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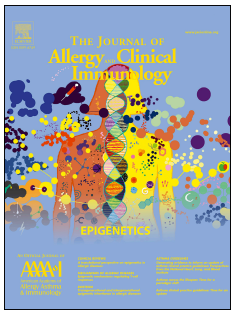

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PII: S0091-6749(19)30934-0

DOI: https://doi.org/10.1016/j.jaci.2019.06.038

Reference: YMAI 14082

To appear in: *Journal of Allergy and Clinical Immunology*

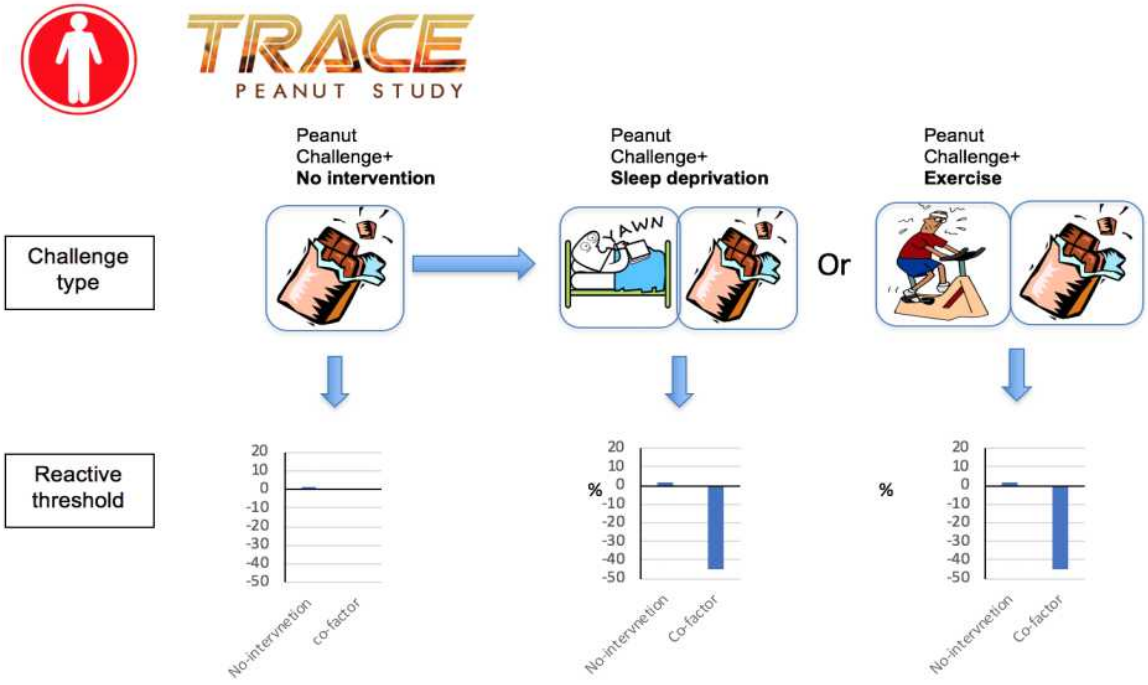
Received Date: 29 October 2018

Revised Date: 11 June 2019

Accepted Date: 17 June 2019

Please cite this article as: Dua S, Ruiz-Garcia M, Bond S, Durham SR, Kimber I, Mills C, Roberts G, Skypala I, Wason J, Ewan P, Boyle R, Clark A, The effect of sleep deprivation and exercise on reaction threshold in peanut-allergic adults: a randomised controlled study, *Journal of Allergy and Clinical Immunology* (2019), doi: https://doi.org/10.1016/j.jaci.2019.06.038.

Exercise and sleep deprivation independently reduce peanut reactive thresholds



The effect of sleep deprivation and exercise on reaction threshold in peanut-allergic adults: a randomised controlled study

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Disclosure statement

Funded by the Food Standards Agency who had no involvement in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. None of the authors had a conflict of interest relevant to this study.

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ABSTRACT

Background

Peanut allergy causes severe and fatal reactions. Current food allergen labelling fails to address these risks adequately against the burden of restricting food choice for allergic individuals because of limited data on thresholds of reactivity and the influence of everyday factors.

Objective

We estimated peanut threshold doses for a UK peanut-allergic population and examined the effect of sleep deprivation and exercise.

Method

In a crossover study, following blinded challenge, peanut-allergic participants underwent three open peanut challenges in random order: with exercise following each dose, with sleep deprivation preceding challenge, and with no intervention. Primary outcome was the threshold dose triggering symptoms (mg protein). Primary analysis estimated the difference

between non-intervention challenge and each intervention in log threshold (as % change). Dose distributions were modelled deriving eliciting doses in the peanut-allergic population.

Results

Baseline challenges were performed in 126 subjects, 100 were randomized and 81 (mean age 25y) completed at least one further challenge. 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. 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.

Conclusion

Exercise and sleep deprivation each significantly reduce the threshold of reactivity in people with peanut allergy, putting them at greater risk of a reaction. Adjusting reference doses using these data will improve allergen risk-management and labelling to optimize protection of peanut-allergic consumers.

ClinicalTrials.gov Identifier: NCT01429896

Keywords

Peanut, allergy, thresholds, exercise and sleep deprivation

Abbreviations

DBPCFC double blind placebo controlled food challenge

ED eliciting dose

ICSA interval censored survival analysis

IgE Immunoglobulin E

LOAEL lowest observed adverse effect level

PAL precautionary allergen labelling

Clinical implications

Exercise and sleep deprivation each individually lower reaction threshold by approximately half; this needs to be accounted for when defining reference doses for food labelling.

Capsule summary

We show that co-factors (sleep deprivation and exercise) cause a reduction in reactivity threshold to peanut by 45% and accounting for this variation in population threshold estimates will more accurately guide reference doses for allergen risk management..

