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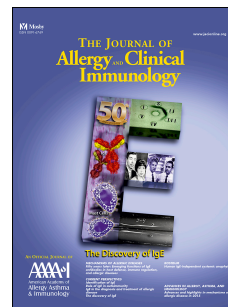
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Peanut Allergen Threshold Study (PATS): Novel single-dose oral food challenge study to validate eliciting doses in peanut allergic children

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1 **Peanut Allergen Threshold Study (PATS): Novel single-dose oral food challenge**
2 **study to validate eliciting doses in peanut allergic children**

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40

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43

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Abbreviations

46	DBPCFC	Double blind, placebo-controlled food challenge
47	ED	Eliciting dose
48	FAQLQ	Food allergy related quality of life questionnaire
49	FEV ₁	Forced expiratory volume in 1 second
50	LOAEL	Lowest adverse effect level
51	OFC	Oral food challenge
52	PA	Peanut allergy
53	PAL	Precautionary allergen labelling
54	PATS	Peanut allergen threshold study
55	spIgE	Specific IgE
56	SPT	Skin prick test
57	VITAL	Voluntary Incidental Trace Allergen Labelling
58	VSEP	VITAL scientific expert panel

59

Capsule summary

61 The derived ED₀₅ for peanut (1.5mg peanut protein) was given in a single dose to 378 peanut
62 allergic subjects. Only 8 subjects (2.1%) met predetermined criteria for an objective reaction,
63 suggesting the derived ED₀₅ could be used as a safe reference dose.

64

Clinical Implications

66 The ED₀₅ for peanut (1.5mg peanut protein) was validated in a multicentre study, using a novel
67 single dose challenge design, which provides a significant quality of life benefit for parents of
68 participants and could be adapted to other research or clinical settings.

69

Keywords

71 Eliciting dose (ED), Food Allergy related Quality of Life Questionnaires-(FAQLQ), Single dose,
72 Peanut thresholds, Oral Food Challenges (OFC), Voluntary Incidental Trace Allergen Labelling
73 (VITAL). Peanut Allergen Threshold Study (PATS)

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79 **Abstract**

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81 **Background**

82 Eliciting doses (ED) of allergenic foods can be defined by the distribution of threshold doses for
83 individuals within a specific population. ED₀₅ is the dose that elicits a reaction in 5% of allergic
84 subjects. The predicted ED₀₅ for peanut (PN) is 1.5 mg of peanut protein (6 mg whole peanut).

85 **Objective**

86 We sought to validate the predicted peanut ED₀₅ (1.5 mg) with a novel single dose challenge.

87 **Methods**

88 Consecutive eligible peanut allergic children in 3 centres were prospectively invited to participate,
89 irrespective of previous reaction severity. Predetermined criteria for objective reactions were used
90 to identify ED₀₅ single dose reactors.

91 **Results**

92 518 children (mean age 6.8 years) were eligible. No significant demographic or clinical differences
93 were identified between 381(74%) participants and 137 (26%) non-participants or between subjects
94 recruited at each centre. 378 children (206 male) completed the study. Almost half the group
95 reported ignoring precautionary allergen labelling. 245 (65%) experienced no reaction to the single
96 dose of peanut. 67 (18%) reported a subjective reaction without objective findings. 58 (15%)
97 experienced signs of a mild and transient nature that did not meet the pre-determined criteria. Only
98 8 subjects (2.1%, 95% CI 0.6%-3.4%) met the pre-determined criteria for an objective and likely
99 related event. No child experienced more than a mild reaction, 4 of the 8 received oral
100 antihistamines only and none received epinephrine. Food allergy related quality of life improved
101 from baseline to 1 month post challenge regardless of outcome (eta squared = 0.2, p <0.0001).
102 Peanut SPT, peanut and Ara h 2 spIgE levels were not associated with objective reactivity to PN
103 ED₀₅.

104

105 Conclusion

106 A single administration of 1.5 mg PN protein elicited objective reactions in fewer than the predicted
107 5% of peanut-allergic subjects. The novel single dose OFC appears clinically safe and patient-
108 acceptable, regardless of the outcome. It identifies the most highly dose-sensitive food allergic
109 population, not otherwise identifiable using routinely available peanut SPT or spIgE levels but this
110 single-dose approach has not yet been validated for risk assessment of individual patients.

