.173,0.838)	+39	(- 16,+131)	0.19
Age (per 10 years)	0.050	(- 0.308,0.408)	+5	(- 27,+50)	0.78

A significant effect of centre was also observed. Compared with Cambridge, London participants had a lower threshold (logged) across post-baseline challenges. In particular with regard to exercise a marginally non-significant difference in effect of exercise challenge vs non-intervention was observed between centres (threshold (logged) -0.78 (95% CI -1.59,0.03) p=0.061). However, the exercise versus non-intervention point estimate was consistent with the overall estimate (i.e. the direction of effect was the same within each centre). Overall, a threshold lowering effect of

both interventions was seen independently at both sites. Pre-specified analysis of the primary outcome was adjusted for both site and challenge order.

Secondary outcome: Population threshold curves

Log-normal, log-logistic and Weibull probabilistic distribution models were fitted to the data and no significant differences were found between the three models. Using the Akaike information criterion (AIC), a value was calculated for each model, a lower AIC suggesting a relatively better fit between candidate models. The log-normal model, which fitted the data best was used. Curves were derived for each challenge type and are shown. Figure 12 displays the threshold distribution curve for the extended analysis population which consisted of all individuals who underwent a baseline challenge and included non-randomised individuals. The mean (95% confidence interval) eliciting doses were $ED_1 = 1.3 \text{ mg} (0.8, 2.0), ED_5 = 3.8 \text{mg} (2.4, 5.7)$ and ED₁₀ =7mg (4.5,10.5) peanut protein. Fourteen participants did not reach challenge stopping criteria during baseline challenge and their data were therefore right censored at the maximum dose. An independent expert reviewed their cases (in blinded fashion) and on the basis of their history, sensitisation patterns and challenge symptoms deemed that they were clinically allergic with likely thresholds greater than 1 gram protein. They were therefore included in the extended analysis population but excluded from randomisation.

Figure 12: Dose distribution curve for extended analysis population (n=123) with 95% confidence intervals Dose is mg peanut protein. Eliciting doses (ED) in mg with 95% CI for 1, 5, 10, 50, 80 and 95% of the extended analysis population are shown as an inset table.



Threshold distribution curves for the Full Analysis Population are shown in Figure 13 and included all individuals who underwent at least 1 baseline challenge. Cumulative EDs were extrapolated from the models and are listed below. The mean (95% confidence interval) eliciting doses for the full-analysis population during nonintervention challenge were ED₁ = 1.5mg (0.8,2.5), ED₅ = 4.0mg (2.4,6.4) and ED₁₀ = 6.7mg (4.1,10.5) peanut protein respectively. Compared with the threshold dose distribution curves (TDC) for the non-intervention challenges, the curves for exercise and sleep deprivation were significantly different and shifted to the left (Figure 13). Thus, during exercise or sleep deprivation challenges, participants reacted at a lower dose than when no intervention was applied. For example, the ED₁ for no intervention was 1.5mg (0.8,2.5), for sleep deprivation was 0.5mg (0.2,0.8) and for exercise was 0.3mg (0.1,0.6). The effect was most pronounced at lower eliciting doses, but not noticeable at higher eliciting doses ($ED_{50} - ED_{95}$) (Figure 14; Table 8).

Figure 13: Threshold dose distribution model Doses given in mg peanut protein, per challenge type, showing cumulative probability of reacting against dose in peanut protein in milligrams. Full analysis population, n=81.


Figure 14: Eliciting dose estimates (mg peanut protein) derived from threshold distribution curve; mean (95% CI) by challenge type for eliciting doses (ED) for 1, 5, 10, 50, 80 and 95% of the full analysis population, n=81 are shown.



Table 8: Predicted dose vs reactions. Predicted dose (and 95% Cl) that gives different probability of reactions (EDx = dose that gives x% probability of reaction), full-analysis set n=81

Dose	Baseline challenge, (n=81)	Non-intervention challenge, (n=71)	Sleep challenge, (n=71)	Exercise challenge, (n=73)
ED ₁	3 (1.7,4.8)	1.5 (0.8,2.5)	0.5 (0.2,0.8)	0.3 (0.1,0.6)
ED ₅	7.6 (4.7,12)	4 (2.4,6.4)	1.3 (0.7,2.2)	1.1 (0.5,1.7)
ED ₁₀	12.8 (8.2,19.8)	6.7 (4.1,10.5)	2.4 (1.4,3.8)	1.9 (1.1,3.1)
ED ₅₀	80.6 (57.9,112)	44.6 (30.8,64.5)	20.4 (12.9,31.9)	19.7 (12,32)
ED ₈₀	255 (180.2,360.8)	156.2 (103.5,235.5)	101.8 (58.4,176.9)	123.6 (65.3,233.3)
ED ₉₅	715.9 (441.9,1159.4)	502 (276.9,909.3)	537 (223.6,1287.6)	894.7 (308.4,2592.2)

Safety

There was a single serious adverse reaction which was reviewed in detail by the IDMC. One participant had a severe reaction following dose 7 (300mg peanut protein) on a non-intervention challenge. The participant developed a persistent dry cough, central chest pain, nausea, lip oedema hypotension and tachycardia. The participant required two doses of intramuscular adrenaline, intravenous fluids, nebulised salbutamol and nebulised adrenaline to stabilise and was admitted to HDU at the Royal Brompton Hospital.

The IDMC reviewed three further cases who suffered unexpectedly severe reactions. Given the severity of their reactions the investigators took the decision to exclude them from the study.

Following these severe cases, the Trial Management Group devised the following measures to improve challenge safety which were the following:

- 1. Investigators could override the stopping criteria at any time for safety reasons
- The current challenge could be interpreted in the context of previous reactions and symptoms to earlier doses
- 3. Caution was applied to updosing after the development of a 'new' cough during challenge
- 4. Participants were treated immediately once the decision had been made to terminate the challenge
- The definition of 'persistent' was changed to '>/=10m /escalating symptoms if <10m'
- 6. If at least two concurrent yellow symptoms, or one lower respiratory (including cough) yellow symptom was present
 - Next dose was delayed for 60m
 - Next dose was not to be given unless symptom-free for 30m
- The standard interval between doses 6 (100mg)- 7 (300mg) and 7-8 (1000mg) was increased to 60m for all participants.

Intramuscular adrenaline use

Intramuscular adrenaline was delivered in 52/342 (15%) challenges. Two doses of intramuscular adrenaline were delivered to stabilise the participants in 6/342 (2%) challenges Nebulised adrenaline was administered in 3/342 (1%) challenges.

