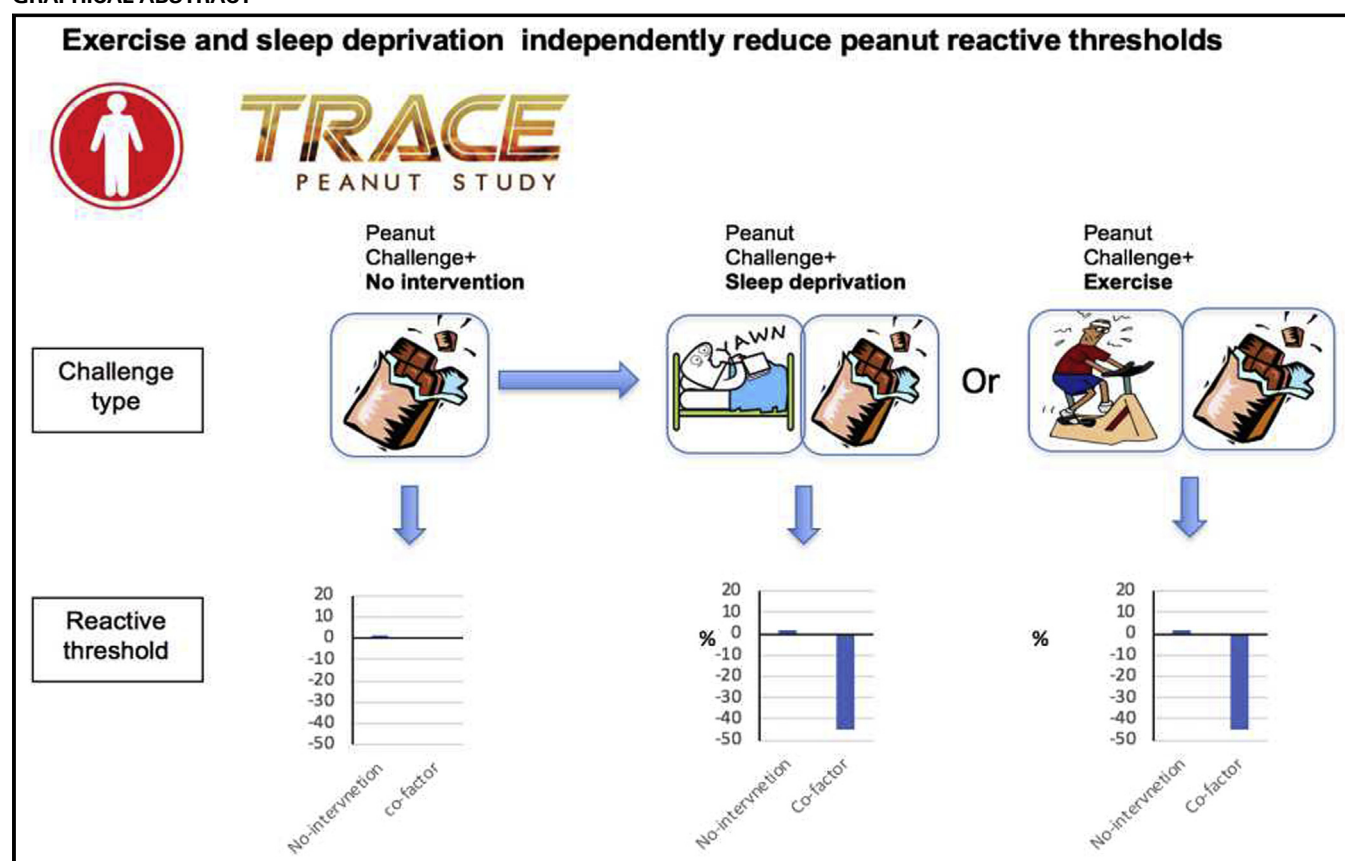


# Effect of sleep deprivation and exercise on reaction threshold in adults with peanut allergy: A randomized controlled study

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## GRAPHICAL ABSTRACT



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**Background:** Peanut allergy causes severe and fatal reactions. Current food allergen labeling does not address these risks adequately against the burden of restricting food choice for allergic patients because of limited data on thresholds of reactivity and the influence of everyday factors.

**Objective:** We estimated peanut threshold doses for a United Kingdom population with peanut allergy and examined the effect of sleep deprivation and exercise.

**Methods:** In a crossover study, after blind challenge, participants with peanut allergy underwent 3 open peanut challenges in random order: with exercise after each dose, with sleep deprivation preceding challenge, and with no intervention. Primary outcome was the threshold dose triggering symptoms (in milligrams of protein). Primary analysis estimated the difference between the nonintervention challenge and each intervention in log threshold (as percentage change). Dose distributions were modeled, deriving eliciting doses in the population with peanut allergy.

**Results:** Baseline challenges were performed in 126 participants, 100 were randomized, and 81 (mean age, 25 years) completed at least 1 further challenge. The mean threshold was 214 mg (SD, 330 mg) for nonintervention challenges, and this was reduced by 45% (95% CI, 21% to 61%;  $P = .001$ ) and 45% (95% CI, 22% to 62%;  $P = .001$ ) for exercise and sleep deprivation, respectively. Mean estimated eliciting doses for 1% of the population were 1.5 mg (95% CI, 0.8–2.5 mg) during nonintervention challenge ( $n = 81$ ), 0.5 mg (95% CI, 0.2–0.8 mg) after sleep, and 0.3 mg (95% CI, 0.1–0.6 mg) after exercise.

**Conclusion:** Exercise and sleep deprivation each significantly reduce the threshold of reactivity in patients with peanut allergy, putting them at greater risk of a reaction. Adjusting reference doses using these data will improve allergen risk management and labeling to optimize protection of consumers with peanut allergy. (J Allergy Clin Immunol 2019;■■■:■■■–■■■.)

**Key words:** Peanut, allergy, thresholds, exercise and sleep deprivation

IgE-mediated peanut allergy is a significant public health concern, being the most common cause of severe and sometimes fatal allergic reactions to food.<sup>1,2</sup> The current standard of care for the management of peanut allergy is complete avoidance of peanut,<sup>3</sup> but this is difficult to achieve, and inadvertent reactions are common.