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130 **Introduction**

131 Food allergic individuals are clinically selected to participate in diagnostic or research oral food
132 challenge (OFC) protocols that use graded, incremental doses administered at short, fixed time
133 intervals. Subjects who have experienced anaphylaxis are often not offered routine clinical OFC
134 and may be excluded from research OFC protocols (1). It is generally not possible from graded
135 protocols to determine whether a reaction has occurred to a *discrete* threshold dose of the allergenic
136 food or alternatively has been the result of the *cumulative* dose consumed by the allergic individual
137 at the time of reaction.

138

139 The eliciting dose (ED) for a peanut allergic reaction in 5% of the peanut allergic population (ED₀₅)
140 has been estimated at 1.5 mg of peanut protein (6 mg of whole peanut) based upon the population
141 distribution of threshold doses (children and adults) from graded, blinded oral challenges of 750
142 peanut allergic individuals (2-4).

143

144 This study aims to assess the precision of the predicted ED₀₅ using a single dose challenge (6 mg
145 peanut = 1.5mg of peanut protein, approximately 1/100th of a peanut kernel) in an unselected group
146 of peanut-allergic children and to validate the processes used to develop the only existing reference
147 doses for peanut, which have been based upon the ED₀₁ (which is the dose which elicits reactions in
148 1% of subjects studied) (2). It is likely that subjects who react only mildly at the ED₀₅ would
149 tolerate the ED₀₁ at least as well (4). This may assist clinicians, regulators and other stakeholders in
150 risk management for peanut allergic subjects.

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155 **Methods**

156 We have already published an in-depth description of the background and methodology of the
157 PATS study (please see reference 5). Additional details are provided below.

158

159 **Recruitment**

160 This multi-centre study involved three geographically diverse teaching centres, set in University-
161 affiliated hospitals, providing local, regional and national allergy services. To minimise recruitment
162 bias, the protocol required that the study was discussed fully with every potentially suitable child
163 and family, met during routine medical encounters in clinic or hospital attendances. Families who
164 chose not to participate were asked to complete a study-specific “non-participant” questionnaire,
165 adapted from Osborne et al (6) and to give written informed consent for their routinely available
166 laboratory data to be examined anonymously in the study. Inclusion and exclusion criteria are
167 shown in Table 1.

168

169 **Food Allergy related Quality of Life Questionnaires-(FAQLQ)**

170 Validated FAQL-Parental form (FAQL-PF) and FAQL Child form (FAQL-CF) questionnaires were
171 self-administered prior to OFC (T1) and 1 month after OFC (T2) to assess the impact of this novel
172 single dose OFC protocol on FAQL (8). FAQL-PF and CF are age appropriate questionnaires that
173 assess the health related quality of life (HRQL) of children with food allergy. The PF version is
174 completed by a parent of the food allergic child (0-12 years) and the CF by the child themselves (8-
175 12 years) on a 7-point scale ranging from not at all (1) to extremely (7). It has been found to have
176 excellent reliability ($\alpha > 0.9$), and construct, cross-cultural, content and longitudinal validity. A
177 higher score on either questionnaire reflects higher burden and poorer FAQL. A lower score
178 reflects lower burden/better FAQL.