Discussion and implications

We have defined a mean reactivity threshold of 214mg peanut protein for an individual, approximately equivalent to one whole peanut seed (15)²³, and have demonstrated that both exercise and sleep deprivation caused a 45% reduction in an individual's threshold. To our knowledge these findings provide the first systematically generated data on peanut allergy thresholds in a UK adult peanut-allergic population, and the first prospectively collected data to show that co-factors significantly reduce allergic thresholds in peanut allergy.

By using a dosing regimen with a low starting dose of 3 micrograms, a NOAEL was confidently established as there were no first (left censored) dose reactors. In addition, by using modified symptom severity grading criteria true thresholds based on objective symptoms were identified.

To determine a population threshold, we used threshold dose distribution modelling, to estimate the amounts of peanut protein that would elicit a reaction in 1, 5 and 10% of the peanut-allergic population. These eliciting doses were 1.5mg, 4mg and 6.7mg peanut protein respectively. Fourteen participants were able to tolerate the top dose (right censored) however this was a minority of subjects. Eliciting dose values in the extended analysis population were not significantly different, even when including the right-censored individuals who had no threshold identified. Several groups have established peanut threshold distribution data on children, although none have been elicited for UK adults. Furthermore, these studies have often included individuals with milder phenotypes, and have excluded participants with a history of anaphylaxis.

In addition, previous studies have often based threshold estimates on subjective symptoms which could lead to an underestimation of the true threshold dose. Our estimate for ED_{10} (6.7mg) was higher when compared to some other previous

²³ Wang M, Tonnis B, Pinnow D, Barkley N, Pederson G. Progress on screening the USDA cultivated peanut germplasm collection for variability in seed weight, seed-coat color, oil content and fatty acid composition) [Internet]. 2015. Available: www.ars.usda.gov/research/publications/publication/?seqNo115=317089

estimates, which range from 0.7-4.42mg.^{24 25 26 27 28} Although some studies have often used subjective symptoms as stopping criteria leading to lower threshold estimates,²⁶ many have not.⁶

The most likely explanation for the higher ED_{10} in this study is the use of more robust stopping criteria employed in our study, where three concurrent objective symptoms were required to stop the challenge and establish the threshold. Klemans at al who used threshold data derived from diagnostic food challenges estimated an ED_{10} of 13.7 (4.37-42.8 95% CI) mg peanut protein in adults, although the confidence intervals were wide.²⁹

²⁵ Ballmer-Weber BK, Fernandez-Rivas M, Beyer K, Defernez M, Sperrin M, Mackie AR, et al. How much is too much? Threshold dose distributions for 5 food allergens. J Allergy Clin Immunol. 2015;135(4):964–71.

²⁶ Blumchen K, Beder A, Beschorner J, Ahrens F, Gruebl A, Hamelmann E, et al. Modified oral food challenge used with sensitization biomarkers provides more reallife clinical thresholds for peanut allergy. J Allergy Clin Immunol [Internet]. Elsevier Ltd; 2014;134(2):390–398.e4. Available from: <u>dx.doi.org/10.1016/j.jaci.2014.03.035</u>

²⁷ Taylor SL, Moneret-Vautrin DA, Crevel RWR, Sheffield D, Morisset M, Dumont P, et al. Threshold dose for peanut: Risk characterization based upon diagnostic oral challenge of a series of 286 peanut-allergic individuals. Food Chem Toxicol. 2010;48(3):814–9.

²⁸ Blom WM, Vlieg-Boerstra BJ, Kruizinga AG, Van Der Heide S, Houben GF, Dubois AEJ. Threshold dose distributions for 5 major allergenic foods in children. J Allergy Clin Immunol [Internet]. Elsevier Ltd; 2013;131(1):172–9. Available: <u>dx.doi.org/10.1016/j.jaci.2012.10.034</u>

²⁹ Klemans RJB, Blom WM, van Erp FC, et al. Objective eliciting doses of peanutallergic adults and children can be combined for risk assessment purposes. Clin Exp Allergy. 2015;45(7):1237-1244. doi:10.1111/cea.12558

²⁴ Zhu J, Pouillot R, Kwegyir-Afful EK, Luccioli S, Gendel SM. A retrospective analysis of allergic reaction severities and minimal eliciting doses for peanut, milk, egg, and soy oral food challenges. Food Chem Toxicol. 2015;80:92–100.

We show for the first time that co-factors lower the reactivity threshold in allergic reactions.

Reports from the literature suggest that exercise as a cofactor plays a role in 0-15.9% of anaphylaxis cases.^{30 31 32} However this estimate is based on retrospective analyses of implicating factors in anaphylactic reactions. Previously it has been shown in a clinically distinct condition, Food Dependent Exercise Induced Anaphylaxis that exercise can act as an augmentation factor in participants who can otherwise tolerate wheat containing products when given alone.³³ However, the effect of exercise in patients who have established allergy and cannot tolerate the allergen under any circumstance has not previously been shown. It is possible that exercise exerts its effect through under-perfusion of the gut resulting in a relative ischaemia with resultant damage to tight junction integrity. This may lead to increased permeability of the gut to food allergens.²¹

Sleep deprivation resulted in a more pronounced lowering of the reactivity threshold compared to exercise. The effect of sleep deprivation, in this case used as a proxy for stress, has never prospectively been studied in allergic reactions. It has been noted in immunotherapy studies that a loss of tolerance to peanut can occur in the maintenance phase when subjects consume peanut doses whilst tired or stressed.³⁴

³³ Scherf KA, Brockow K, Biedermann T, Koehler P, Wieser H. Wheat-dependent exercise-induced anaphylaxis. Clin Exp Allergy. 2016. doi:10.1111/cea.12640

³⁴ Anagnostou K, Islam S, King Y, Foley L, Pasea L, Bond S, et al. Assessing the efficacy of oral immunotherapy for the desensitisation of peanut allergy in children (STOP II): a phase 2 randomised controlled trial. Lancet [Internet]. Anagnostou et al. Open Access article distributed under the terms of CC BY-NC-ND;

³⁰ De Swert LFA. Risk factors for allergy. Eur J Pediatr. 1999. doi:10.1007/s004310051024

³¹ Hompes S, Dölle S, Grünhagen J, Grabenhenrich L, Worm M. Elicitors and cofactors in food-induced anaphylaxis in adults. 2013:2-5.