Introduction

IgE-mediated peanut allergy is a significant public health concern, being the commonest cause of severe and sometimes fatal allergic reactions to food.(1,2) The current standard of care for the management of peanut allergy is complete avoidance of peanut(3) but this is difficult to achieve and inadvertent reactions are common. To assist peanut-allergic individuals in the safe management of their allergy, the presence of food allergen can be indicated on food labelling. The labelling of deliberately added allergens as ingredients is legally mandated in the European Union and United States. However, allergens can also accidentally contaminate foods during production methods and some manufacturers utilise voluntary precautionary allergen labelling (PAL), such as 'May contain traces of…', warning patients about allergen contamination. Studies show that PAL may bear no relationship to the presence of allergen, with some PAL labelled foods containing no allergen at all, and other unlabelled foods containing residual amounts of allergen.(4,5) These confusing and vague statements affect peanut-allergic individuals, restricting their food choices and impairing their quality of life.(6)

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,(7) but concerns remain about the general applicability of such levels and how they might be modified by everyday lifestyle factors (co-factors).(8) The involvement of sleep deprivation as a co-factor in modulating allergic reactions has so far relied on anecdotal

reports as well as retrospective surveys of individuals suffering from anaphylaxis which is subject to recall bias. There is good evidence that exercise may exacerbate allergic reactions to wheat and other foods although this has not been formally explored in relation to peanut(9),(10) There are also indications from peanut immunotherapy studies that co-factors may be responsible for a loss of tolerance during maintenance therapy.(11) Food challenges from which threshold data are derived are usually performed under 'ideal' test conditions that do not reflect everyday exposure conditions.(12) Furthermore, 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 co-factors, exercise and sleep deprivation.

Methods

Trial design

A multicentre randomised crossover 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). Following confirmation of allergy by a double-blind, placebo-controlled (DBPC) peanut challenge (baseline challenge), eligible participants underwent three further open peanut challenges in a randomly assigned, balanced order: one with exercise, one with sleep deprivation on the night preceding the challenge and one with neither intervention (termed non-intervention).

Participants

Participants were recruited from the UK general adult peanut-allergic population both nationally (through advertisements in the media and through national patient support groups, (Anaphylaxis Campaign and Allergy UK)) and locally (allergy clinics and local media). Interested participants registered on the study website where they were asked initial screening questions about their allergy. Eligible participants underwent telephone screening. If they fulfilled criteria on pre-screening, they were invited for face to face screening visit. 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. Sensitisation was defined as a positive skin prick test to peanut (extract ALK-Abello, Hørsholm, Denmark), skin weal of ≥3mm greater than the negative control or serum specific IgE to peanut >0.35 kUA/L (ImmunoCAP). Volunteers were excluded if they gave a history suggestive solely of oral allergy syndrome to peanut (a different, milder disorder). They were also excluded if

they had previous life-threatening reactions to peanut, poorly controlled asthma, a significant drop in lung function with exercise or a diagnosis of mastocytosis. A full list of inclusion and exclusion criteria is included in the supplementary material (Table E1).

The study was approved by the national research ethics (NRES) committee East of England (12 EE02/89) and performed with each participant's written informed consent. The UK Food Standards Agency funded the study.

Randomisation

The baseline challenge consisted of one active peanut and one placebo challenge on separate days, at an interval of one week. The order of these challenges was randomly assigned, and both the participant and investigator were blinded to the order. Participants then underwent three further challenges at three-month intervals in a randomised open fashion. Two of the challenges were interventional; one combined with exercise and one following sleep deprivation prior to the day of the challenge. A third challenge with no intervention was also undertaken, and termed the 'non-intervention' challenge. The randomised challenge sequence for each patient was determined using a secure online tool with audit trail (randomizer.au). Randomization was to one of six blocks containing all permutations of challenge combinations. Randomization was stratified according to centre, age and presence of asthma.

Food challenges

Prior to the commencement of challenges participants were physically examined and the control of co-existent atopic conditions was assessed using the Asthma Control Test(13) and spirometry for asthma, the POEM score for eczema(14) and Total Nasal Symptom score for rhinitis. Challenges were postponed if these conditions were inadequately controlled or if the patient was unwell with an infective illness. The challenges were undertaken using a

harmonised protocol in accordance with best practice where participants ingested increasing doses of the validated Europrevall dessert food matrix(12) 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). 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. During the active and intervention challenges participants consumed increasing doses of peanut protein in the form of partially defatted peanut flour in a challenge matrix. The dosing regimen was: 3µg, 30µg, 300µg, 3mg, 30mg, 100mg, 300mg and 1g peanut protein (1 gram peanut protein is equivalent to approximately 8 peanuts) (15). The doses were delivered at 30-minute intervals although the investigator could extend the interval to a maximum of 1 hour if symptoms were evolving. A dose could be repeated if the participant was nearing their threshold and the investigator deemed it appropriate not to escalate by a full dose increment. Challenges were performed in a harmonised manner across both centres using a common approach to score and stop challenges with site training. Using a modified version of the PRACTALL criteria (11), symptoms were assigned a green, yellow or red colour code (Table E2). Challenges were stopped when participants reached an objective threshold of 3 concurrent yellow symptoms or one red symptom. After piloting the established PRACTALL challenge criteria on 6 participants (data not shown) it was decided by Trial Steering Committee consensus that further refinement of the criteria was needed to enhance safety. Greater discrimination was added to lower respiratory symptoms defining milder airway symptoms and peak flow was incorporated as a functional measurement to detect rapid progression to severe symptoms. Gastrointestinal symptoms were also further defined in terms of their persistence (>/=30minutes). Detail on the modification of the criteria are shown in Table E2. Participants who developed symptoms were given treatment as

appropriate. The intervention challenges were run in the same way, and modified as follows. The exercise challenge regimen, optimised during pilot testing, consisted of a 10-minute bout of exercise on a static bike at an intensity of 85% VO_2 max (maximum exercise capacity, determined during screening) 5 minutes after each dose. In London the investigator supervised the challenge and exercise, whereas in Cambridge the investigator supervised the challenge and a physiologist supervised the exercise. For the sleep deprivation challenge, participants were admitted to the research ward on the night preceding the challenge and were allowed to sleep for a maximum of 2 hours and then kept awake until the challenge. Prior to the challenges, participants were encouraged to keep a sleep diary and if they had experienced a disruption to their normal sleep pattern (<30% normal sleep in the two weeks preceding the challenge) appointments were postponed. The non-intervention challenge was run in exactly the same way as the initial challenges, except that, like the Interventional Challenges, the challenge was open, with only one 'active' challenge taking place (see Protocol Changes section).

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 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- $ED_{x\%}$). The number and type of adverse events were reported. A summary of terms and their definitions are detailed in Table I.