To assist patients with peanut allergy in the safe management of their allergy, the presence of food allergen can be indicated on food labeling. The labeling 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 use voluntary precautionary allergen labeling (PAL), such as “May contain traces of...,” warning patients about allergen contamination. Studies show that PAL might bear no relationship to the presence of allergen, with some PAL-labeled foods containing no allergen at all and other unlabeled foods containing residual amounts of allergen.<sup>4,5</sup> These confusing and vague statements affect patients with peanut allergy, restricting their food choices and impairing their quality of life.<sup>6</sup>

#### Abbreviations used

DBPC: Double-blind, placebo-controlled  
ED: Eliciting dose  
PAL: Precautionary allergen labeling  
UK: United Kingdom

The identification of reference doses for food allergens considered safe for the majority of patients with food allergy 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 that elicit reactions in allergic patients have been performed, and attempts have been made using dose distribution modeling 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,<sup>7</sup> but concerns remain about the general applicability of such levels and how they might be modified by everyday lifestyle factors (cofactors).<sup>8</sup> The involvement of sleep deprivation as a cofactor in modulating allergic reactions has thus far relied on anecdotal reports, as well as retrospective surveys of patients with anaphylaxis, which is subject to recall bias. There is good evidence that exercise can exacerbate allergic reactions to wheat and other foods, although this has not been formally explored in relation to peanut.<sup>9,10</sup> There are also indications from peanut immunotherapy studies that cofactors might be responsible for a loss of tolerance during maintenance therapy.<sup>11</sup> Food challenges from which threshold data are derived are usually performed under “ideal” test conditions that do not reflect everyday exposure conditions.<sup>12</sup> Furthermore, the effects of cofactors have not been investigated in a prospective study. If cofactors can affect the threshold dose at which allergic reactions are elicited, then there is a need to account for this in population threshold modeling. 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 2 important cofactors: exercise and sleep deprivation.

## METHODS

### Trial design

A multicenter randomized crossover study was performed between 2013 and June 2016 at the National Institute for Health Research/Welcome Trust Cambridge Clinical Research Facility (Cambridge, United Kingdom) and the Royal Brompton & Harefield NHS Foundation Trust Clinical Research Facility (London, United Kingdom). After confirmation of allergy by using a double-blind, placebo-controlled (DBPC) peanut challenge (baseline challenge), eligible participants underwent 3 further open peanut challenges in a randomly assigned and balanced order: one with exercise, one with sleep deprivation on the night preceding the challenge, and one with neither intervention (termed nonintervention).

### Participants

Participants were recruited from the United Kingdom (UK) general adult population with peanut allergy 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 Web site, where they were asked initial screening questions about their allergy. Eligible participants underwent

telephone screening. If they fulfilled criteria on prescreening, they were invited for a face-to-face screening visit.

Participants were included in the study if they were aged 18 to 45 years with a history of an immediate systemic allergic reactions after peanut ingestion with evidence of sensitization to peanut and a diagnosis confirmed by a positive DBPC peanut challenge result. Sensitization was defined as a positive skin prick test response to peanut extract (ALK-Abelló, Hørsholm, Denmark), a skin wheal of 3 mm or larger than that elicited by the negative control, or a serum specific IgE level to peanut of greater than 0.35 kU<sub>A</sub>/L (ImmunoCAP; Phadia, Uppsala, Sweden). Volunteers were excluded if they provided 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 decrease in lung function with exercise, or a diagnosis of mastocytosis. A full list of inclusion and exclusion criteria is included in Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).

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

## Randomization

The baseline challenge consisted of 1 active peanut and 1 placebo challenge on separate days at an interval of 1 week. The order of these challenges was randomly assigned, and both the participant and investigator were blind to the order. Participants then underwent 3 further challenges at 3-month intervals in a randomized open fashion. Two of the challenges were interventional: one combined with exercise and one after sleep deprivation before the day of the challenge. A third challenge with no intervention was also undertaken and termed the nonintervention challenge. The randomized challenge sequence for each patient was determined by using a secure online tool with an audit trail ([randomizer.au](http://randomizer.au)). Randomization was to one of 6 blocks containing all permutations of challenge combinations. Randomization was stratified according to center, age, and presence of asthma.

## Food challenge

Before commencement of challenges, participants were physically examined, and control of coexistent atopic conditions was assessed by using the Asthma Control Test<sup>13</sup> and spirometry for asthma, the Patient Oriented Eczema Measure score for eczema,<sup>14</sup> and the 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. Challenges were undertaken with a harmonized protocol in accordance with best practice in which participants ingested increasing doses of the validated EuroPrevall dessert food matrix<sup>12</sup> either alone (placebo) or containing peanut allergen (active, 12.5% fat, light-roast peanut flour; Golden Peanut Company, Alpharetta, Ga) until they had an objective allergic reaction (definition below). An unblinded scientist with no interaction with the participants or the study team was responsible for the randomization of participants 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 as follows: 3 µg, 30 µg, 300 µg, 3 mg, 30 mg, 100 mg, 300 mg, and 1 g of peanut protein (1 g of peanut protein is equivalent to approximately 8 peanuts).<sup>15</sup> 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 participants were nearing their thresholds and the investigator deemed it appropriate not to escalate by a full dose increment.

Challenges were performed in a harmonized manner across both centers by using a common approach to score and stop challenges with site training. Using a modified version of the PRACTALL criteria,<sup>11</sup> symptoms were assigned a green, yellow, or red color code (see Table E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Challenges were stopped when participants reached an objective threshold of 3 concurrent yellow symptoms or 1 red symptom. After piloting the established PRACTALL challenge criteria on 6 participants (data not shown), it was decided by Trial Steering Committee

**TABLE I.** Terms and their definitions

Term	Definition
Primary outcome	Peanut threshold or lowest observed adverse effect level (LOAEL), which is defined as the lowest cumulative dose causing an objective allergic reaction determined for each participant in milligrams of peanut protein after each challenge
Primary analysis	Difference in log-threshold between nonintervention challenge and each intervention challenge also expressed as percentage change
Secondary outcome	Eliciting dose (ED <sub>x</sub> ) or population threshold: cumulative ED predicted to provoke a reaction in a defined proportion of the population (x)
Extended analysis population	All participants who received a baseline peanut challenge
Baseline challenge	Initial DBPC challenge to confirm diagnosis of peanut allergy
Nonintervention challenge	Open challenge to determine threshold when no intervention applied
Intervention challenge	Open challenge to determine threshold with either exercise or sleep deprivation intervention