³² Uguz A, Lack G, Pumphrey R, et al. Allergic reactions in the community: A questionnaire survey of members of the anaphylaxis campaign. Clin Exp Allergy. 2005. doi:10.1111/j.1365-2222.2005.02257.x

The underlying mechanism may also due to enhanced gastrointestinal permeability. It has been shown in animal models of inflammatory bowel disease that stress results in enhanced intestinal permeability.³⁵ Both acute and chronic stress have been shown to increase ion and water secretion and intestinal permeability in the jejunum and colon of laboratory animals.³⁶ These changes were associated with a significant increase in the permeability of the epithelium to macromolecules.

It is well known that under stressful circumstances such as acute sleep loss, corticotrophin releasing factor (CrF) is released signalling the first step in the activation of the HPA. This hormone has potent effects on the gut via inflammation, increase in gut permeability and modulation of gut motility.³⁷ The translation of stress signals to gut mast cells may also play a pivotal role. Mast cells posess surface receptors for corticotrophin releasing factor which may be an important indication of the link between stress and these cells. Mast cells in the gastrointestinal tract serve as end effectors of the brain-gut-axis (BGA). When the brain gut axis is activated mast cells release a wide range of mediators including mast cell tryptase, histamine, heparin and PAF.³⁸ Tryptase can activate PAR2 receptors on epithelial cells resulting in modulation of tight junction proteins and increases in permeability through

2014;383(9925):1297–304. Available: linkinghub.elsevier.com/retrieve/pii/S0140673613623016

³⁵ Million M, Taché Y, Anton P. Susceptibility of Lewis and Fischer rats to stressinduced worsening of TNB-colitis: protective role of brain CRF. Am J Physiol. 1999.

³⁶ Gue M, Bonbonne C, Fioramonti J, et al. Stress-induced enhancement of colitis in rats: CRF and arginine vasopressin are not involved. AmJPhysiol. 1997.

³⁷ Santos J, Yang PC, Söderholm JD, Benjamin M, Perdue MH. Role of mast cells in chronic stress induced colonic epithelial barrier dysfunction in the rat. Gut. 2001. doi:10.1136/gut.48.5.630

³⁸ Rijnierse A, Nijkamp FP, Kraneveld AD. Mast cells and nerves tickle in the tummy. Implications for inflammatory bowel disease and irritable bowel syndrome. Pharmacol Ther. 2007. doi:10.1016/j.pharmthera.2007.06.008 paracellular pathways in the intestinal epithelium.³⁹ PAR2 receptors have also been found on mast cells, thus activation of PAR2 can propagate the release of proinflammatory mediators from nerve endings, potentiating mast cell degranulation and creating a positive feedback loop.⁴⁰ Stress did not affect gut permeability in MCdeficient rats³⁷ supporting the critical role that mast cells play in orchestrating the stress response in the gut (Figure 15).

³⁹ Jacob C, Yang P-C, Darmoul D, et al. Mast cell tryptase controls paracellular permeability of the intestine. Role of protease-activated receptor 2 and beta-arrestins. J Biol Chem. 2005. doi:10.1074/jbc.M506338200

⁴⁰ He SH, He YS XH. Activation of human colon mast cells through proteinase activated receptor-2. World J Gastroenterol. 2004;10(3):327-331. www.ncbi.nlm.nih.gov/pubmed/14760751

Figure 15: The effect of stress on mucosal mast cells and intestinal permeability.



Co-factors such as exercise, alcohol and non-steroidal anti-inflammatory drugs, are increasingly being implicated in food anaphylaxis.

There is evidence of an effect on threshold with an increasing number of visits. A significant lowering of threshold in the final intervention visit compared to the first intervention visit was seen. Indeed, in a study by Wainstein et al where follow up oral food challenges were performed in peanut allergic children who had been challenged at 35.5 (mean) months earlier, a decrease in threshold was noted in 10/13 patients, probably reflecting a high rate of natural resolution in their cohort of patients.⁴¹

This study is the first to establish population eliciting doses for peanut when participants are deliberately subjected to the co-factors sleep deprivation and exercise. Furthermore, we are able to relate these to a reference threshold when no co-factor (non-intervention) is applied to calculate the magnitude of the effect.

⁴¹ Wainstein BK, Saad RA. Repeat oral food challenges in peanut and tree nut allergic children with a history of mild/moderate reactions. Asia Pac Allergy. 2015. doi:10.5415/apallergy.2015.5.3.170

Current allergen risk assessment by food industry and regulators involves defining an eliciting dose (e.g. ED₁ or ED₅) representing an exposure that is likely to be safe for the population. Hourihane et al have recently validated the proposed ED₅ for peanut of 1.5mg peanut protein by performing single dose peanut challenges on 378 children and observed that only eight participants (2.1%) experienced objective symptoms (all mild), only half of whom required treatment with oral antihistamines.⁸ Further studies are required to validate proposed ED₅ and ED₁ doses, particularly in the adult population. The food industry can then use these validated eliciting doses to develop guidelines for the use of voluntary precautionary food labelling (reference doses).

Previously a reference dose of 0.2mg peanut protein, based on the ED₁, has been proposed by the VITAL group.⁹ However, the group acknowledge in their study that further application of an uncertainty or safety factor to this reference dose may be necessary to account for individual factors which may potentially affect this dose estimate. Due to a paucity of clinical data, the application of safety factors has traditionally followed toxicology practice accounting for (10-fold) inter-species (for thresholds defined in non-human models) and (a further 10-fold) intra-individual variation in response. In practice, such large safety factors result in very low reference doses which, being near or below the limit of detection of available assays, are difficult to measure with accuracy, rendering them impractical for the food industry to implement. This results in over-cautious food labelling. We show in this study, that such safety factors can be many magnitudes smaller and hence the reference doses could be within the operating range of routinely available assays.

Sleep deprivation lowers the ED₁ from 1.5mg (for the non-intervention dose distribution) to 0.5mg; this is equivalent to applying a safety factor of 0.33 to the ED₁ calculated from the non-intervention dose distribution. Similarly, exercise lowers the ED₁ from 1.5mg (non-intervention) to 0.3mg equivalent to a safety factor of 0.2. The derivation of reference doses which use evidence-based safety factors such as those which are provided by our study will enhance the allergen risk assessment process. This should encourage better industry engagement with evidence-based voluntary food labelling reducing excessive, overly cautious precautionary allergen labelling and provide allergic consumers with greater assurance that foods without precautionary allergen labelling are safe for the majority to consume.