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 in a separate manuscript.

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 post-baseline challenges were also performed (data not shown). The extended analysis set consisted of all patients who received a baseline challenge. The safety population consisted of all participants who underwent at least one challenge.

Sample size

As there were no published data on intra-individual variation in thresholds over time from repeat challenges, we considered different scenarios (described in protocol), with power assessed by simulation. In the most conservative scenario investigated (within-person correlation=0.5 and variance=4), 72 participants would mean 80% power (5% two-sided significance level) to detect a minimum change in threshold (logged) of -0.9 (i.e. a 60% reduction in threshold from baseline).

Protocol changes

The initial protocol specified blinded food challenge (DBPC) for all challenges. However, in view of the complexity of the protocol and excessive time burden on participants a decision was made by the Trial Steering Committee to change to open challenges for the final three challenges for each participant. Eighteen blinded challenges with interventions were performed, and a sensitivity analysis showed no difference in threshold between challenges with and without placebo.

Statistical analyses

All analyses were planned prospectively and detailed in a statistical analysis plan. The primary outcome was expressed as a mean (SD). The primary analysis estimated the difference in log-threshold between the non-intervention challenge and each intervention challenge (exercise and sleep deprivation) using a linear mixed-effects model along with 95% confidence interval and p-value for whether the difference in log threshold was significant. Changes in threshold were also expressed as percentage change. Fixed effects included the challenge type (exercise, sleep deprivation, with non-intervention as reference), age, sex, order of challenge, baseline log threshold, presence of asthma, centre and baseline Ara h 2. non-intervention. . For the secondary outcome of constructing the population threshold curves, a parametric interval-censored survival analysis method described by Taylor(16) was used. The threshold values were included as interval censored data between the threshold dose one below and at which the reaction occurred. Thresholds were expressed as cumulative doses unless otherwise specified. If a participant reacted on the first dose of the challenge the data was left censored at the first dose. If no reaction took place for any dose the data were right censored at the final dose. The Survival package (‘survreg’ function in ’R’)(17) was used to fit log-normal, log-logistic and Weibull distributions. The model that fitted the data best according to the Akaike information criterion was chosen. The model was used to find the eliciting dose (and 95% CI) predicted to provoke reactions in different proportions (as percentage) of the peanut-allergic population ($EDx_{\%}$). For example, the ED_{10} is the dose which provokes a reaction in 10% of the peanut-allergic population. For the baseline population threshold curve the extended analysis population was used which included all participants who underwent a baseline challenge (excluding those who were subsequently determined to be non-allergic). All other population threshold curves were based on the full analysis population.

Results

Participants

We screened 222 participants aged 18-45y (Figure 1). Of these, 123 underwent both baseline challenges and 100 participants were randomised to undergo the interventional challenges (median 25.0 years F:53). The most frequent reason for non-randomisation was tolerance of all challenge doses (14 subjects) and hence inability to identify a threshold, with other reasons being severity of reactions, non-compliance and quota of randomised patients already being complete (9). During placebo challenges the majority of symptoms experienced by participants were mild green symptoms or infrequently yellow symptoms usually abdominal pain or persistent nausea occurring in isolation however none of these symptoms met the stopping criteria in any participant. The baseline characteristics of the randomised participants are listed in Table II. The full analysis population completed at least one post-baseline challenge and consisted of 81 participants. Sixty-four participants completed all three post-baseline challenges (per-protocol set) (Data not shown).

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 (n=81). 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 2 and Table III. No patient reacted on the first dose (3µg protein), therefore there were no left-censored participants.

Secondary outcomes: Threshold distribution modelling for peanut

Full analysis population

The mean (95% confidence interval) eliciting doses for the full-analysis population during non-intervention challenge were ED_1 = 1.5mg (0.8,2.5), ED_5 = 4.0mg (2.4,6.4) and ED_{10} = 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 3). Thus, during exercise or sleep deprivation challenges, participants reacted at a lower dose than when no intervention was applied. For example, the ED_1 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 4; Table IV).

Extended analysis population

The dose distribution curve for the extended analysis population, which included all individuals who received a baseline challenge, is shown in Figure 5 (n=123). The mean (95% confidence interval) eliciting doses were ED_1 =1.3 mg (0.8,2.0), ED_5 =3.8mg (2.4,5.7) and ED_{10} =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 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.

Covariates

No significant effects on threshold were observed for other variables including presence of asthma, sex, age, or IgE against Ara h 2 levels (Table III and Figure 2).

There was a trend towards reduction in threshold for each successive intervention visit 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.

A post-hoc descriptive responder analysis was undertaken on those the participants who undertook an exercise and non-intervention challenge or sleep deprivation and non-intervention challenge (n=66) (Figure 6).

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 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.

Safety

There was a single serious adverse reaction, one patient was admitted overnight following a challenge after developing hypotension and required two doses of adrenaline and intravenous fluids. 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

We have defined a mean reactivity threshold of 214mg peanut protein for an individual, approximately equivalent to one peanut(15), 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.

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. Eliciting dose values for 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. Our estimate for ED_{10} (6.7mg) was higher when compared to some other previous estimates, which range from 0.7-4.42mg(18)(19)(20)(21) (22) . Although some studies have often used subjective symptoms as stopping criteria leading to lower threshold estimates(19), many have not(21,23) .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(23).

We show for the first time that co-factors lower the reactivity threshold in allergic reactions. Sleep deprivation may exert its effect at least partly through a stress response affecting the immune and gastrointestinal systems. In animal models of inflammatory bowel disease, stress results in enhanced intestinal permeability (24,25) potentially associated with a significant increase in permeability of the epithelium to macromolecules, which may account for the reduction in threshold. Similarly under-perfusion of the gut may occur during exercise leading to ischaemia with resultant damage to tight junction integrity and increased permeability to food allergens. (26) Co-factors such as exercise, alcohol and non-steroidal anti-inflammatory drugs, are increasingly being implicated in food anaphylaxis. (27)

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. Further, 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. Current allergen risk assessment by food industry and regulators involves defining an eliciting dose (e.g. ED_1 or ED_5) representing an exposure that is likely to be safe for the population. Hourihane et al have recently validated the proposed ED_5 for peanut of 1.5mg peanut protein by performing single dose peanut challenges on 378 children and observed that only 8 participants (2.1%) experienced objective symptoms (all mild), only half of whom required treatment with oral antihistamines(7). Further studies are required to validate proposed ED_5 and ED_1 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 ED_1, has been proposed by the VITAL group. (8) 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 a safety factor can be many magnitudes smaller. 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.