179

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181

182 Single dose Oral Food Challenge (OFC)

183 The shelf-stable single-dose challenge cookies were manufactured at University of Nebraska-
184 Lincoln, USA and then distributed to participating clinic centres. Peanut content was determined
185 using the Neogen® Veratox® Quantitative Peanut Allergen Test (Neogen Corporation, Lansing
186 MI). This assay was also used to establish a validated mixing method to achieve a homogeneous
187 incorporation of peanut flour into the formulation as well as determining that all ingredients in the
188 formulation were below the limit of quantitation (2.5 ppm). The stability of product was established
189 by meeting acceptable criteria for water activity and microbial load. To maintain taste and texture
190 cookies were stored frozen until use. The single-dose cookie (6 mg whole peanut = 1.5 mg peanut
191 protein) consisted of granulated sugar, brown sugar, all-purpose wheat flour, vegetable shortening,
192 salt, baking soda and light roast, partially defatted peanut flour (Golden Peanut Company,
193 Alpharetta, Georgia USA). The cookie was eaten under standard open OFC conditions in hospital.
194 For subjects allergic to other cookie ingredients (e.g. wheat), the peanut dose of 1.5 mg peanut
195 protein was administered as the same light roast, partially defatted peanut flour in a vehicle food of
196 the subject's choice. Routine OFC monitoring was performed, according to local clinical practice.
197 Children were observed until 2 hours after OFC if no symptoms and signs were elicited or until 2
198 hours after such symptoms and signs had resolved, with or without treatment.

199 Criteria for a positive OFC result.

200 A highly liberal, inclusive strategy was used to capture clinical data during the OFC. Staff were
201 encouraged to make extensive notes, recording *any* physical or behavioural changes observed or
202 self-reported during the single-dose OFC. Predetermined objective criteria were used because the
203 ED₀₅ was predicted on the basis of challenge-associated objective responses only (1-4). The prior
204 agreed upon objective criteria for a positive OFC result occurring within 2 hours of ingestion were:

205 3 or more concurrent noncontact urticaria persisting for at least 5 minutes; or perioral or periorbital
206 angioedema; or rhinoconjunctivitis including sneezing; or diarrhoea; or vomiting (excluding gag
207 reflex); or anaphylaxis (with evidence of circulatory or respiratory compromise, e.g. persistent
208 cough, wheeze, change in voice, stridor, difficulty breathing, and collapse) (9).

209 Subjective symptoms were also recorded, such as: palatal itch, headache, dizziness, bloating,
210 abdominal pain, cramps, muscle aches, aching joints, anxiety, tension, and agitation.

211

212 **Case definition**

213 When the clinical study was completed all co-investigators met in person and reviewed all clinical
214 comments written by staff in each centre during the study. The above criteria were applied and
215 cases were designated “objective” or “subjective” and then as having met or not met the
216 predetermined objective criteria as above.

217

218 **Blood test**

219 A blood sample was taken for peanut sIgE component analysis (local hospital laboratories, using
220 ThermoFisher Immucaps®, according to manufacturer’s instructions) and quantitative peanut-
221 specific IgE fluoroenzyme immunoassays 20 minutes after OFC.

222

223 **Sample size estimation**

224 Assuming that the observed proportion of the sample that react to the single dose OFC is 5%, a
225 sample size of 375 corresponds to a 95% confidence interval for the population proportion with a
226 lower limit of 3.1% and an upper limit of 7.8% using the properties of the binomial distribution.

227 The investigators felt that this degree of precision in estimation was sufficient to rule out gross
228 incompatibility between the predicted and observed proportion of participants reacting to the single
229 dose.

230 **Statistics**

231 Data were analysed using SPSS Version 22(IBM, Evanston, Illinois, USA). Two sample t-tests for
232 continuously valued variables and Pearson's chi- square test or Fisher's exact test (for low
233 prevalences) for binary variables were conducted to determine the extent of any covariate
234 imbalance between participants and non-participants. Differences in means and proportions
235 between centres were also examined using similar statistical methods. The impact of the single dose
236 protocol on FAQL was analysed using multivariable regression analysis.

237 Partial Eta-squared' (η_p^2), also known as 'R-Squared', was the effect size produced by the
238 statistical tests used in this study. There are many advantages to including effect size when
239 reporting significant results. Effect size is not influenced by sample size or number of variables.
240 While a significant result (p value) shows whether an effect exists, it does not reflect the size of the
241 effect. Therefore both the magnitude (effect size) and significance (*P* value) are essential results to
242 be reported (10-12). A small effect is less than 0.08, a medium effect is less than 0.24 and a large
243 effect size is 0.25 and above (11).