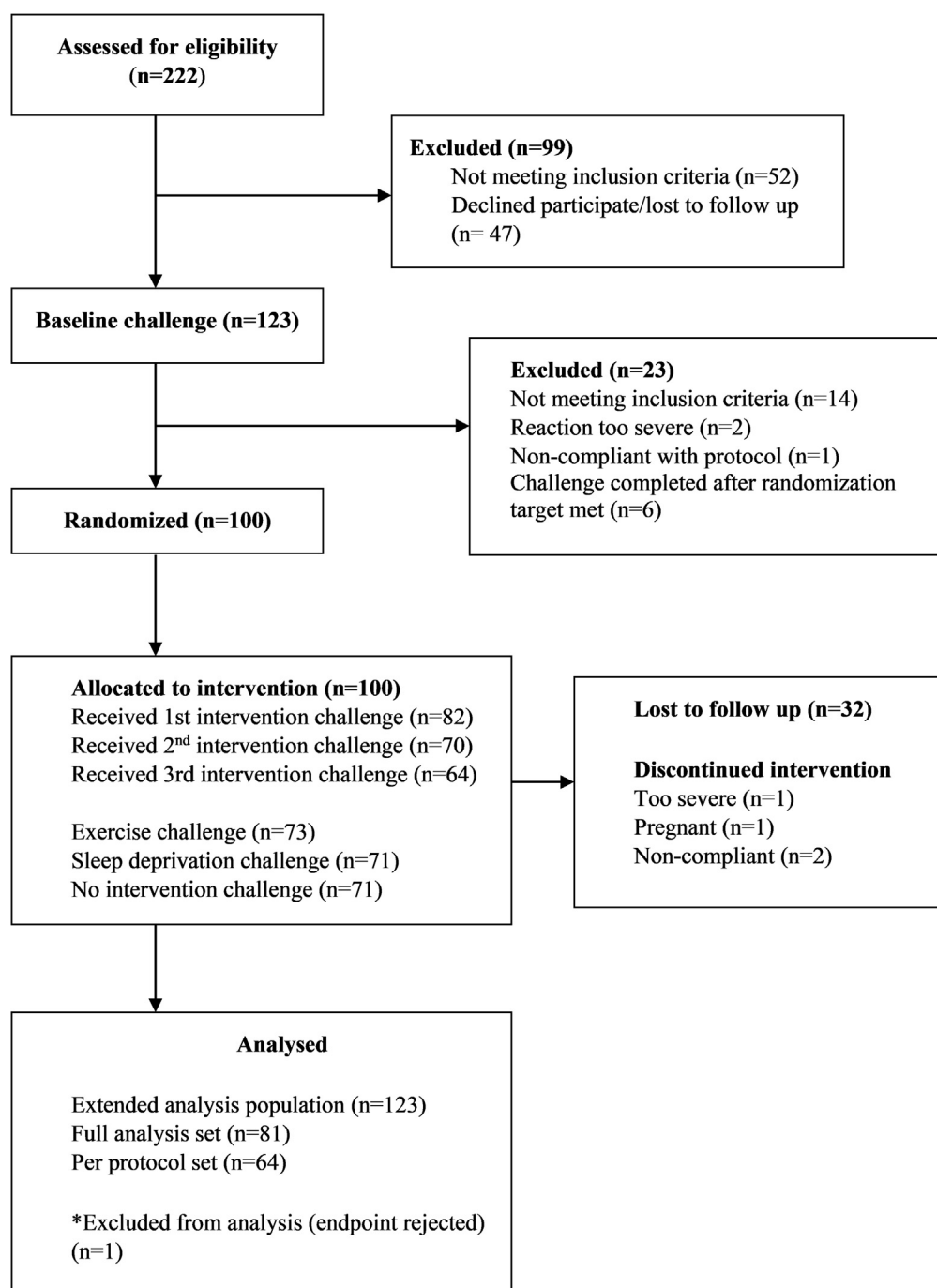
consensus that further refinement of the criteria was needed to enhance safety. Greater discrimination was added to lower respiratory symptoms defining milder airway symptoms, and peak flow was incorporated as a functional measurement to detect rapid progression to severe symptoms. Gastrointestinal symptoms were also further defined in terms of their persistence ( $\geq 30$  minutes). Details on the modification of the criteria are shown in Table E2. Participants with symptoms were given treatment as appropriate.

The intervention challenges were run in the same way and modified as follows. The exercise challenge regimen, which was optimized during pilot testing, consisted of a 10-minute bout of exercise on a static bike at an intensity of 85% maximal oxygen intake (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 allowed to sleep for a maximum of 2 hours and then kept awake until the challenge. Before 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 2 weeks preceding the challenge), appointments were postponed. The nonintervention 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 the "Protocol changes" section).

## Outcomes

The primary outcome was the peanut threshold in each participant (or dose triggering symptoms), which was defined as the lowest observed adverse effect level, the lowest cumulative dose that causes an objective allergic reaction (defined below). This was measured in milligrams of peanut protein and summarized by challenge type and timing of challenge. As secondary outcomes, threshold dose distribution curves were derived for the different challenge types, and probability distribution modeling was used to determine population thresholds, the cumulative dose of peanut protein predicted to provoke reactions in different percentages of the population with peanut allergy (eliciting dose [ED]<sub>x%</sub>). The number and type of adverse events were reported. A summary of terms and their definitions are detailed in Table I.



**FIG 1.** CONSORT diagram. \*One analysis was excluded after review on the grounds that it had been stopped prematurely, resulting in a full analysis population of 81 participants.

Reaction severity was not measured as a preplanned 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 article.

### Analytic populations

The primary analytic population was the full analysis set, which was defined as all participants who had completed at least 1 postbaseline challenge. Analyses on the per-protocol population, which was defined as participants

who completed all 3 postbaseline 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 1 challenge.

### Sample size

Because there were no published data on intraindividual variation in thresholds over time from repeat challenges, we considered different scenarios (described in protocol), with power assessed by means of simulation. In the



most conservative scenario investigated (within-person correlation, 0.5; variance, 4), 72 participants would mean 80% power (5% two-sided significance level) to detect a minimum change in threshold (logged) of  $-0.9$  (ie, a 60% reduction in threshold from baseline).

## Protocol changes

The initial protocol specified DBPCs 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 3 challenges for each participant. Eighteen blind 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 nonintervention challenge and each intervention challenge (exercise and sleep deprivation) by using a linear mixed-effects model along with a 95% CI and *P* value for whether the difference in log threshold was significant. Changes in threshold were also expressed as percentage change. Fixed effects included challenge type (exercise and sleep deprivation, with nonintervention as reference), age, sex, order of challenge, baseline log threshold, presence of asthma, center, and baseline Ara h 2 level.

For the secondary outcome of constructing the population threshold curves, a parametric interval-censored survival analysis method described by Taylor et al<sup>16</sup> was used. Threshold values were included as interval-censored data between the threshold dose 1 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 were 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 software)<sup>17</sup> was used to fit log-normal, loglogistic, 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 ED (and 95% CI) predicted to provoke reactions in different proportions (as percentages) of the population with peanut allergy ( $ED_{x\%}$ ). For example, the  $ED_{10}$  is the dose that provokes a reaction in 10% of the population with peanut allergy. 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 nonallergic). All other population threshold curves were based on the full analysis population.

## RESULTS

### Participants

We screened 222 participants aged 18 to 45 years (Fig 1). Of these, 123 underwent baseline challenges, and 100 participants were randomized to undergo interventional challenges (median, 25.0 years; female participants, 53). The most frequent reason for nonrandomization was tolerance of all challenge doses (14 participants) and hence inability to identify a threshold, with other reasons being severity of reactions, noncompliance, and quota of randomized patients already being complete.<sup>9</sup> 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 randomized participants are listed in Table II. The full analysis population completed at least 1 postbaseline challenge and consisted of 81 participants. Sixty-four participants completed all 3 postbaseline challenges (per-protocol set, data not shown).