The safety data in this trial show that the overall adrenaline use across all challenges was 15%, 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(28) and Lieberman in 9% of positive food challenges.(29) The use of multiple doses of adrenaline was infrequent, and only occurred in 2% of challenges.

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.(20)

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 did not differ significantly from those participants with histories of milder reactions.(21) Our study population had a low average age of 25 years. Fatal anaphylaxis episodes occur more commonly in this age group (30) perhaps due to more risk taking behaviour(31), thus in defining a threshold for the whole population, it is of benefit that the model is based on this age group.

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. (32) All analyses were adjusted for centre.

Another potential weakness was the use of 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 co-factors 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, 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.

This study was commissioned by the UK Food Standards Agency (FSA), UK Government.

Acknowledgements

The authors thank the members of the study Trial Steering Committee: Moira Austin, Phillipa Cauldwell, Victoria Cornelius, Hazel Gowland, Laura Pasea, Steve Till and Yvonne King for their assistance in study design and study oversight, the study team extend particular thanks to Dr Chris Palmer for statistical help during the design of the study. In addition the study team are grateful to the members of the TRACE IDMC: Tina Dixon, David Luyt, Abdel Douri, Gillian Vance for reviewing patient safety and data, the Anaphylaxis Campaign for

their assistance with study design and patient recruitment and providing a lay member for the Trial Steering Committee, Sabita Islam, Loraine Foley and Frankie Saunders for their help with challenge meal preparation, all members of the Cambridge NIHR/Wellcome Trust Clinical Research Facility and the Royal Brompton and Harefield Biomedical Research Unit, staff at the University of Manchester in particular Matthew Machin and Kathleen Haigh-Hutchinson for their assistance with data management and the database build and Carol-Ann Costello for her help with challenge meal production and supply, Paul Turner and finally the Food Standards Agency, in particular, Chun-Han Chan, Joelle Buck, Sarah Hardy, Sue Hattersley, Paul Tossell, Ruth Willis and Ross Yarham.

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Figure 1: 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

Figure 2: Percentage change in threshold (logged) for each covariate. Full-analysis population n=81. Visits 1-3 refer to the chronological order of post-baseline challenge days. LOAEL = lowest observed adverse effect level is the reactive threshold in mg peanut protein during baseline challenge

Figure 3: 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 4: 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.

Figure 5: 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.

Figure 6 Descriptive analysis of participants whose dose threshold increased, decreased or remained the same following exercise and sleep deprivation (n=66). Numbers show percentage of participants in each group (of a total of n=66 who undertook an exercise and non-intervention challenge, or sleep deprivation and non-intervention challenge). This was a post –hoc analysis therefore no statistical test was applied.

Table I: Terms and their definitions

Term	Definition
Primary Outcome	Peanut threshold or lowest observed adverse effect level (LOAEL) which is the lowest cumulative dose causing an objective allergic reaction. Determined for each individual in mg peanut protein following each challenge
Primary Analysis	Difference in log-threshold between non-intervention challenge and each intervention challenge also expressed as percentage change
Secondary outcome	*Eliciting dose (EDx) or population threshold* cumulative eliciting dose (ED) predicted to provoke a reaction in a defined proportion of the population (x)
Full analysis population	Individuals who received at least one post baseline intervention challenge
Extended analysis population	All individuals who received a baseline peanut challenge
Baseline challenge	Initial double-blind placebo-controlled challenge to confirm diagnosis of peanut allergy
Non-intervention challenge	Open challenge to determine threshold when no intervention applied
Intervention challenge	Open challenge to determine threshold with either exercise or sleep deprivation intervention

Table II: 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)
$V0_2$ max (ml/min/kg)	34.5 (11)	34 (10)
Peanut specific IgE (kU_A/L)	30 (34)	31.6 (35)
Ara h 2 specific IgE (kU_A/L)	20.6 (28)	21.3 (29)
FEV_1 (l)	3.9 (0.8)	3.9 (0.78)
FEV_1 (l, % 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 (l/min)	511.8 (110)	506.7 (110)

Table III: Estimated effect shown in log and percentage scale, 95% confidence interval and p-value for each term in the linear mixed effects model. Full-analysis population, n=81. Visits 1-3 refer to the chronological order of post-baseline 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)**	**CI**	**Estimate (absolute change in %)**	**CI**	**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
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

Table IV: Predicted dose (and 95% CI) 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)
ED1	3 (1.7,4.8)	1.5 (0.8,2.5)	0.5 (0.2,0.8)	0.3 (0.1,0.6)
ED5	7.6 (4.7,12)	4 (2.4,6.4)	1.3 (0.7,2.2)	1.1 (0.5,1.7)
ED10	12.8 (8.2,19.8)	6.7 (4.1,10.5)	2.4 (1.4,3.8)	1.9 (1.1,3.1)
ED50	80.6 (57.9,112)	44.6 (30.8,64.5)	20.4 (12.9,31.9)	19.7 (12,32)
ED80	255 (180.2,360.8)	156.2 (103.5,235.5)	101.8 (58.4,176.9)	123.6 (65.3,233.3)
ED95	715.9 (441.9,1159.4)	502 (276.9,909.3)	537 (223.6,1287.6)	894.7 (308.4,2592.2)