Furthermore, this study has also been instructive in terms of advice to patients for example who are receiving peanut immunotherapy. Patients can be advised to leave an interval of 2 hours between ingestion of their peanut doses and subsequent exercise. Furthermore, if the patients suffer a period of sleep loss (for example due to jet lag or sleepovers) they should be advised to omit the peanut dose. In general allergy clinic, we have also observed that cofactors may be implicated in reactions in patients who are allergic to lipid transfer protein. Allergy to lipid transfer protein is widely reported in the Mediterranean population and also in northern European countries such as the UK.⁴² This advice may also be relevant in these cases.

The safety data in this trial show that the overall adrenaline use across all challenges was 15% and nebulised adrenaline 2%, broadly reflecting the rate of adrenaline use in positive food challenges in other studies. Jarvinen et al reported its use in 11% of positive food challenges ⁴³ and Lieberman in 9% of positive food challenges.⁴⁴ Yanagida et al reported a rate of 23% for IM administration in patients undergoing oral food challenges prior to the commencement of immunotherapy. The rate of use of inhaled adrenaline was 13%.⁴⁵ In contrast, Noone *et al* reported a much higher rate of adrenaline use in their study, again screening subjects for food therapeutic

⁴² Ballmer-Weber BK, Lidholm J, Fernández-Rivas M, et al. IgE recognition patterns in peanut allergy are age dependent: perspectives of the EuroPrevall study. Allergy. 2015;70(4):391-407. doi:10.1111/all.12574

⁴³ Järvinen KM, Amalanayagam S, Shreffler WG, Noone S, Sicherer SH, Sampson HA, et al. Epinephrine treatment is infrequent and biphasic reactions are rare in foodinduced reactions during oral food challenges in children. J Allergy Clin Immunol. 2009;124(6):1267–72.

⁴⁴ Lieberman JA, Cox AL, Vitale M, Sampson HA. Outcomes of office-based, open food challenges in the management of food allergy. J Allergy Clin Immunol. 2011;1120–2.

⁴⁵ Yanagida N, Sato S, Asaumi T, Ogura K, Ebisawa M. Risk Factors for Severe Reactions during Double-Blind Placebo-Controlled Food Challenges. Int Arch Allergy Immunol. 2017:173-182. doi:10.1159/000458724

trials.⁴⁶ In their study, intramuscular adrenaline was administered in 39.2% cases, however the higher rate may be accounted for by differences in physician practice for example, the use of adrenaline to treat severe abdominal cramping which was not an indication in the TRACE study protocol. In food challenge studies the rate of multiple doses ranges from 0.68-6.5%.^{43,45} Of course studies focussed on community reactions presenting to the ED department report higher rates of repeated epinephrine use 13-16%.^{47 48}

We found no association between threshold and other factors such as the presence of asthma, the level of peanut specific IgE, Ara h 2 or gender. Previous studies have noted an inverse correlation between Ara h 2 specific IgE and elicitation threshold, but we did not replicate this finding in our study.⁴⁹

⁴⁶ Noone S, Ross J, Sampson HA, Wang J. Epinephrine Use in Positive Oral Food Challenges Performed as a Screening Test for Food Allergy Therapy Trials. J Allergy Clin Immunol Pract. 2015. doi:10.1016/j.jaip.2014.10.008

⁴⁷ Manivannan V, Campbell RL, Bellolio MF, Stead LG, Li JTC, Decker WW. Factors associated with repeated use of epinephrine for the treatment of anaphylaxis. Ann Allergy, Asthma Immunol. 2009. doi:10.1016/S1081-1206(10)60358-4

⁴⁸ Oren E, Banerji A, Clark S, Camargo CA. Food-induced anaphylaxis and repeated epinephrine treatments. Ann Allergy, Asthma Immunol. 2007. doi:10.1016/S1081-1206(10)60568-6

⁴⁹ Blumchen K, Beder A, Beschorner J, Ahrens F, Gruebl A, Hamelmann E, et al. Modified oral food challenge used with sensitization biomarkers provides more reallife clinical thresholds for peanut allergy. J Allergy Clin Immunol [Internet]. Elsevier Ltd; 2014;134(2):390–398.e4. Available: <u>dx.doi.org/10.1016/j.jaci.2014.03.035</u>

Limitations

A potential limitation of this study is that our eliciting dose estimate is based on a volunteer peanut-allergic population. Although participants with a history of anaphylaxis and historical adrenaline use were included, those who have suffered the most severe reactions in the community may be under-represented, being possibly reluctant to volunteer for the study. This could introduce bias only if participants who suffered more severe reactions in the community represent the more sensitive (i.e. lower dose) reactors. However, a previous study has shown that minimum eliciting dose distributions for participants with histories of more severe reactions. Our study population had a low average age of 25 years, our study population was predominantly students and a broader demographic would have been preferable. However, fatal anaphylaxis episodes occur more commonly in this age group perhaps due to more risk-taking behaviour ⁵⁰, thus in defining a threshold for the whole population, it is of benefit that the model is based on this age group.

Furthermore, community exposures to peanut could be larger and more sudden than the gradual incrementally increasing allergen exposure in our protocol thus further data is needed on individual consumption patterns of high-risk foods. Food matrix is known to have an effect on threshold dose, with higher fat matrices delaying absorption of allergen and ultimately resulting in higher cumulative doses of allergen.⁵¹ However, an ultimate aim is to combine these data with data from other studies using a variety of matrices which will average out the differences between the challenge vehicles. In this study partially defatted roasted peanut flour was used and previously authorities have questioned whether this differs significantly from whole

⁵⁰ Marrs T, Lack G. Why do few food-allergic adolescents treat anaphylaxis with adrenaline? - Reviewing a pressing issue. Pediatric Allergy and Immunology. 2013. p. 222–9.

⁵¹ Grimshaw KEC, King RM, Nordlee JA, Hefle SL, Warner JO, Hourihane JOB. Presentation of allergen in different food preparations affects the nature of the allergic reaction - A case series. Clin Exp Allergy. 2003;33(11):1581-1585. doi:10.1046/j.1365-2222.2003.01795.x

peanut. Allen at al had sufficient data to allow a comparison or ED5 values for challenges using pulverised peanut and others using partially defatted peanut flour and found no significant difference between the two sources.⁹

A significant centre effect was observed with participants in London having overall lower thresholds than those in Cambridge, though a threshold lowering effect of both interventions was seen independently at both sites, reinforcing the generalisability of our findings. No differences were observed in the baseline characteristics of the study populations to account for the centre effect. The most likely explanation is variation between investigators in the interpretation of clinical symptoms and decision about when to stop the challenge and administer treatment. Attempts were made to standardise practice across both sites through common stopping criteria for challenges and cross-site training to minimise this. Variability in the interpretation of clinical symptoms by clinical experts is known to occur in food challenges and has been reported in another study.⁵² All analyses were adjusted for centre.