244

245 ***Ethical approval***

246 This Study was approved by Cork University Hospital Research Ethics Committee (ECM 4 g),
247 Melbourne Royal Children's Hospital Human Research Ethics Committee (HRECApp 32166A),
248 and the Partners Human Research Committee (2012P002475). Written, informed parental and
249 adolescent consent and younger children's assent (according to local IRB age-related
250 requirements) were obtained.

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253

254 **Results**

255 Between October 2013 and February 2015, 518 patients were serially approached for participation
256 (Figure). One hundred thirty-seven individuals were deemed either ineligible or did not wish to take
257 part in the study. Three hundred seventy-eight completed the challenge protocol. Three subjects did
258 not complete the protocol. Comparisons of participants and non-participants in each centre are
259 shown in Table 2. Univariate analysis of variance showed no significant age differences between
260 participants and non-participants ($p = 0.62$), controlling for centre location ($p=0.84$). Sixty
261 percent of the overall sample was male. Twenty-two percent of females approached did not
262 participate, compared to 30% of males ($\chi^2= 6.7$, $p=0.035$). There was no difference in participant
263 sex between centres ($\chi^2= 2.6$, $p=0.63$).

264

265 A significant association was found between entry criteria and study centre location. Twenty-seven
266 percent of Irish subjects had been diagnosed with peanut allergy by the most stringent criterion
267 (positive OFC), compared to 11% in Australia and only 2.5% in the US, ($p<0.001$). However,
268 diagnostic method did not significantly differ between participants and non-participants ($\chi^2= 3.6$,
269 $p=0.17$) or between sexes ($\chi^2= 6.17$, $p=0.19$).

270

271 **Reactions to single dose ED₀₅ OFC**

272 381 participants took part in this stage of the study; two were excluded due to incomplete ingestion
273 of the peanut cookie. One subject was excluded before starting the protocol due to inter-current
274 illness, evident on clinical examination on the day of study. 378 subjects completed the protocol.
275 362 subjects (96%) received the single dose in the cookie. The remaining 16 subjects received
276 peanut flour instead in another vehicle food of their choice. There were no significant differences
277 in reaction type between the 362 children who ate the standard cookie and the 16 children who ate
278 the peanut flour in another vehicle ($\chi^2= 2.21$, $p=0.53$).

279

280 245 subjects showed no reaction to the cookie single dose OFC (Table 3). For 133 subjects, some
281 comment indicative of a possible reaction was recorded in the written OFC records. Sixty-seven
282 reported subjective symptoms only. Sixty-six events were considered objective, but 58 of these did
283 not meet the predetermined criteria. The very mild and transient objective symptoms that did not
284 meet the predetermined criteria included non-persistent usually single sneeze, non-persistent
285 usually single cough, small areas of transient erythema, and fewer than 3 hives lasting <5 minutes.
286 Eight participants experienced objective events that met the predetermined criteria (Table 4). All
287 eight subjects who met the pre-determined criteria consumed the cookie not an alternative vehicle.
288 No participant experienced more than a mild reaction; four of the 8 most objectively reacting
289 subjects were treated with oral antihistamines. No other subject was treated and none received
290 epinephrine.

291

292 Multivariable regression analysis showed no significant differences for age and centre, reaction
293 type or participant/ non participant status. The eight subjects who met the predetermined objective
294 criteria were no different in age to others included in the study (Table 4).

295

296 Study centre and reaction type were not significantly related to diagnostic entry criterion ($\chi^2=3.39$,
297 $p= 0.76$). Subjects' sex was not significantly related to reaction type ($\chi^2=4.76$, $p= 0.19$).

298

299 Univariate analyses showed peanut spIgE, Ara h1, Ara h2, Ara h3, Ara h8, Ara h9 spIgE levels and
300 total IgE levels had no effect on inclusion criterion met or participant/non-participant status,

301 (p 0.21 - 0.99) (Table 5). Peanut SPT differed between study centre location ($\eta_p^2 = 0.02$, $p = 0.03$)
302 with a small effect size (11), but not for reaction type ($p=0.25$). Irish subjects had the lowest mean
303 wheal size ($M= 9.50$ mm, $SD= 2.66$) and Australia the highest ($M=15$ mm, $SD=6.47$). No other skin
304 or blood tests were significant for either type of reaction or location ($p>0.05$).