**TABLE II.** Baseline characteristics for study populations

Characteristic	All randomized (n = 100)	Full analysis set (n = 81)
Age (y)	24.7 (6.6)	25.2 (7)
Female sex	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)
Maximal oxygen intake (mL/min/kg)	34.5 (11)	34 (10)
Peanut-specific IgE (kU <sub>A</sub> /L)	30 (34)	31.6 (35)
Ara h 2-specific IgE (kU <sub>A</sub> /L)	20.6 (28)	21.3 (29)
FEV <sub>1</sub> (L)	3.9 (0.8)	3.9 (0.78)
FEV <sub>1</sub> (L, % predicted)	105.8 (12)	106 (13)
No. of historical reactions	8.6 (3.4)	8.7 (3.5)
Baseline LOAEL (mg of protein)	304 (410)	330.1 (420)
PEFR (L/min)	511.8 (110)	506.7 (110)

For binary variables, numbers and percentages (in parentheses) are shown; for continuous variables, means and SDs (in parentheses) are shown.

LOAEL, Lowest observed adverse effect level; PEFR, peak expiratory flow rate; SPT, skin prick test.

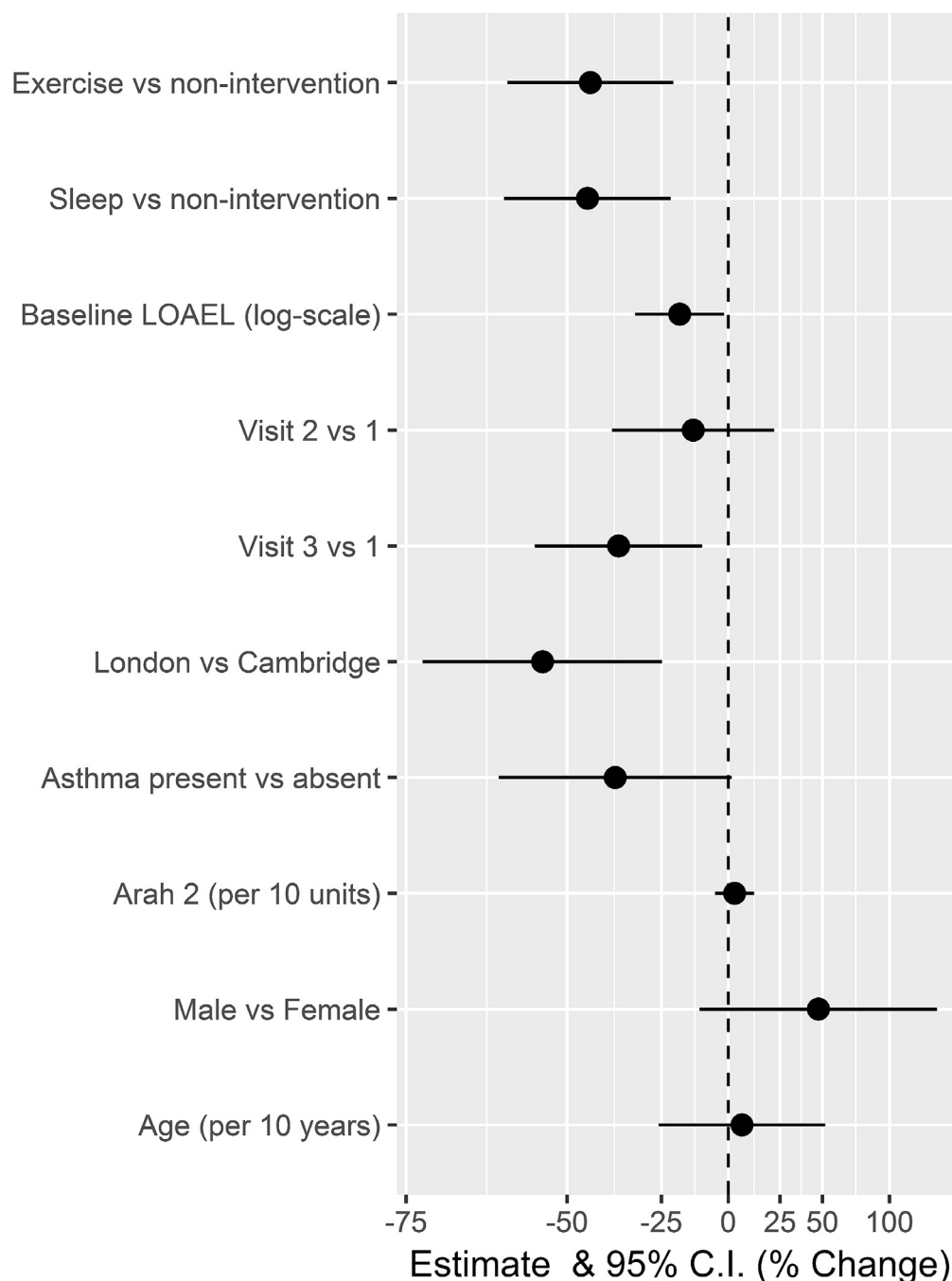
### Primary outcome: Peanut thresholds and the effect of cofactors

The mean cumulative threshold for baseline challenges was 330 mg (SD, 424 mg) of peanut protein for the full analysis population, 191 mg (SD, 358 mg) for exercise challenges, 157 mg (SD, 300 mg) for sleep deprivation challenges, and 214 mg (SD, 330 mg) for nonintervention challenges (*n* = 81). When assessing the effect of each intervention on threshold, the estimated change in (natural) log threshold for the sleep deprivation challenge compared with the nonintervention challenge was  $-0.61$  (95% CI,  $-0.97$  to  $-0.25$ ; *P* = .0011), and that for the exercise challenge was  $-0.60$  (95% CI,  $-0.95$  to  $-0.24$ ; *P* = .0013). Both changes equate to a reduction in threshold of 45%, as shown in Fig 2 and Table III. No patient reacted on the first dose (3  $\mu$ g of protein), and therefore there were no left-censored participants.

### Secondary outcomes: Threshold distribution modeling for peanut

**Full analysis population.** Mean EDs for the full analysis population during nonintervention challenge were an  $ED_1$  of 1.5 mg (95% CI, 0.8-2.5 mg), an  $ED_5$  of 4.0 mg (95% CI, 2.4-6.4 mg), and an  $ED_{10}$  of 6.7 mg (95% CI, 4.1-10.5 mm) of peanut protein, respectively. Compared with the threshold dose distribution curves for nonintervention challenges, the curves for exercise and sleep deprivation were significantly different and shifted to the left (Fig 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 1.5 mg (95% CI, 0.8-2.5 mg), that for sleep deprivation was 0.5 mg (95% CI, 0.2-0.8 mg), and that for exercise was 0.3 mg (95% CI, 0.1-0.6 mg). The effect was most pronounced at lower EDs but not noticeable at higher EDs ( $ED_{50}$ - $ED_{95}$ , Fig 4 and Table IV).