Another potential weakness was the change in protocol to use open challenges following the blinded baseline food challenge. We observed an apparent lowering of threshold linked with an increasing number of challenges. Although this may be a true phenomenon it is also possible that the open study design may have contributed to this, by participants and investigators 'learning' reactions over time and anticipating the development of more severe symptoms. However, the study was designed to minimise this bias by ensuring that the participant was deemed to have reached their reaction threshold with only the appearance of pre-specified objective symptoms, and the balanced design means that the two interventions were spread equally across the order of challenge days.

In conclusion, our study identified eliciting dose estimates from a well characterised adult peanut-allergic population. Also, for the first time it has been shown that cofactors such as sleep deprivation and exercise lower allergen reactivity thresholds, and the magnitude of their effect has been defined. This study, funded by the FSA,

⁵² Erp FC Van, Knulst AC, Meijer Y, Gabriele C, Ent CK Van Der. Standardized food challenges are subject to variability in interpretation of clinical symptoms. 2014;1–6.

has important public health implications helping food policy makers and the food industry provide harmonised guidance on allergen labelling, which will ultimately benefit all peanut allergic individuals.

Appendices

Appendix 1: TRACE study website registration portal



Appendix 2: Screening Visit Form

Centre:

Participant initials:

subject code (patient's unique identifier):

Date of examination (dd/mm/yyyy):

Date of written consent (dd/mm/yyyy):

The written consent should be kept with the hard copy of the CRF Patient's demographic data:

Birth date (dd/mm/yyyy):

Age at visit to clinic (years): [18-45]

Sex: Male/Female

1. Age at onset of the first adverse reaction to peanut : (0-45)

2. Number of adverse reactions: [0-20]

Regarding the most severe reaction induced by peanut:

3. Type of food:

4. Minimum intake to trigger the first complaint:

- A bite / a swallow
- ¼ helping
- 1/2 helping
- One normal helping (according to patient's age)
- Unknown

5. Interval between the food intake and the onset of symptoms:

- < 5 minutes
- 5-15 minutes

- >15- 30 minutes
- >30 60 minutes
- 1-2 hours
- 2 hours
- Unknown

6. Symptoms associated with the most severe reaction induced by peanut

- A. Complaints of the oral cavity
 - Oral allergy syndrome only
 - Oral itching

B. Skin complaints

- Urticaria
- Angioedema
- Erythema / flushing
- Itching

C. Digestive complaints

- Nausea
- Vomiting
- Stomach pain
- Cramps
- Diarrhoea
- Dysphagia

D. Airway complaints

- Asthma (dyspnea, wheezing, cough, chest tightness)
- Rhinitis
- Dysphonia
- Tightness of the throat
- E. Eye complaints

Conjunctivitis

F. Cardiovascular complaints

- Cardiac arrhythmia
- Myocardial ischaemia (angina, infarction)
- Hypotension

G. Neurologic complaints

- Disorientation, confusion
- Dizziness
- Seizures
- Incontinence
- Loss of consciousness
- H. Anaphylaxis (tick all the applicable)
 - with severe bronchospasm
 - with severe laryngeal oedema
 - with hypotension (anaphylactic shock)
 - EIA (exercise induced anaphylaxis)

7. Medication received to control the reaction:

Yes:

- Antihistamines
- corticosteroids
- adrenaline
- intravenous fluids
- vasopressors
- oxygen
- mechanical ventilation

No/unknown

8. Emergency care assistance and/or hospitalization after the reaction:

- Yes
- No/unknown

9. Time elapsed since the last (any) reaction to peanut (until today):

- Up to 1 month
- 6 months
- >6-12 months
- 12-24 months
- >2-5 years
- >5 years
- Unknown

10. Does the patient have any other food allergies (including any of the matrix components?)

- Yes
- No

If yes denote which ones below and complete additional food adverse reactions form for each.

Foods involved in immediate (≤ 2 hours) adverse reactions.

	Yes	No
Cow's milk		
Hen's egg		
Brazil nut		
Almond		
Hazelnut		

	Yes	No
Walnut		
Cashew		
Pistachio		
Pine nut		
Sesame seeds		
Pecan nut		

Other associated conditions - Asthma

10. Do you have asthma? Yes No Unknown

Asthma Control Test score..... [0-25]

11a. Triggers:

- Dust
- Pollen
- Animal dander
- Fungal spores
- NSAIDS
- Infection
- Exercise
- Cold air

11b. If pollen related asthma present denote period of symptoms:

January	
February	
March	
April	
Мау	
June	
July	
August	
September	
October	
November	
December	

13. Current treatment:

- Short acting B2 agonist
- Inhaled corticosteroid
- Long acting B2 agonist
- Combination device
- Systemic corticosteroids
- Additional agents

14. Number of courses of oral corticosteroids with the last 2 years [0-10]

15. Number of previous asthma related hospital admissions [0-10]

16. Number of previous ITU/HDU admissions [-0-5]

Rhinitis/Rhinoconjunctivitis

17. Do you suffer from rhinitis/rhinoconjunctivitis?

- Yes
- No

If yes, Total Nasal Symptom Score [0-12]

18. Triggers:

- Dust
- Pollen
- Animal dander
- Fungal spores

19a. Seasonal

- Yes
- No

19b. Perennial

- Yes
- No

20. If seasonal denote period of symptoms

January	
February	
March	
April	
May	
June	
July	
August	
September	
October	
November	
December	

21. Treatment required:

- Antihistamines
- Nasal spray/drops
- Eye drops
- Oral steroids
- Leukotriene antagonists

Eczema and skin conditions

22. Associated atopic dermatitis:

- Yes
- No
- Unknown

POEM score [0-28]

23. Associated urticaria/angioedema

- Yes
- No

Past medical history

24. Do you suffer from any major illnesses or conditions including:

Gastric or duodenal ulcer

- Yes
- No

Eosinophilic oesophagitis

- Yes
- No

Coronary artery disease

- Yes
- No

A past medical history of clinically significant ECG abnormalities

- Yes
- No

Other significant illness which may prevent inclusion

Are you currently pregnant?

- Yes
- No

Current medication

25. Any drug allergies

- Yes
- No

26. Are you on any current medication including:

Systemic corticosteroids

- Yes
- No

Immunosuppressants

- Yes
- No

Beta blockers

- Yes
- No

ACE inhibitors

- Yes
- No

Antacid medication

• Yes

• No

Tricyclic antidepressants

- Yes
- No

Sedatives

- Yes
- No

Other ...