305

306 Adherence to precautionary labelling at study entry was significantly lower in Australia where 76%
307 ignore labelling compared to Ireland (33%) and US (36%) ($\chi^2 = 66.21, p < 0.001$). Proxy and self-
308 reported adherence to precautionary allergen labelling did not significantly change from T1-T2 and
309 was unaffected by age of child, study centre or diagnostic criteria met ($p = 0.82-0.42$).

310

311 **Food allergy-related quality of life**

312 Baseline scores (before OFC) in the FAQL-PF predicted likelihood of reporting subjective vs
313 objective symptoms (after OFC) ($p = 0.001$). In effect, children who later experienced subjective
314 symptoms to the single dose of peanut had the most adverse impact on FAQL at baseline (Mean
315 $= 2.6, SD = 1.4$). Those who did not experience any reaction had the best FAQL (lowest burden) at
316 baseline (Mean $= 1.8, SD = 1.3$). This provides further evidence of the association between clinical
317 and psychological factors in food allergy.

318

319 There was a significant main effect for time from T1 to T2 for parent reported proxy FAQL-PF (η_p^2
320 $= 0.24, p = 0.014$), with a medium to large effect size (11), where parents reported an improvement in
321 FAQL for their children from baseline to 1 month post protocol. There was a significant three-way
322 interaction between age, sex and time ($\eta_p^2 = 0.11, p = 0.014$) with a medium effect size (11).

323 Regardless of age or sex of child, parents reported improved FAQL at T2. Younger boys
324 experienced a higher impact, whereas as age increased, parents reported more adverse impact for
325 girls. Diagnostic criteria and type of reaction elicited in the single dose study were not significant.

326

327 Children's self-reported FAQL-CF also improved from baseline (T1) to 1 month post protocol (T2)
328 ($\eta^2_p=0.5$, $p=0.001$) with a very large effect size (11). Again there was no effect on FAQL by
329 inclusion criteria met or type of reaction ($p=0.158$).

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352 Discussion

353 The novel single-dose PATS findings strongly support the safety of the statistically determined
354 ED₀₅ based upon population dose-distribution modelling (2) for administration to a non-selected
355 patient population. The protocol was very acceptable to families and was clinically very safe. This
356 approach offers the opportunity to identify the most dose-sensitive peanut allergic population in a
357 safe and efficient manner. It could be adapted for other major allergenic foods.

358

359 Population EDs can be estimated by statistical dose-distribution modelling of individual patient
360 threshold doses (2-4). ED estimates can vary depending upon the choice of model. The single-dose
361 PATS approach serves as a useful way to validate the ED estimates and select the best parametric
362 model. In this single-dose PATS, the percentage of patients reacting with the predetermined
363 objective criteria (2.1%) was lower than predicted from the log-normal model (5%; 95% CI of 3.1-
364 7.8%). Several reasons could explain the observed difference between the predicted 5% versus the
365 observed 2.1% rate. First, selection bias toward more highly sensitive patients could have occurred
366 with the 750 peanut-allergic subjects in the modelled dataset as many of the patients included in the
367 dataset were from tertiary allergy clinics which could contribute to a bias toward a more sensitive
368 peanut-allergic population (2,3), though this study group of consecutive patients was also recruited
369 in tertiary centres. Second, although objective responses were used in the clinics conducting
370 threshold challenges and the PATS, the objective criteria used to establish the lowest observed
371 adverse effect level (LOAEL) for some of the patients may not have been as stringent as the criteria
372 established for the PATS. In particular and among the mild transient reactions that did not meet the
373 predetermined objective criteria, 13 additional patients experienced hives (a single hive in 8 cases,
374 2 hives in 4 cases, and 3 hives in 1 case, all lasting less than the stipulated 5 minutes). Had these 13
375 cases been counted as positive response to the single-dose challenge, the reaction rate would have

376 been 5.5%. Given these possibilities, the log-normal model used appears to be reasonable and
377 appropriately conservative for use in the estimation of EDs for peanut.