**Extended analysis population.** The dose distribution curve for the extended analysis population, which included all



**FIG 2.** Percentage change in threshold (logged) for each covariate. The full analysis population included 81 participants. Visits 1 to 3 refer to the chronological order of postbaseline challenge days. The lowest observed adverse effect level (LOAEL) is the reactive threshold in milligrams of peanut protein during baseline challenge.

participants who received a baseline challenge, is shown in [Fig 5](#) ( $n = 123$ ). Mean EDs were an ED<sub>1</sub> of 1.3 mg (95% CI, 0.8-2.0 mg), an ED<sub>5</sub> of 3.8 mg (95% CI, 2.4-5.7 mg), and an ED<sub>10</sub> of 7 mg (95% CI, 4.5-10.5 mg) of peanut protein. Fourteen participants did not reach challenge-stopping criteria during baseline challenge, and therefore their data were right censored at the maximum dose. An independent expert reviewed their cases, and based on their history, sensitization patterns and challenge symptoms deemed that they were clinically allergic, with likely

thresholds greater than 1 g of protein. Therefore they were included in the extended analysis population but excluded from randomization.

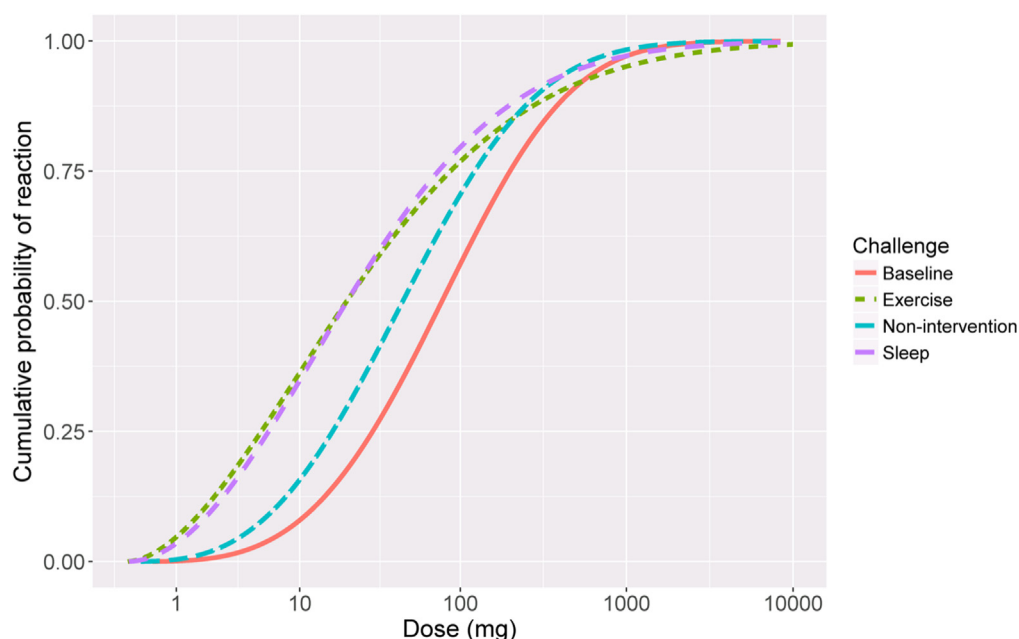
**Covariates.** No significant effects on threshold were observed for other variables, including presence of asthma, sex, age, or IgE to Ara h 2 ([Fig 2](#) and [Table III](#)).

There was a trend toward reduction in threshold for each successive intervention visit, which became significant only for the third postbaseline challenge versus the first postbaseline

**TABLE III.** Estimated effect shown in log and percentage scale, 95% CI, and *P* value for each term in the linear mixed-effects model

Variables	Estimate (log scale)	95% CI	Estimate (absolute change in percentage)	95% CI	<i>P</i> value
Baseline LOAEL (log scale)	−0.244	−0.436 to −0.052	−22	−35 to −5	.014
Nonintervention	Reference				
Exercise	−0.596	−0.953 to −0.239	−45	−61 to −21	.0013
Sleep	−0.599	−0.959 to −0.239	−45	−62 to −21	.0013
Postbaseline visit 1	Reference				
Postbaseline visit 2	−0.148	−0.497 to 0.2	−14	−39 to +22	.40
Postbaseline visit 3	−0.469	−0.83 to −0.107	−37	−56 to −10	.011
Cambridge	Reference				
London	−0.820	−1.33 to −0.309	−56	−74 to −27	.002
No asthma at baseline	Reference				
Asthma at baseline	−0.456	−0.963 to 0.051	−37	−62 to +5	.077
Ara h 2 (per 10 units)	−0.039	−0.133 to 0.055	−4	−12 to +6	.41
Female	Reference				
Male	0.332	−0.173 to 0.838	+39	−16 to +131	.19
Age (per 10 y)	0.050	−0.308 to 0.408	+5	−27 to +50	.78

The full analysis population included 81 participants. Visits 1 to 3 refer to the chronological order of postbaseline challenge days. The lowest observed adverse effect level (LOAEL) is the reactive threshold in milligrams of peanut protein during baseline challenge. Estimates for binary variables indicate the modeled difference from the reference category in log LOAEL (and absolute percentage change). Estimates for continuous variables (Ara h 2, age, and baseline LOAEL) indicate the modeled change in log LOAEL per 1-unit increase.

**FIG 3.** Threshold dose distribution model. Doses are given in milligrams of peanut protein per challenge type, showing the cumulative probability of reacting against the dose of peanut protein in milligrams. The full analysis population included 81 participants.

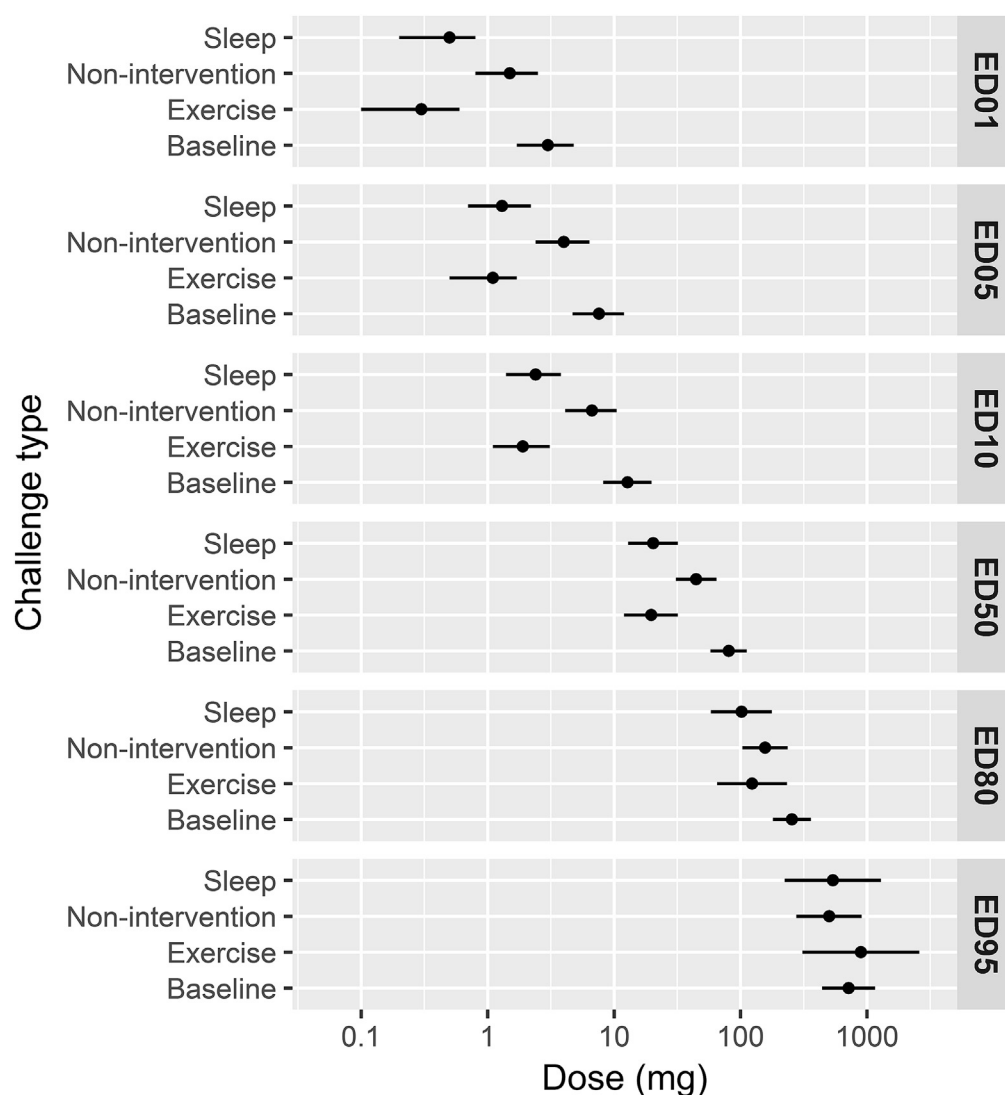
challenge (threshold [logged] =  $-0.47$ ; 95% CI,  $-0.83$  to  $-0.11$ ;  $P = .011$ ).