Social

27. Alcohol consumption: units/week [0-40]

28. Smoker: pack year history [0-60]

29. Occupation:

Night shift worker

- Yes
- No

Family history

30. Family background of atopy

Mother

- Yes
- No

Father

• Yes

• No

Sibling(s)

- Yes
- No

Investigations

Skin prick tests

	Wheal (mm)	Flare (mm)
Negative control	[0-5]	[0-50]
Histamine	[0-30]	[0-50]

Nut

	Wheal (mm)	Flare (mm)
Peanut	[0-30]	[0-50]
Brazil	[0-30]	[0-50]
Almond	[0-30]	[0-50]
Hazelnut	[0-30]	[0-50]
Walnut	[0-30]	[0-50]
Cashew	[0-30]	[0-50]
Pistachio	[0-30]	[0-50]
Macadamia	[0-30]	[0-50]
Pecan	[0-30]	[0-50]

Other foods

	Wheal (mm)	Flare (mm)
Milk	[0-30]	[0-50]
Wheat	[0-30]	[0-50]
Egg (white)	[0-30]	[0-50]
Soya	[0-30]	[0-50]
Sesame	[0-30]	[0-50]
Lupine flour	[0-30]	[0-50]
Cod	[0-30]	[0-50]
Shrimp	[0-30]	[0-50]
Peach	[0-30]	[0-50]

Aeroallergens

	Wheal (mm)	Flare (mm)
5 grasses	[0-30]	[0-50]
Dermatophagoides farina	[0-30]	[0-50]
Dermatophagoides pteronyssinus	[0-30]	[0-50]
Alternaria alternate	[0-30]	[0-50]
Aspergillus	[0-30]	[0-50]
Cladosporium (Cladosporoides, herbarum)	[0-30]	[0-50]
Alder	[0-30]	[0-50]
Birch	[0-30]	[0-50]
Hazel	[0-30]	[0-50]
Plane	[0-30]	[0-50]
Cat	[0-30]	[0-50]
Dog	[0-30]	[0-50]

Investigations (continued)

ECG ok for challenge?

- Yes
- No

Spirometry

Pre exercise FEV1 litres/minute [2.0-5.5]

Post VO2 max exercise FEV1 litres/minute [2.0-5.5]

Fall in FEV1 >15% suggesting possible exercise induced asthma

- Yes
- No

Exercise test

VO2 max test [0-100] mL/kg/min

Maximum heart rate achieved 100-250 bpm

Target heart rate for exercise challenge [85% maximal heart rate] 100-250 bpm

Blood test results

FBC normal?

- Yes
- No

Renal function normal?

- Yes
- No

Test	Result	Range
Baseline tryptase		[2.0-25.0] ng/ml
lgE		[0-10000] KU/L
Peanut specific Ige		[0-5000] KUa/L
Arah1		[0-500] KUa/L
Arah2		[0-500] KUa/L
Arah3		[0-500] KUa/L
Arah8		[0-500] KUa/L
Arah9		[0-500] KUa/L

Checklist [paper crf only]

Appointment Date	
Time	
Visit No	
Medical/Dr	
Consent	
Notes	
Observations/Physical exam	
Bloods	
Spirometry	
V02 Max	
ECG	
SPT	
POEM	
Pregnancy Test	
IV Access	
Asthma CQ	
Comments	

Appendix 3: Modification of existing PRACTALL criteria, with explanation

The colour coded symptom grading system was used as follows:

Green (mild) symptoms were not an indication to alter dosing.

Yellow (moderate) symptoms if present singly would be an indication for the investigator to proceed with caution. If three yellow symptoms were present concurrently within the same organ or across different organ systems then this was an indication to stop.

Existing PRACTALL criteria	Modified PRACTALL criteria	Explanation of modification made
Mild, occasional scratching [Green]	Pruritus -Occasional scratching [Green]	
Moderate -scratching continuously for > 2 minutes at a time [Green] Severe hard continuous scratching excoriations [Yellow]	Pruritus- scratching continuously for >2 mins at a time [Green] Hard continuous scratching causing excoriations [Yellow]	
Mild < 3 hives, or mild lip edema [Yellow] Moderate - < 10 hives but >3, or significant lip or face edema [Red]	Urticaria-<3 hives or mild lip oedema [Yellow] Urticaria- <10 hives ≥ 3or significant lip or face oedema [Red]	

Red (severe) symptoms if present singly was an immediate indication to stop.

Existing PRACTALL criteria	Modified PRACTALL criteria	Explanation of modification made
Severe generalized	Urticaria-generalised	
involvement [Red]	involvement [Red]	
Mild few areas of faint	Rash- Few areas of faint	
erythema [Green]	erythema [Green]	
Moderate areas of erythema	Rash- Areas of erythema	
[Yellow]	[Yellow]	
Severe generalized marked	Rash- Generalised marked	
erythema (>50%) [Red]	erythema>50% [Red]	
Mild rare bursts, occasional	Itching in inner ear canal	Itching in inner ear canal was
sniffing [Green]	[green]	added as it was a common mild
Moderate bursts < 10,	Rare bursts of sneezing	symptom identified by many
intermittent rubbing of nose,	occasional sniffing [Green]	patients during piloting.
and/or eyes or frequent sniffing	Puroto < 10 intermittent	Rhinitis symptoms downgraded
[Yellow]	rubbing of nose and/or eves or	from red to yellow. These were
Severe continuous rubbing of	frequent sniffing [Yellow]	not regarded by the study team as severe enough symptoms
nose and/or eyes, periocular swelling and/or long bursts of sneezing, persistent rhinorrhea	Continuous rubbing of nose and/or eyes, [Yellow]	singly to warrant stopping challenge.
[Red]	Periocular swelling and/or	
	long bursts of sneezing,	
	[Yellow]	
	Persistent rhinorrhoea [Yellow]	
Existing PRACTALL criteria	Modified PRACTALL criteria	Explanation of modification made
--	---	---
Mild expiratory wheezing to auscultation [Red] Moderate inspiratory and expiratory wheezing [Red] Severe use of accessory muscles, audible wheezing [Red]	atory wheezing to on [Red]Chest tightness without any fall in PEFR [Green]In the existing Practall criter study team felt that there needed to be representation milder respiratory symptoms the existing criteria escalate rapidly to wheeze which is a clear objective symptoms. Therefore to enhance safety	In the existing Practall criteria study team felt that there needed to be representation of milder respiratory symptoms as the existing criteria escalate too rapidly to wheeze which is a clear objective symptoms. Therefore to enhance safety and
	Chest tightness with a >20% fall in PEFR [Red] Expiratory or inspiratory wheeze [Red] Use of accessory muscles [Red]	aid detection, the gradation of lower respiratory symptoms was extended adding milder ones and incorporating functional measurement of PEFR.
Mild >3 discrete episodes of throat clearing or cough, or persistent throat tightness/pain [Yellow] Moderate hoarseness, frequent dry cough [Red] Severe stridor [Red]	Throat tingling/altered sensation in throat [Green] > 3 discrete episodes of throat clearing or cough [Yellow] Persistent throat tightness [Yellow] Hoarseness or frequent dry cough [Red]	Mild oropharyngeal symptoms added Definition of persistence added and defined as symptom present for ≥30 minutes
	Stridor [Red]	