378

379 Population modelling of individual threshold doses can be used to establish public health measures
380 such as the control of precautionary allergen labelling (PAL). In Australia, a Reference Dose for
381 peanut of 0.2 mg peanut protein was established from estimates of the ED₀₁ (2). The ED₀₁ was
382 selected by the VITAL Scientific Expert Panel (VSEP) because it is predicted to protect 99% of the
383 peanut-allergic population. However, based on the mild and transient responses encountered in
384 PATS, the use of the ED₀₅ as the basis for the peanut Reference Dose would be a more reasonable
385 and implementable risk management decision.

386

387 PAL abounds in many marketplaces but stakeholders find fault with the approach because use of
388 PAL bears little relationship to actual risk (13,14). Almost 50% of the study population were
389 routinely ignoring precautionary labelling. PATS has validated the ED₀₅, so the medical and food
390 science communities, manufacturing industry, and public health authorities should consider
391 adopting this model. This would assist in establishing an ED₀₅-based peanut Reference Dose to be
392 used in quantitative risk assessment to underpin PAL, backed by sound scientific evidence, that
393 protects the vast majority of the peanut allergic community.

394

395 No centre appeared to have a uniquely more sensitive study population than the other two,
396 suggesting this protocol and the predetermined criteria used for assessing single dose OFC could be
397 used in other centres. Ireland had far more challenge-proven cases than the other centres but lower
398 average ages than the US centre, and Australian patients had larger peanut SPT and paid less
399 attention to precautionary advisory labels. These inter-centre demographic and diagnostic
400 differences did not influence the primary or secondary outcomes of the study.

401 The predetermined approach to offer the study to all peanut allergic subjects in 3 distinct
402 geographical regions, the comparison of characteristics of participants and non-participants, the
403 permissive entry criteria and the pre-determined conservative case definition combine to address
404 the most common criticism of OFC studies: How representative of the general peanut allergic
405 population are the subjects who volunteered? This study showed peanut allergic children in each
406 centre were broadly similar, that severe reactors were included and, critically, that participants
407 appeared not to differ clinically from non-participants. While we did not prospectively record
408 previous reaction severity, all subjects were recruited from referred populations seen for their
409 peanut allergy in tertiary/national referral centres, so it is likely the representation of the severe end
410 of the clinical spectrum of peanut allergy in this study population is at least similar than that
411 reported peanut allergy norms.

412

413 **Limitations of the study**

414 Many of the patients recruited were diagnosed without the gold standard double-blind placebo
415 controlled food challenge. However, the intended recruitment strategy was to recruit relatively
416 unselected but near-certain cases, to capture the whole spectrum of cases, which is often not
417 included in incremental dose challenge studies. Our data show no differences in demographic
418 details or serological findings between participant and non-participants or between reactors and
419 non-reactors or between the 8 most certain objective reactors and other groups. The inclusion and
420 exclusion criteria appear to have been well constructed, based on established clinical methods used
421 elsewhere, clinical history and SPT and sIgE levels above determined decision points (7).

422

423 Subjects did not undergo placebo challenges, just an active-dose cookie, given once. Placebo doses
424 would have required doubling attendances to more than 700 visits and we considered the projected
425 likelihood of significant reactivity of around 5% in the single dose study did not justify a placebo

426 arm. It is notable that 65% of subjects reported no reaction at all to the ED₀₅ cookie, despite
427 knowing it was an “active” dose. Intentionally liberal documentation of reported symptoms and
428 having a set of fixed, pre-test criteria for an objective reaction allowed *post hoc* distinction of
429 subjective from objective reactors, though relatedness of any reaction to the single dose was
430 difficult in real time due to the lack of options normally available in routine OFC, such as waiting
431 longer between doses and repeating doses (1,7). Subjective reactors had lower pre-test FAQL than
432 objective reactors and non-reactors which suggests anxiety may play a role in reports of
433 mild/subjective reactions at low doses in the community and in DBPCFC (15) and also possibly in
434 reactions to placebo doses during DBPCFC (16).