A *post hoc* descriptive responder analysis was undertaken on those participants who undertook an exercise and nonintervention challenge or sleep deprivation and nonintervention challenge ( $n = 66$ , Fig 6).

A significant effect of center was also observed. Compared with Cambridge, London participants had a lower threshold (logged) across postbaseline challenges. In particular, with regard to exercise, a marginally nonsignificant difference in effect of

exercise challenge versus nonintervention was observed between centers (threshold [logged] =  $-0.78$ ; 95% CI,  $-1.59$  to  $0.03$ ;  $P = .061$ ). However, the exercise versus nonintervention point estimate was consistent with the overall estimate (ie, the direction of effect was the same within each center). Overall, a threshold-decreasing effect of both interventions was seen independently at both sites. Prespecified analysis of the primary outcome was adjusted for both site and challenge order.

**Safety.** There was a single serious adverse reaction: 1 patient was admitted overnight after a challenge and after having



**FIG 4.** ED estimates (in milligrams of peanut protein) derived from the threshold distribution curve. Means (95% CIs) by challenge type for EDs for 1%, 5%, 10%, 50%, 80%, and 95% of the full analysis population are shown. The full analysis population included 81 participants.

**TABLE IV.** Predicted dose (95% CI) that produces a different probability of reactions

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

The full analysis set included 81 participants.

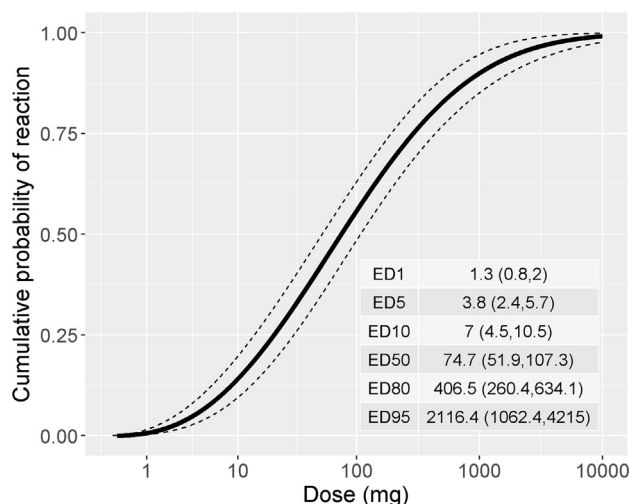
ED<sub>x</sub>, Dose that produces x% probability of reaction.

hypotension and required 2 doses of adrenaline and intravenous fluids. Intramuscular adrenaline was delivered in 52 (15%) of 342 challenges. Two doses of intramuscular adrenaline were delivered to stabilize the participants in 6 (2%) of 342 challenges. Nebulized adrenaline was administered in 3 (1%) of 342 challenges.

## DISCUSSION

We have defined a mean reactivity threshold of 214 mg of peanut protein for a participant, which is approximately equivalent to 1 peanut,<sup>15</sup> and have demonstrated that both exercise and sleep deprivation caused a 45% reduction in a participant's threshold. To our knowledge, these findings provide the first





**FIG 5.** Dose distribution curve for the extended analysis population ( $n = 123$ ) with 95% CIs. The dose is in milligrams of peanut protein. EDs in milligrams with 95% CIs for 1%, 5%, 10%, 50%, 80%, and 95% of the extended analysis population are shown as an inset table.

systematically generated data on peanut allergy thresholds in a UK adult population with peanut allergy and the first prospectively collected data to show that cofactors significantly reduce allergic thresholds in patients with peanut allergy.

To determine a population threshold, we used threshold dose distribution modeling to estimate the amounts of peanut protein that would elicit a reaction in 1%, 5%, and 10% of the population with peanut allergy. These EDs were 1.5, 4, and 6.7 mg of peanut protein, respectively. ED values for the extended analysis population were not significantly different, even when including the right-censored participants 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 participants with milder phenotypes and have excluded participants with a history of anaphylaxis. Our estimate for ED<sub>10</sub> (6.7 mg) was greater when compared with some other previous estimates, which range from 0.7 to 4.42 mg.<sup>18–22</sup> Although some studies have often used subjective symptoms as stopping criteria, leading to lower threshold estimates,<sup>19</sup> many have not.<sup>21,23</sup> The most likely explanation for the greater ED<sub>10</sub> values in this study is the use of more robust stopping criteria in our study, in which 3 concurrent objective symptoms were required to stop the challenge and establish the threshold. Klemans et al,<sup>23</sup> who used threshold data derived from diagnostic food challenges, estimated an ED<sub>10</sub> of 13.7 mg (95% CI, 4.37–42.8 mg) of peanut protein in adults, although the CIs were wide.