Existing PRACTALL criteria	Modified PRACTALL criteria	Explanation of modification made
Mild complaints of nausea or abdominal pain, itchy	Oral itching [Green]	Milder and transient abdominal symptoms downgraded
mouth/throat [Yellow]	Transient nausea [Green]	Incorporated duration of abdominal symptoms as a
Moderate frequent c/o nausea or pain with normal activity	Transient abdominal pain [Green]	defined as symptom present ≥30 minutes
[Yellow]	Persistent nausea [Yellow]	
Severe - notably distressed due to GI symptoms with decreased activity [Yellow]	Persistent abdominal pain [Yellow]	
Objective	Emesis/diarrhoea (1 episode) [Yellow]	
Mild 1 episode of emesis or diarrhea [Yellow]	Emesis/diarrhoea (more than 1 episode) [Red]	
Moderate 2-3 episodes of emesis or diarrhea or 1 of each [Red]		
Severe >3 episodes of emesis or diarrhea or 2 of each [Red]		
Mild-subjective response	Weak/dizzy or tachycardia	
[Yellow]		
moderate-drop in blood	brop in BP and/or >20% from baseline [Red]	
pressure and/or >20% from	Cardiovascular collapse/signs of impaired circulation [Red]	

Existing PRACTALL criteria	Modified PRACTALL criteria	Explanation of modification made
baseline, or significant change	Altered level of consciousness	
in mental status. [Red]	[Red]	
severe-cardiovascular		
collapse, signs of impaired		
circulation (unconscious) [Red]		

Appendix 4: TRACE study case record form



Baseline Peanut DBPCFC:

Study Participant UID:

Supervising clinician:

Nurse:

Challenge Day 1

Date:

Challenge SOP version used:

1. Has the participant given consent to continue?

- Yes
- No

2. Type of challenge

- Baseline
- No intervention
- Sleep
- Exercise

3. Pre-challenge history

Asthma control:

Is FEV1 >80% predicted

- Yes
- No

Asthma control test score above 20?

- Yes
- No

A significant clinical reaction to peanut within the previous three months

- Yes
- No

Significant illness with systemic features (e.g. fever >37.5 degrees Celcius) within two (2) weeks prior to challenge

- Yes
- No

Any current symptoms of allergic disease (urticaria, angioedema, eczema, rhinitis, asthma)

- Yes
- No

Musculoskeletal disease which could impair the participant's ability to perform the exercise challenge

- Yes
- No

Any stomach pain, sickness, diarrhoea, bloating?

• Yes

• No

Has subject fasted for at least 2 hours?

- Yes
- No

Has intense exercise been avoided for 12 hours?

- Yes
- No

No caffeine intake in last 12 hours

- Yes
- No

Has alcohol been avoided for 24 hours?

- Yes
- No

Alcohol or drug misuse

- Yes
- No

Night shift working within the last month

- Yes
- No

Drugs that may alter reactivity and influence the outcome of the DPT if taken concomitantly: Guidance provided in study SOP:

- Yes
- No

Corticosteroids (systemic) in previous 2 weeks

- Yes
- No

Antihistamine in previous

3 days (short-acting e.g. chlorpheniramine)

- Yes
- No

5 days (long acting e.g. cetirizine, fexofenadine)

- Yes
- No

Regular treatment with: systemic immunosuppressants, beta blockers, ACE inhibitor, antacid medication, antidepressant (tricyclic) or sedatives

- Yes
- No

Contraindication to the administration of adrenaline (e.g., ischaemic heart Disease, poorly controlled hypertension or cardiac arrhythmia)

- Yes
- No

Any clinically significant disease that can affect patient's safety or can make implementation of the protocol or interpretation of the results difficult, and has arisen subsequent to the screening visit?

- Yes
- No

Pregnancy (if applicable)

- Yes
- No

Date of last period (if applicable):|

Pregnancy test (dipstick) result, if applicable

- Positive
- Negative

Does the patient have rhinitis?

- Yes
- No

Score each symptom below 1 (mild) 2(moderate) 3 (severe) (Total score 12)

- Runny nose
- Sneeze
- Nasal itch
- Congestion

Does the patient have eczema

- Yes
- No

Patient oriented eczema measure [0-28]

Sleep details

Average number of hours sleep per night in 2 weeks prior to challenge? [0-12]

Has the patient received 3 hours sleep or less the night before the challenge?

- Yes
- No

If no, record how many hours of sleep the patient has had [0-10]

4. Pre-challenge examination

Baseline observations (Pre-Dose1): Time:

Temperature °C [36.0-42.0]

• If above 37.5 no challenge

Blood pressure (mmHg) systolic [60-200] diastolic [30-120]

Heart rate beats/minute [30-150]

Respiratory rate /minute [4-40]

SpO2 % [90-100]

Peak expiratory flow rate (PEFR) litres/minute [300-800]

% of predicted PEFR % [0-150]

• If less than 80% no challenge predicted

FEV1 litres/minute [2.0-5.5]

Percentage predicted % [0-150]

Vital signs stable (SO2, PEFR, BP, Pulse, respiratory rate)

- Yes
- No

Examination

If abnormal provide details

Oral cavity

Normal

Abnormal

Skin

- Normal
- Abnormal

Nasal passages

- Normal
- Abnormal

Respiratory system

- Normal
- Abnormal

Cardiovascular system

- Normal
- Abnormal

Gastrointestinal system

- Normal
- Abnormal

Room temperature °C [36.0-42.0]

5. Challenge Scheduling

DBPCFC to be rescheduled due to abnormal examination finding

- Yes
- No

6. Pre-Challenge Set-up

i.v. access

- Yes
- No

Emergency medications available in challenge room?