435

436 PATS was an assessment of low-dose sensitivity in a population of peanut allergic subjects at a
437 single time point and further studies are needed to assess both population-level and individual
438 subjects’ variation in low-dose sensitivity over time. Standard, incremental DBPCFC does not
439 correlate well with reported severity of community reactions (17) and dose is only one variable to
440 be considered in the difficult assessment of severity of food allergy. (18)

441

442 The PATS study offers a new clinical paradigm and methodology with regards to assessing clinical
443 risk; this current study may potentially define the 5% of patients who are most dose-sensitive. It
444 confirms previous findings that validated questionnaires assessing FAQL show patients gain nearly
445 as much from a “failed” OFC as they do from a “passed” OFC, probably due to decreased
446 uncertainty about the next and future reactions. (13). This tangible impact could promote adoption
447 of PATS single dose peanut challenges in units not currently performing diagnostic multi-dose
448 OFC.

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450 The single dose protocol does not replace current clinical food challenges which are critical for
451 definitive diagnosis of food allergy but would provide extra clinical information of patients' level
452 of risk, related to dose, and could help inform consumer choices and physician advice to patients
453 regarding PAL (14, 15); single dose challenges could be done before starting a progressive clinical
454 food challenge to identify the most highly sensitive patients and reduce any risks associated with
455 the use of higher doses used in clinical food challenges. PATS suggests clinical validation of other
456 allergenic food sources could be addressed in similar studies, where the population dose-
457 distribution has been modelled using sufficient threshold data. Clinicians may be able to use PATS
458 single dose OFCs widely as they are easier to perform than routine diagnostic OFC or DBPCFC.

459

460 ***Conclusion***

461 The novel single dose OFC, based upon the statistical dose-distribution analysis of past challenge
462 trials, is a clinically safe and efficient approach to identify the most highly dose-sensitive
463 population of food-allergic people and it improves food allergy related quality of life. The
464 validation of the ED₀₅ will also assist regulators, public health agencies and manufacturers in the
465 establishment of approaches to allergen management that will protect the vast majority of food-
466 allergic consumers/patients.

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475 **Author's contributions**

476 JOBH is the guarantor of the study and wrote manuscript drafts.

477 JOBH, KA and SLT conceived the study initially.

478 WS and KA contributed to study design, clinical supervision and data analysis.

479 JN produced OFC materials, monitored the study and contributed to data analysis.

480 JB and ADG contributed to study design and data analysis.

481 GDG performed data analysis.

482 GZ and LG contributed to study design, selection of statistical methods and challenge performance.

483 All authors have contributed to manuscript drafts and have seen and approved the final version

484

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489

490 **Conflicts of Interest**

491 JOBH, KJA, LCG, GZ, JN, GDG, ADG none to declare. WGS receives consulting fees from

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1 **Legends**

2 Figure Flow diagram of subject recruitment and participation

3 Table 1. Inclusion and Exclusion Criteria

4 Table 2. Demographic comparison of participants to non-participants

5 Table 3. Primary Outcomes (reaction to single dose food challenge) per centre.

6 Table 4. Participants who met the predetermined objective reactivity criteria/case definition

7 Table 5. Reaction type vs. Mean values for Skin and Blood Tests

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29 **Table 1. Inclusion and Exclusion Criteria**

Inclusion Criteria	Exclusion Criteria
Age between one to eighteen years old inclusively.	Medically unfit for challenge according to local unit OFC guidelines/protocol (e.g. high fever, unwell with intercurrent illness)
<u>Evidence of peanut allergy by one of the following:</u>	Oral corticosteroids within 14 days prior to challenge
History of unequivocal exposure (including accidental) and typical acute allergic reaction within the preceding 2 years and positive peanut SPT (performed according to local clinical protocols) /specific IgE.	Episode of anaphylaxis of any cause in the 4 weeks prior to challenge
Positive oral food challenge with peanut performed within 2 years - either open oral food challenge or DBPCFC (Double-blind, placebo-controlled food challenge)	Use of antihistamines within 5 days of oral food challenge
Peanut never ingested, but sensitisation to peanut above the 95% positive predictive value (PPV) for clinical allergy, i.e. peanut serum IgE \geq to 15kU/L (by CAP FEIA) and/or peanut SPT wheal size \geq to 8mm (7) within 2 months of the single dose challenge.	Asthma that is not well controlled as demonstrated by FEV ₁ <85% of predicted best.