For the first time, we show that cofactors decrease the reactivity threshold in allergic reactions. Sleep deprivation can 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<sup>24,25</sup> potentially associated with a significant increase in permeability of the epithelium to macromolecules, which might account for the reduction in threshold. Similarly, underperfusion of the gut can occur during exercise, leading to ischemia with resultant damage to tight junction integrity and increased permeability to food allergens.<sup>26</sup> Cofactors, such as exercise,

alcohol, and nonsteroidal anti-inflammatory drugs, are increasingly being implicated in food-induced anaphylaxis.<sup>27</sup>

This study is the first to establish population EDs for peanut when participants are deliberately subjected to the cofactors of sleep deprivation and exercise. Furthermore, we are able to relate these to a reference threshold when no cofactor (nonintervention) is applied to calculate the magnitude of the effect. Current allergen risk assessment by the food industry and regulators involves defining an ED (eg, ED<sub>1</sub> or ED<sub>5</sub>) representing an exposure that is likely to be safe for the population. Hourihane et al<sup>7</sup> have recently validated the proposed ED<sub>5</sub> for peanut of 1.5 mg of peanut protein by performing single-dose peanut challenges on 378 children and observed that only 8 (2.1%) participants experienced objective symptoms (all mild), only half of whom required treatment with oral antihistamines. Further studies are required to validate proposed ED<sub>5</sub> and ED<sub>1</sub> doses, particularly in the adult population. The food industry can then use these validated EDs to develop guidelines for the use of voluntary precautionary food labeling (reference doses). Previously, a reference dose of 0.2 mg of peanut protein, based on the ED<sub>1</sub>, was proposed by the VITAL group.<sup>8</sup> However, the group acknowledge in their study that further application of an uncertainty or safety factor to this reference dose might be necessary to account for individual factors that could affect this dose estimate.

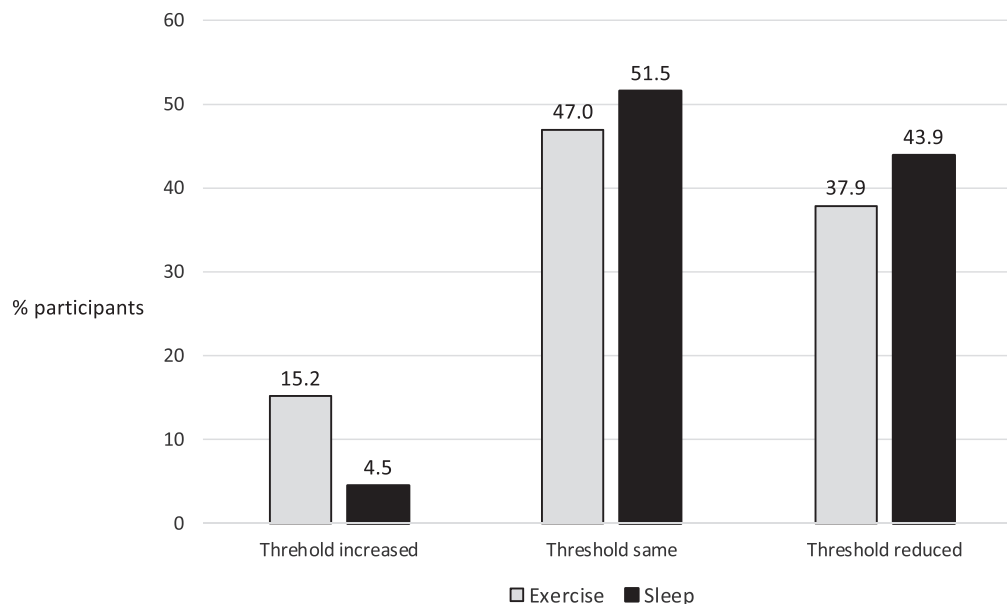
Because of a paucity of clinical data, the application of safety factors has traditionally followed toxicology practice, accounting for (10-fold) interspecies (for thresholds defined in nonhuman models) and (a further 10-fold) intraindividual variation in response. In practice, such large safety factors result in very low reference doses that, being near or less than 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 overcautious food labeling.

We show in this study that a safety factor can be many magnitudes smaller. Sleep deprivation decreased the ED<sub>1</sub> from 1.5 mg (for the nonintervention dose distribution) to 0.5 mg; this is equivalent to applying a safety factor of 0.33 to the ED<sub>1</sub> calculated from the nonintervention dose distribution. Similarly, exercise decreases the ED<sub>1</sub> from 1.5 mg (nonintervention) to 0.3 mg equivalent to a safety factor of 0.2. The derivation of reference doses that use evidence-based safety factors, such as those provided by our study, will enhance the allergen risk assessment process. This should encourage better industry engagement, with evidence-based voluntary food labeling reducing excessive and overly cautious PAL and provide allergic consumers with greater assurance that foods without PAL are safe for the majority to consume.

The safety data in this trial show that overall adrenaline use across all challenges was 15%, broadly reflecting the rate of adrenaline use in positive food challenges in other studies. Järvinen et al<sup>28</sup> reported its use in 11% of positive food challenge results, and Lieberman et al<sup>29</sup> reported its use in 9% of positive food challenge results. 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 sex. 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.<sup>20</sup>

A potential limitation of this study is that our ED estimate is based on a volunteer population with peanut allergy. Although



**FIG 6.** Descriptive analysis of participants whose dose threshold increased, decreased, or remained the same after exercise and sleep deprivation ( $n = 66$ ). Numbers show percentages of participants in each group (of a total of  $n = 66$  who undertook an exercise and nonintervention challenge or sleep deprivation and nonintervention challenge). This was a *post hoc* analysis, and therefore no statistical test was applied.

participants with a history of anaphylaxis and historical adrenaline use were included, those with the most severe reactions in the community might be underrepresented, being possibly reluctant to volunteer for the study. This could introduce bias only if participants with more severe reactions in the community represent the more sensitive (ie, lower dose) reactors. However, a previous study has shown that minimum ED distributions for participants with histories of more severe reactions did not differ significantly from those participants with histories of milder reactions.<sup>21</sup> Our study population had a low average age of 25 years. Fatal anaphylaxis episodes occur more commonly in this age group,<sup>30</sup> perhaps because of more risk-taking behavior.<sup>31</sup> Thus in defining a threshold for the whole population, it is of benefit that the model is based on this age group.

A significant center effect was observed, with participants in London having overall lower thresholds than those in Cambridge, although a threshold-decreasing effect of both interventions was seen independently at both sites, reinforcing the generalizability of our findings. No differences were observed in the baseline characteristics of the study populations to account for the center effect. The most likely explanation is variation between investigators in interpretation of clinical symptoms and the decision about when to stop the challenge and administer treatment. Attempts were made to standardize practice across both sites through common stopping criteria for challenges and cross-site training to minimize 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.<sup>32</sup> All analyses were adjusted for center.

Another potential weakness was the use of open challenges after the blind baseline food challenge. We observed an apparent threshold decrease linked to an increasing number of challenges. Although this might be a true phenomenon, it is also possible that the open study design could 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 minimize this bias by ensuring that the participant was deemed to have reached their reaction threshold with only the appearance of prespecified objective symptoms, and the balanced design means that the 2 interventions were spread equally across the order of challenge days.

In conclusion, our study identified ED estimates from a well-characterized adult population with peanut allergy. Also, for the first time, it has been shown that cofactors, such as sleep deprivation and exercise, decrease allergen reactivity thresholds, and the magnitude of their effect has been defined. This study, funded by the UK Food Standards Agency, has important public health implications, helping food policy makers and the food industry provide harmonized guidance on allergen labeling, which will ultimately benefit all patients with peanut allergy.