- Yes
- No

Challenge meal batch number and expiry date:

Challenge randomization code:

7. Challenge dose:

Any persistent symptoms from previous dose?

- Yes
- No

If yes which?

Pre-dose observations [DOSE]:

Temperature °C [36.0-42.0]

Blood pressure (mmHg) systolic [60-200] diastolic [30-120]

Heart rate beats/minute [30-150]

Respiratory rate /minute [4-40]

(PEFR) litres/minute [300-800]

SpO2 % [90-100]

Dose double checked

- Yes
- No

Time Dose given:

Whole dose ingested?

- Yes
- No

If no: specify ingested amount in g:

Water ingestion – specify volume

Symptoms after dose (Refer to key)	Time of Onset	Time of resolutio n	Examination findings	Treatment given
Drop down list from table 1				Drop down list from treatment list below

Whole exercise period undertaken?

- Yes
- No

If no please state how many minutes were undertaken [0-10]

Target heart rate maintained during exercise

- Yes
- No

Table 1 Symptom table

Symptom	Percentage area
Pruritus -Occasional scratching [Green]	
Pruritus- scratching continuously for >2 mins at a time [Green]	
Hard continuous scratching>excoriations [Yellow]	
Urticaria-<3 hives or mild lip oedema [Yellow]	
Urticaria- <10 hives ≥ 3or significant lip or face oedema [Red]	
Urticaria-generalised involvement [Red]	
Rash- Few areas of faint erythema [Green]	
Rash- Areas of erythema [Yellow]	
Rash- Generalised marked erythema>50% [Red]	

Upper respiratory [Total Nasal Symptom Score] 0-12	
Itching in inner ear canal [Green]	
Rare bursts of sneezing occasional sniffing [Green]	
I Bursts < 10, intermittent rubbing of nose, and/or eyes or frequent sniffing [Yellow]	
Continuous rubbing of nose and/or eyes, [Yellow]	
Periocular swelling and/or long bursts of sneezing, [Yellow]	
Persistent rhinorrhoea [Yellow]	
Throat tingling/altered sensation in throat [Green]	
> 3 discrete episodes of throat clearing or cough [Yellow]	
Persistent throat tightness [Yellow]	
Hoarseness or frequent dry cough [Red]	

Stridor [Red]	
Chest tightness without any fall in PEFR [Green]	
Chest tightness with a 10% fall in PEFR [Yellow]	
Chest tightness with a 20% fall in PEFR [Red]	
Expiratory or inspiratory wheeze [Red]	
Use of accessory muscles [Red]	
Oral itching [Green]	
Transient nausea [Green]	
Persistent nausea [Yellow]	
Transient abdominal pain [Green]	
Persistent abdominal pain [Yellow]	
Emesis/diarrhoea (1 episode) [Yellow]	

Emesis/diarrhoea (more than 1 episode) [Red]	
Cardiovascular	
Weak/dizzy or tachycardia [Yellow]	
Drop in BP and/or >20% from baseline [Red]	
Cardiovascular collapse/signs of impaired circulation [Red]	
Neurological	
Altered level of consciousness [Red]	

Stopping criteria applied?

- Yes
- No

If yes, tick the criteria used:

- Green symptoms >120 minutes
- Three or more yellow symptoms
- One red symptom
- Participant request

8. Post challenge

Day 1- Post last dose observations:

Time (hour/min)

Temperature °C [36.0-42.0]

Blood pressure (mmHg) systolic [60-200] diastolic [30-120]

Heart rate beats/minute [30-150]

Respiratory rate /minute [4-40]

SpO2 % [90-100]

Peak expiratory flow rate (PEFR) litres/minute [200-800] % predicted [0-150]

PEFR 20% drop litres/minute

FEV1 litres/minute [2-5.5] % predicted [0-150]

9. Treatment given during challenge

Oral antihistamine Dose 1 Dose 2 and time of doses

IV antihistamine Dose 1 and time of dose

IM adrenaline Dose 1 Dose 2 Dose 3 Dose 4 and time of doses

Nebulised adrenaline Dose 1 Dose 2 Dose 3 Dose 4 and time of doses

IV Saline bolus 1 Litre Dose 1 Dose 2 Dose 3 Dose 4 and time of administration

IV adrenaline infusion Dose 1 time infusion started

Other inotrope infusion Dose 1 time infusion started

High flow oxygen

- Yes
- No

Other treatment

10. Summary of observations during challenge

Record the peak symptom severity during the first 2 hours of the allergic reaction as below:

Lowest blood pressure recorded during reaction systolic [60-200] / diastolic [30-120]

Highest heart rate recorded during reaction /minute [30-150]

Lowest peak expiratory flow rate recorded during reaction litres/minute % [50-800]

Highest respiratory rate recorded during reaction /minute [4-40]

Lowest SaO2 recorded during reaction % [0-100]

Time to complete resolution of symptoms [hours] [0-72]

11. Disposal:

Home

Admitted to hospital

Where

Admitted to intensive care

Where

Treatment plan given?

- Yes
- No

12. Post-challenge examination

examination of oral cavity, skin, lung performed

- Yes
- No

withdraw i.v. access

- Yes
- No

Blood pressure (mmHg) systolic [60-200] diastolic [30-120]

Heart rate beats/minute [30-150]

* post-challenge PEF [200-800] (..... % of predicted) [0-150]

* post-challenge FEV1 [2-5.5] (...... % of predicted) [0-150] * assessment of either FEV1 or PEF

Outcome of Day 1 challenge

- Reactive
- Nonreactive
- Inconclusive

Late onset reactions

Did the patient report a late onset reaction after challenge day 1?

- Yes
- No
- Unknown

Did the patient report a late onset reaction after challenge day 2?

- Yes
- No

• Unknown

Keep a record of the late reactions together with the hard copy of the DBPCFC form in the CRF

Insert Day 2 (complete repeat of record)

13. Decryption of DBPCFC [link to randomisation]

Challenge of day 1

- Active
- Placebo
- Meal code

Challenge of day 2

- Active
- Placebo
- Meal code

Appendix 5: Karolinska Sleepiness Scale

Grade	Description
1	Extremely alert
2	Very alert
3	Alert
4	Rather alert
5	Neither alert not sleepy
6	Some signs of sleepiness
7	Sleepy, but no difficulty remaining awake
8	Sleepy, some effort to keep awake

Appendix 6: Per protocol results

Figure 1: Dose reached in mg of peanut protein by challenge (per protocol population)









Figure 3: Dose in mg of peanut protein reached by visit number (per protocol)

Figure 4: Log dose of peanut protein reached by visit number (per protocol)