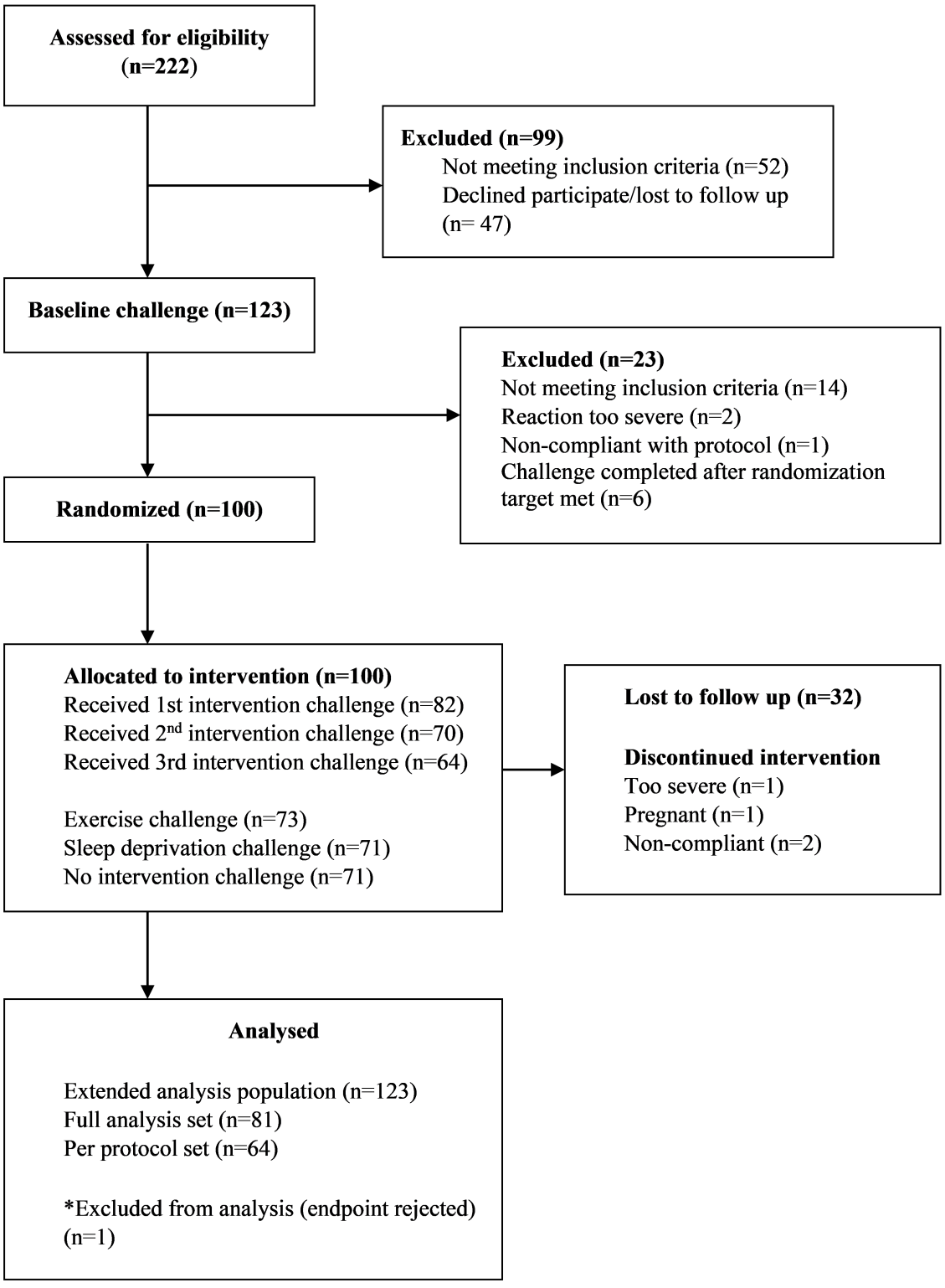
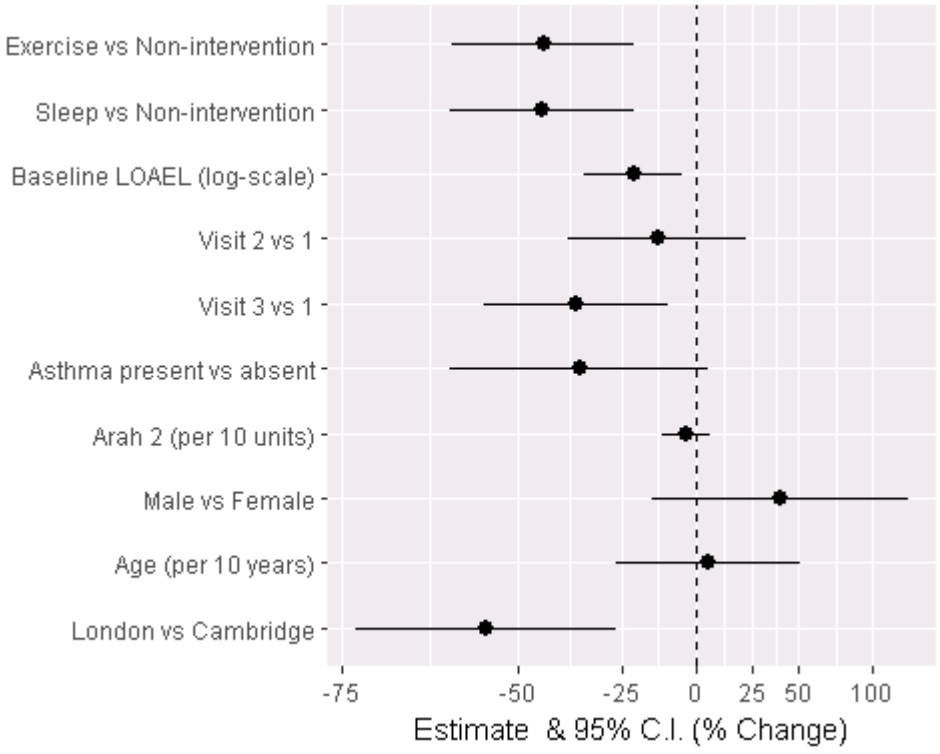
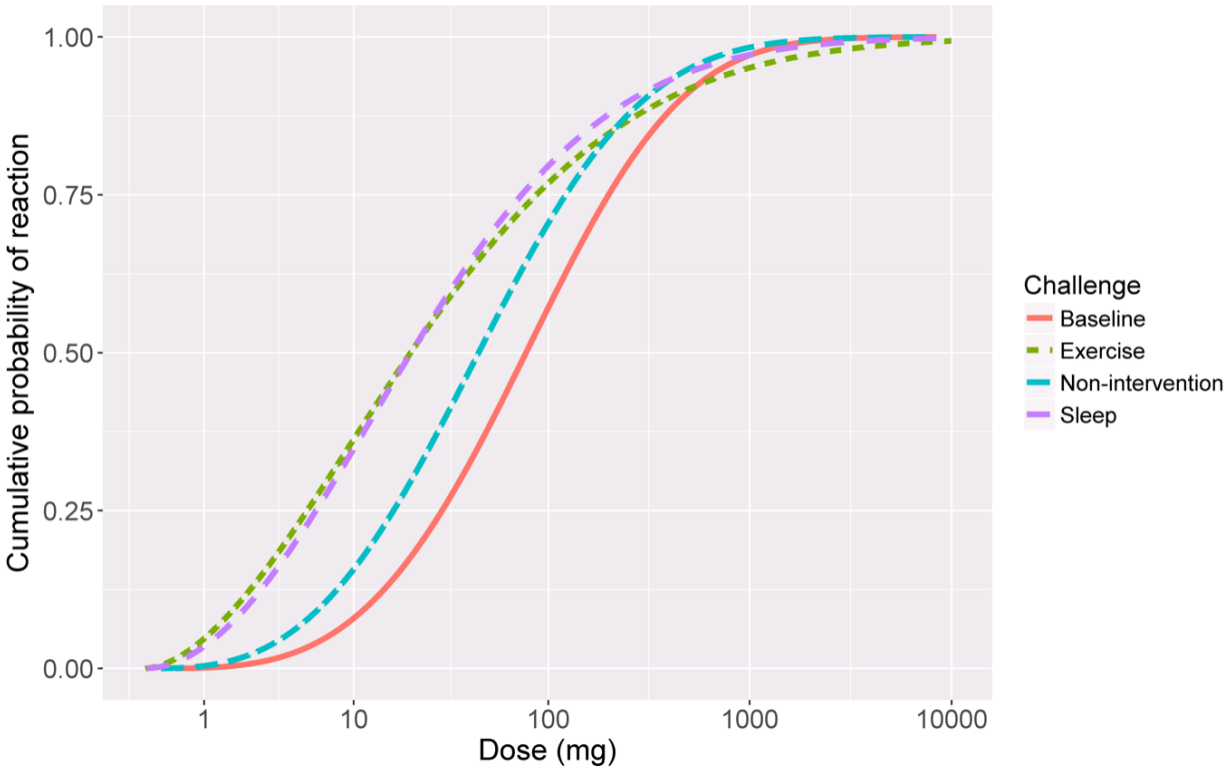