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**Clinical implications: Exercise and sleep deprivation each individually decrease the reaction threshold by approximately half; this needs to be accounted for when defining reference doses for food labeling.**

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**TABLE E1.** Study inclusion and exclusion criteria**Inclusion criteria**

- Male and female subjects who are 18-45 y of age at the time of study entry (visit 1) and 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 2 h)
- A positive peanut DBPC food challenge result 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 placebo. Eligibility to the DBPC food challenge requires fulfillment of all other eligibility criteria at visit 1.
- Subjects must be able to comply with the study procedures.
- Sensitization to peanut demonstrated by skin prick tests or serum specific IgE measurements

**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 pre-10 homologues of peanut [Ara h 8] and low levels of serum IgE to Ara h 1, 2, and 3)
- Monosensitization to Ara h 9
- Use of investigational drugs at the time of enrollment or within 30 days or 5 half-lives of enrollment, whichever is longer
- History of hypersensitivity to any of the matrix components used within the material for the oral food challenge
- Poorly controlled asthma. Asthma control will be assessed by using the Asthma Control Questionnaire (ACQ). Patients with a score of less than 20 will not be eligible for the study. Also, patients should have an FEV<sub>1</sub> of greater than 80% of their predicted value.
- History of significant and repeated exercise-induced asthma attacks requiring treatment independent of food ingestion or a decrease in FEV<sub>1</sub> of greater than 15% during screening maximal oxygen intake exercise session
- Musculoskeletal disease that, in the opinion of the investigator, could impair the participant's ability to perform the exercise challenge
- A sleep or psychiatric disorder that, in the opinion of the investigator, could impair the participant's ability to perform the study procedures
- Pregnancy
- Alcohol or drug misuse
- Night-shift worker
- Concomitant use of:
  - systemic immunosuppressant
  - $\beta$ -blockers
  - angiotensin-converting enzyme inhibitor or other hypertensive drug
  - sedative drugs
  - antacid medication (either proton pump inhibitors or H<sub>2</sub>-antagonists)
- History of any of the following:
  - severe anaphylaxis to peanut, as defined by hypoxia (peripheral pulse oximetry < 92%) or hypotension (>30% decrease in systolic blood pressure) with or without neurological compromise
  - previous reaction to peanut that in the opinion of the investigator (or Trial Management Group) was life-threatening
  - mastocytosis
  - coronary artery disease
  - eosinophilic esophagitis
  - gastric or duodenal ulcer
- Past medical history of clinically significant electrocardiographic abnormalities or identified during the study (visit 1)
- Recent (within the last 3 years) and/or recurrent history of autonomic dysfunction (eg, recurrent episodes of fainting or palpitations)
- Hematologic parameters (total WBC count or hemoglobin level and 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)	Pruritus: occasional scratching (green)	
Moderate: scratching continuously for >2 min at a time (green)	Pruritus: scratching continuously for >2 min at a time (green)	
Severe: hard continuous scratching excoriations (yellow)	Hard continuous scratching causing excoriations (yellow)	
Mild: <3 hives or mild lip edema (yellow)	Urticaria: <3 hives or mild lip edema (yellow)	
Moderate: <10 but >3 hives or significant lip or face edema (red)	Urticaria: <10 or ≥3 hives or significant lip or face edema (red)	
Severe: generalized involvement (red)	Urticaria: generalized involvement (red)	
Mild: few areas of faint erythema (green)	Rash: Few areas of faint erythema (green)	
Moderate: areas of erythema (yellow)	Rash: Areas of erythema (yellow)	
Severe: generalized marked erythema (>50% [red])	Rash: generalized marked erythema >50% (red)	
Mild: rare bursts, occasional sniffing (green)	Itching in inner ear canal (green)	Itching in inner ear canal was added because it was a common mild symptom identified by many patients during piloting.
Moderate: <10 bursts, intermittent rubbing of the nose and/or eyes or frequent sniffing (yellow)	Rare bursts of sneezing, occasional sniffing (green)	Rhinitis symptoms were downgraded from red to yellow. These were not regarded by the study team as severe enough symptoms singly to warrant stopping challenge.
Severe: continuous rubbing of nose and/or eyes, periocular swelling and/or long bursts of sneezing, persistent rhinorrhea (red)	<10 Bursts, 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 rhinorrhea (yellow)	
Mild: expiratory wheezing to auscultation (red)	Chest tightness without any decrease in PEFR (green)	In the existing PRACTALL criteria study, the team believed that there needed to be representation of milder respiratory symptoms because the existing criteria escalate too rapidly to wheeze, which is a clear objective symptom. Therefore to enhance safety and aid detection, the gradation of lower respiratory symptoms was extended, adding milder ones and incorporating functional measurement of PEFR.
Moderate: inspiratory and expiratory wheezing (red)	Chest tightness with a <10% decrease in PEFR (green)	
Severe: use of accessory muscles, audible wheezing (red)	Chest tightness with a 10% to 20% decrease in PEFR (yellow) Chest tightness with a >20% decrease in PEFR (red) Expiratory or inspiratory wheeze (red) Use of accessory muscles (red)	
Mild: >3 discrete episodes of throat clearing or cough or persistent throat tightness/pain (yellow)	Throat tingling/altered sensation in throat (green)	Mild oropharyngeal symptoms were added. Definition of persistence was added and defined as symptoms present for ≥30 min.
Moderate: hoarseness, frequent dry cough (red)	>3 Discrete episodes of throat clearing or cough (yellow)	
Severe: stridor (red)	Persistent throat tightness (yellow) Hoarseness or frequent dry cough (red) Stridor (red)	
Mild: complaints of nausea or abdominal pain, itchy mouth/throat (yellow)	Oral itching (green)	Milder and transient abdominal symptoms were downgraded.
Moderate: frequent c/o nausea or pain with normal activity (yellow)	Transient nausea (green)	Incorporated duration of abdominal symptoms was a marker of severity. Persistent was defined as a symptom present for ≥30 min.
Severe: notably distressed because of gastrointestinal symptoms with decreased activity (yellow)	Transient abdominal pain (green)	
<i>Objective</i>	Persistent nausea (yellow)	
Mild: 1 episode of emesis or diarrhea (yellow)	Persistent abdominal pain (yellow)	
Moderate: 2-3 episodes of emesis or diarrhea or 1 of each (red)	Emesis/diarrhea (1 episode [yellow])	
Severe: >3 episodes of emesis or diarrhea or 2 of each (red)	Emesis/diarrhea (>1 episode [red])	
Mild: subjective response (weak, dizzy) or tachycardia (yellow)	Weak/dizzy or tachycardia (yellow)	
Moderate: decrease in blood pressure and/or >20% from baseline or significant change in mental status	Decrease in blood pressure and/or >20% from baseline (red)	
Severe: cardiovascular collapse, signs of impaired circulation (unconscious [red])	Cardiovascular collapse/signs of impaired circulation (red) Altered level of consciousness (red)	

c/o, Complains of; PEFR, peak expiratory flow rate.