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43 **Table 2. Demographic comparison of participants to non-participants**

	Participants			Non-Participants		
	Cork	Melbourne	Boston	Cork	Melbourne	Boston
Initial Number	124	126	128	63	24	53
Sex (%Male)	61%	56.3%	55.5%	60.3%	70.8%	71.7%
Age (Mean yrs)	6.36	7.63	6.55	6.78	8.54	6.65
Final Number*	124	126	128	63	24	35
<u>Inclusion criterion met**:</u>						
Typical reaction<2years	68	60	74	38	12	19
Positive OFC<2years	43	16	2	8	1	2
SPT/spIgE > 95% PPVs	13	50	52	17	11	14

44 *18 participants in Boston did not wish to participate immediately after initial recruitment and therefore no
 45 diagnostic information was gathered.

46 ** Many subjects met both entry criteria 1 and 2 but only the single one entered in the restricted data file option
 47 is reported here.

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68 **Table 3 Primary Outcome (reaction to single dose food challenge) per centre**

	Total	Cork	Melbourne	Boston
	<u>Participants</u>			
Active Eligible Participants (completed OFC)	378	124	126	128
	<u>Outcome Group</u>			
Total	378	124	126	128
Non-reactors	245	94	65	86
Reactors	133	30	61	42
<u>Subjective Reactors</u>	67	19	30	18
<u>Objective Reactors</u>				
Total Objective	66	11	31	24
Meeting predetermined criteria	8	1	3	4

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91 **Table 4. Details of 8 subjects who met the predetermined objective reactivity criteria/case definition**

Participant Number	Location	Age (yrs)	Sex	Diagnostic method	Peanut Wheal (mm)	Peanut SpIgE kUA/L	SpIgE rArah1	SpIgE Arah2	Outcome
35	Ireland	11	Female	History of typical exposure & reaction & positive SPT/ spIgE	15	69.10	11.20	59.20	Rhinoconjunctivitis
40	Australia	15	Male	History of typical exposure & reaction & positive SPT/ spIgE	13	2.06	0.53	1.74	Urticaria
43	Australia	9	Male	History of typical exposure & reaction & positive SPT/ spIgE	18	N/A	N/A	N/A	Vomiting
95	Australia	2	Female	Peanut never ingested but positive SPT/spIgE > 95% PPVs	13	N/A	N/A	N/A	Vomiting
31	U.S.	9	Male	Peanut never ingested but positive SPT/spIgE > 95% PPVs	11	0.36	0.10	0.14	Urticaria
97	U.S.	2	Male	History of typical exposure & reaction & positive SPT/ spIgE	N/A	100.00	14.80	100.00	Urticaria
109	U.S.	1	Male	History of typical exposure & reaction & positive SPT/ spIgE	N/A	57.70	0.10	49.60	Urticaria
124	U.S.	4	Male	History of typical exposure & reaction and positive SPT/ spIgE	N/A	46.70	14.70	16.20	Rhinorrhoea

92 **Table 5. Reaction type vs. Mean values for Skin and Blood Tests**

	Total IgE	Peanut spIgE	Peanut SPT Wheal (mm)	rAra h1 spIgE	rAra h2 spIgE	rAra h3 spIgE	rAra h8 spIgE	rAra h9 spIgE
<u>Type of reaction</u> (n)								
Non-reactor (245)	490.46	28.18	11.69	11.11	22.52	4.88	1.49	0.74
Subjective (67)	1164.89	46.07	15.23	23.42	32.86	9.33	0.74	0.11
Objective (66)	1130.80	39.46	13.60	14.87	31.90	3.13	1.21	0.19
Satisfies pre- determined criteria (8)	290.67	45.99	14.00	8.18	45.03	2.35	0.13	0.31

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Figure X. Flow of participants through study