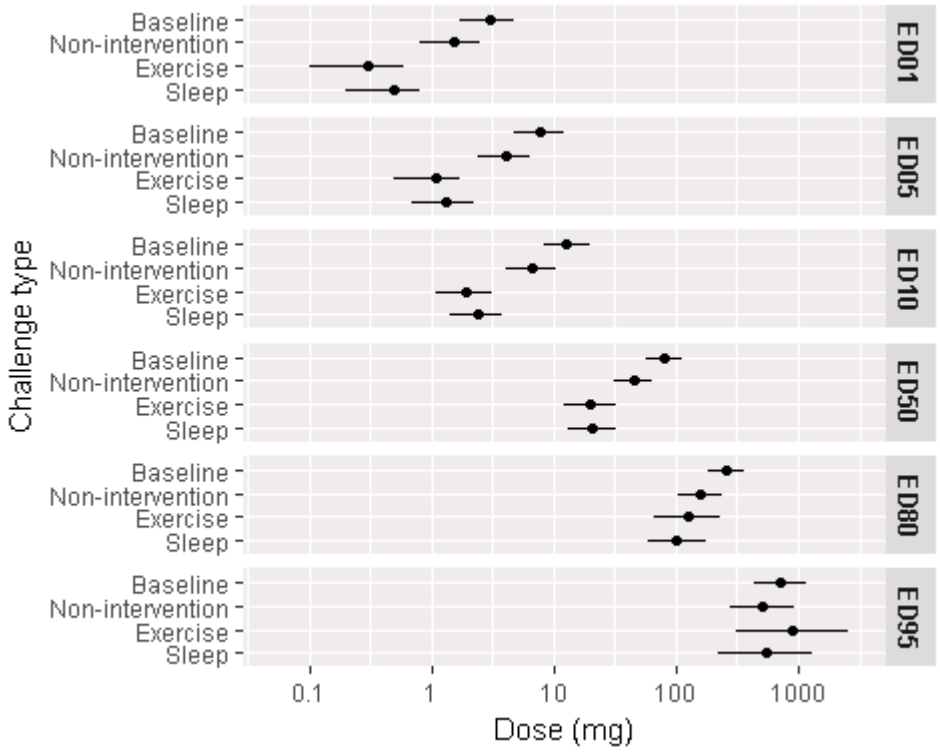
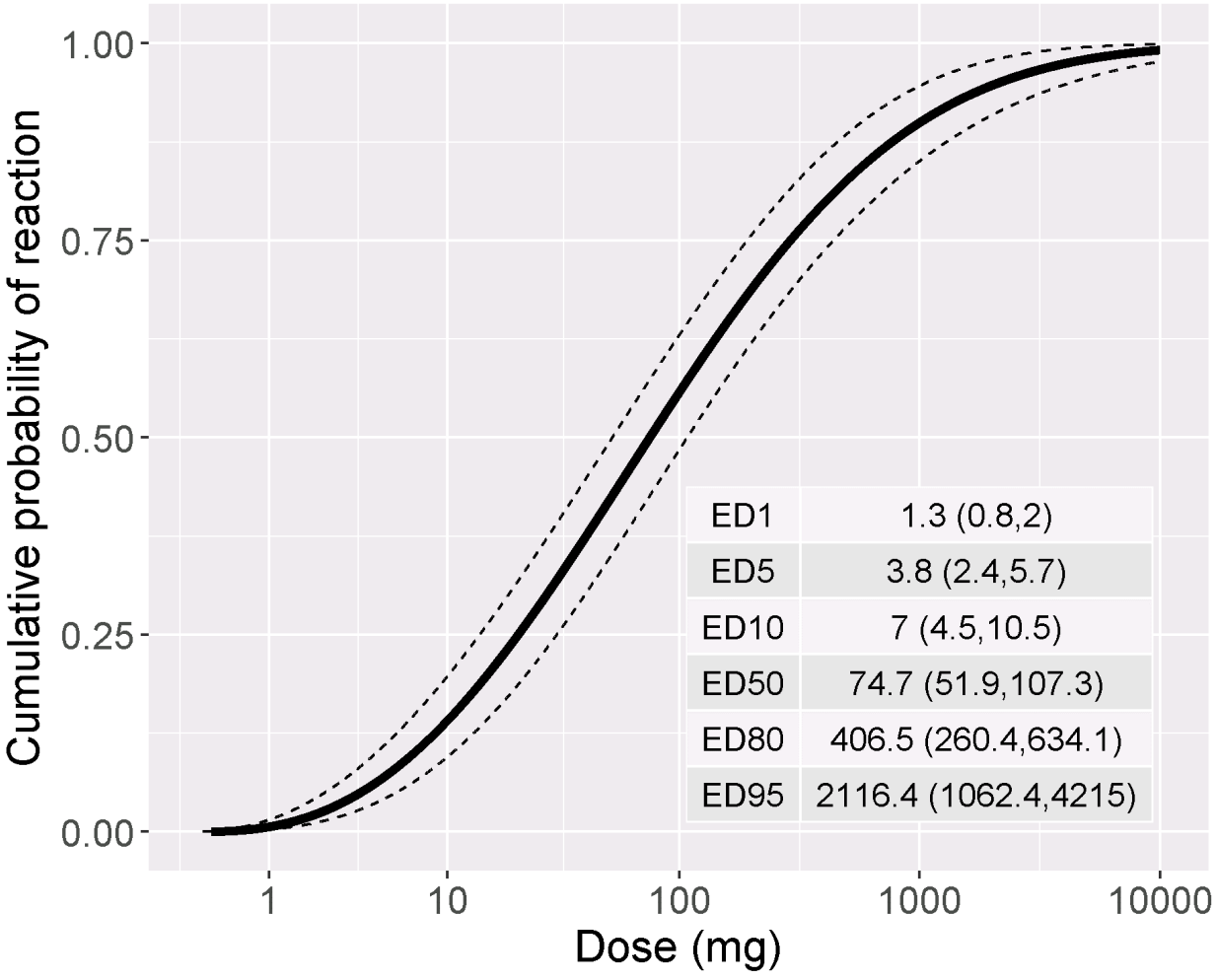

Assessed for eligibility (n=222)
Excluded (n=99)
Not meeting inclusion criteria (n=52)
Declined participate/lost to follow up (n= 47)
Baseline challenge (n=123)
Excluded (n=23)
Not meeting inclusion criteria (n=14)
Reaction too severe (n=2)
Non-compliant with protocol (n=1)
Challenge completed after randomization target met (n=6)
Randomized (n=100)
Allocated to intervention (n=100)
Received 1st intervention challenge (n=82)
Received 2nd intervention challenge (n=70)
Received 3rd intervention challenge (n=64)
Exercise challenge (n=73)
Sleep deprivation challenge (n=71)
No intervention challenge (n=71)
Lost to follow up (n=32)
Discontinued intervention
Too severe (n=1)
Pregnant (n=1)
Non-compliant (n=2)
Analysed
Extended analysis population (n=123)
Full analysis set (n=81)
Per protocol set (n=64)
*Excluded from analysis (endpoint rejected) (n=1)

Figure 1









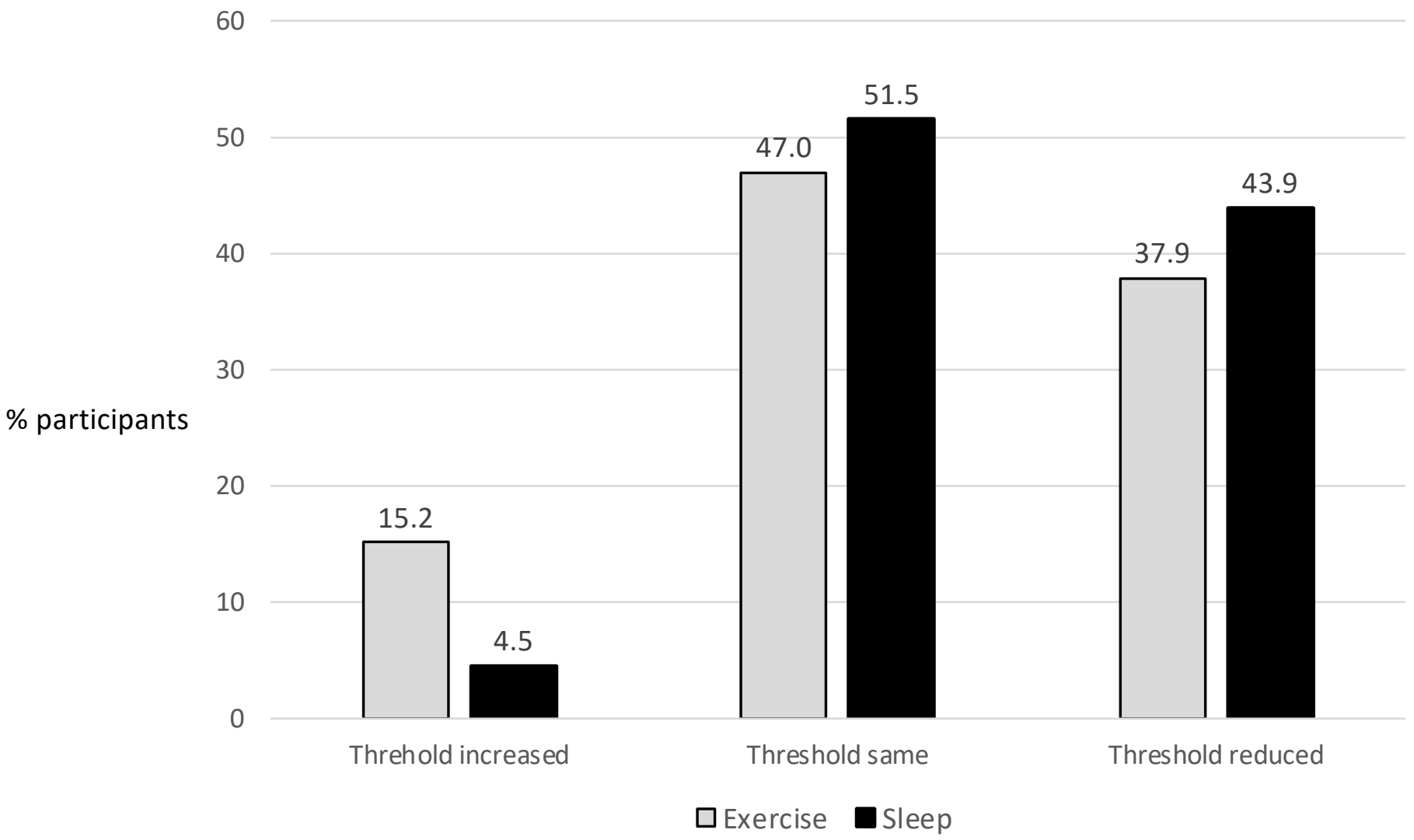


Table E1: Study inclusion and exclusion criteria

Inclusion criteria

- Male and female subject who are 18-45 years of age at the time of study entry (Visit 1) who have a diagnosis of acute 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 is defined as the onset of objective or significant subjective allergic events after ingestion of peanut protein but not to the placebo. Eligibility to the DBPCFC requires fulfillment of all other eligibility criteria at visit 1.
- Subjects must be able to comply with the study procedures.
- 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 pr-10 homologues of peanut (Ara h 8), and low level serum IgE to Ara h 1, 2, 3).
- Mono-sensitisation to Ara h 9
- Use of investigational drugs at the time of enrolment, or within 30 days or 5 half lives of enrolment, whichever is longer.
- History of hypersensitivity to any of the matrix components used within the material for the OFC.
- Poorly controlled asthma. Asthma control will be assessed by the Asthma Control Questionnaire (ACQ). Patients with a score <20 or higher won't be eligible for the study. Also, patients should have FEV1 >80% of their predicted value.
- 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
- Musculo-skeletal disease which in the opinion of the investigator could impair the participants ability to perform the exercise challenge
- A sleep or psychiatric disorder which in the opinion of the investigator could impair the participants ability to perform the study procedures
- Pregnancy
- Alcohol or drug misuse
- Night-shift worker

- Concomitant use of
    - systemic immunosuppressant.
    - beta blocker use.
    - ACE inhibitor or other hypertensive drug use
    - sedative drugs
    - antacid medication (either proton pump inhibitors or H2-antagonists)
- 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 (or Trial Management Group) was life-threatening
    - mastocytosis
    - coronary artery disease
    - eosinophilic oesophagitis
    - gastric or duodenal ulcer
- A past medical history of clinically significant ECG abnormalities or identified during study (Visit1)
- Recent (within the last three (3) years) and/or recurrent history of autonomic dysfunction (e.g., recurrent episodes of fainting, palpitations, etc.).
- Hematological parameters (total WBC count or Hb level, platelet counts) that fall outside the normal reference range of the laboratory at screening and are clinically significant.

Table E2: Modification and explanation of existing PRACTALL CRITERIA

Existing PRACTALL CRITERIA	Modified PRACTALL CRITERIA	Explanation of modification made
Mild, occasional scratching [Green] Moderate -scratching continuously for > 2 minutes at a time [Green] Severe hard continuous scratching excoriations [Yellow]	Pruritus -Occasional scratching [Green] 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] Severe generalized involvement [Red]	Urticaria-<3 hives or mild lip oedema [Yellow] Urticaria- <10 hives ≥ 3or significant lip or face oedema [Red] Urticaria-generalised involvement [Red]	
Mild few areas of faint erythema [Green] Moderate areas of erythema [Yellow] Severe generalized marked erythema (>50%) [Red]	Rash- Few areas of faint erythema [Green] Rash- Areas of erythema [Yellow] Rash- Generalised marked erythema>50% [Red]	
Mild rare bursts, occasional sniffing [Green] Moderate bursts < 10, intermittent rubbing of nose, and/or eyes or frequent sniffing [Yellow] Severe continuous rubbing of nose and/or eyes, periocular swelling and/or long bursts of sneezing, persistent rhinorrhea [Red]	Itching in inner ear canal [green] Rare bursts of sneezing occasional sniffing [green] 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 l ong bursts of sneezing, [Yellow] Persistent rhinorrhoea [Yellow]	Itching in inner ear canal was added as it was a common mild symptom identified by many patients during piloting. Rhinitis symptoms downgraded from red to yellow. These were not regarded by the study team as severe enough symptoms singly to warrant stopping challenge.
Mild expiratory wheezing to auscultation [Red] Moderate inspiratory and	Chest tightness without any fall in PEFR [Green] Chest tightness with a <10% fall in PEFR [green]	In the existing Practall criteria study team felt that there needed to be representation of milder respiratory symptoms as

expiratory wheezing [Red] Severe use of accessory muscles, audible wheezing [Red]	Chest tightness with a 10-20% fall in PEFR [yellow] Chest tightness with a >20% fall in PEFR [red] Expiratory or inspiratory wheeze [Red] Use of accessory muscles [Red]	the existing criteria escalate too rapidly to wheeze which is a clear objective symptoms. Therefore to enhance safety and 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] Stridor [Red]	Mild oropharyngeal symptoms added Definition of persistence added and defined as symptom present for ≥30 minutes
Mild complaints of nausea or abdominal pain, itchy mouth/throat [Yellow] Moderate frequent c/o nausea or pain with normal activity [Yellow] Severe - notably distressed due to GI symptoms with decreased activity [Yellow] Objective Mild 1 episode of emesis or diarrhea [Yellow] 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]	Oral itching [Green] Transient nausea [green] Transient abdominal pain [green] Persistent nausea [yellow] Persistent abdominal pain [yellow] Emesis/diarrhoea (1 episode) [Yellow] Emesis/diarrhoea (more than 1 episode) [Red]	Milder and transient abdominal symptoms downgraded Incorporated duration of abdominal symptoms as a marker of severity. Persistent defined as symptom present ≥30 minutes
Mild-subjective response (weak, dizzy), or tachycardia [Yellow] moderate-drop in blood	Weak/dizzy or tachycardia [Yellow] Drop in BP and/or >20% from baseline [Red]	

pressure and/or >20% from baseline, or significant change in mental status. severe-cardiovascular collapse, signs of impaired circulation (unconscious) [Red]	Cardiovascular collapse/signs of impaired circulation [Red] Altered level of consciousness [Red]	