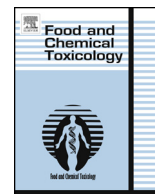




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A retrospective analysis of allergic reaction severities and minimal eliciting doses for peanut, milk, egg, and soy oral food challenges

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ABSTRACT

Food allergy is a public health concern, affecting up to 6% of children and 2% of adults. The severity of allergic reactions can range from mild to potentially life-threatening. In addition, the minimum amount of protein needed to provoke an allergic reaction in an individual patient (the minimal eliciting dose (MED)) ranges from a few micrograms to several grams. To determine whether a retrospective analysis of published data from oral food challenges could be used to assess the potential relationship between MEDs and reaction severities at the MEDs, a three class (mild, moderate, severe) reaction grading system was developed by integrating previously published reaction grading systems. MEDs and symptoms were collected from food challenge studies and each reaction was graded using the integrated grading system. Peanut allergic patients who experienced severe reactions had significantly higher MEDs and threshold distribution doses than those who experienced mild and moderate reactions. No significant differences in threshold distributions according to the severity grading were found for milk, egg and soy. The relationship between threshold dose distribution and reaction severity based on these grading criteria differed between peanut and other allergens, and severe reactions occurred in some patients at low MEDs for all of these food allergens.

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1. Introduction

Food allergy is a growing public health concern in industrialized countries, affecting up to 6% of children and 2% of adults (Baral and Hourihane, 2005; Liu et al., 2010; Sicherer and Sampson, 2010). Many affected children will “outgrow” their allergy with age (Sampson and Scanlon, 1989). However, food allergy can be a lifelong concern especially for peanut-allergic patients (Busse et al., 2002; Fleischer et al., 2004). There is some evidence that the incidence of food allergy may have increased since 1997 in United States and in other countries (Branum and Lukacs, 2008; Grundy et al., 2002).

Food allergy is usually diagnosed based on a combination of history, clinical examination, measurement of allergen-specific IgE levels and skin prick tests (SPTs) (Sampson and Albergo, 1984). However, the double-blind, placebo-controlled oral food challenge (DBPCFC) is considered to be the “gold standard” for diagnosing food allergy because it removes patient and observer bias (May, 1976;

Sampson and Albergo, 1984). Open or single-blind oral food challenges are considered useful when the challenge outcome is negative or when obvious objective symptoms are elicited, especially in young children (Boyce et al., 2010). In addition, oral food challenges (OFCs) may be used to determine whether a food allergy has resolved and to characterize an individual patient's sensitivity to an allergen (Taylor et al., 2004).

The minimum amount of food that elicits an allergic reaction in a sensitized individual in an OFC is often referred to as the “minimum eliciting dose” (MED) (Flinterman et al., 2006b; Peeters et al., 2007). It is well established that there is a wide range in MEDs among individuals with IgE-mediated food allergies (Crevel et al., 2007). However, limited data are available on the correlation between the MED and reaction severity at the MED for individual patients. Anecdotal clinical observations suggest that reactions occurring at low allergen doses during challenge are relatively mild. However, DBPCFC studies have shown that allergenic food doses as low as 0.3 to 1 mg of protein can cause serious reactions of laryngeal edema or throat closing (Anagnostou et al., 2011; Wensing et al., 2002b). In some studies serious reactions occurred at the lowest challenge dose used (Knight et al., 2006; Nicolaou et al., 2010; Sicherer et al., 2000). Thus, it remains possible that serious reactions could have occurred at even lower doses in those patients. Several studies found no correlation between MED and reaction severity during the challenge (Flinterman et al., 2006b; Glaumann et al., 2012; Lam et al., 2008; Sicherer et al., 2000). One study found that patients with a history

Abbreviations: AIC, Akaike information criterion; AP, abdominal pain; CI, confidence interval; DBPCFC, double-blind placebo-controlled food challenge; ED, eliciting dose; GI, gastrointestinal; OAS, oral allergy syndrome; LOAEL, the lowest observed adverse effect level; NOAEL, the no observed adverse effect level; OFC, oral food challenges; RC, rhinoconjunctivitis; SPTs, skin prick tests.

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of severe reactions or patients with moderate-to-severe reactions during challenge had significantly lower MEDs than patients with milder reactions (Wensing et al., 2002b), while another study found no significant difference between the threshold distributions of patients with histories of more severe or less severe reactions (Taylor et al., 2010). All of these studies had confounding factors such as testing of a small number of individuals, differences in geographic location, use of different test material, use of different dosing protocol, etc., that create uncertainty as to how the results apply to the general food allergic population. In addition, standards for classifying the severity of allergic reactions varied among the studies, leading to difficulties in evaluating and comparing results.

The aim of our study was to determine whether a retrospective analysis of published data from OFCs with peanut, milk, egg and soy allergic individuals could be used to assess the potential relationship between MEDs and reaction severities at the MEDs. Because there is no generally accepted system for evaluating allergic reaction severity, and because the terminology used to describe the observed reactions differed between studies, we developed an integrated reaction severity grading system based on eight previously published grading systems. The observed reactions from published OFC studies were then graded based on this integrated grading system and the relationships between MEDs and reaction severities at the MEDs for the four allergens were analyzed by statistical modeling.

2. Materials and methods

2.1. Development of the integrated grading system

Multiple published classification schemes or grading systems for allergic reaction severities were reviewed and compared (Table 1 and Supplementary Table S1) (Brockow and Ring, 2009; Brown, 2004; Cox et al., 2010; Ewan and Clark, 2001;

Flinterman et al., 2007; Mueller, 1966; Rank et al., 2008; Sampson, 2003). These systems were developed independently, are widely recognized, and in some cases have been used by health agencies. Our system for classifying the severity of food allergic reactions was developed by integrating these eight schemes into a three class system (severity grades: mild, moderate or severe) as shown in Table 2.

Since these schemes varied in number of grades (3 to 5), purpose for the grading system (i.e., defining OFC responses vs classifying anaphylaxis or systemic reactions vs establishing priority allergens) and in the types of symptoms reported (Table 1 and Supplementary Table S1), the following criteria were used to integrate these different schemes into a unified three grade scoring system. First, because some of the published systems were developed to classify all types of allergic reactions, not just food allergies, only symptoms that were reported in the clinical studies we reviewed were included (Table 2). Second, for those published grading systems with 3 severity grades (Brown, 2004; Flinterman et al., 2007), their defined mild, moderate or severe reactions were considered as mild, moderate or severe in our systems; for the systems that defined 4 severity grades (Cox et al., 2010; Mueller, 1966; Rank et al., 2008) (Cox et al., 2010 defined 5 grades but the last grade is “death”, so only the first 4 grades were considered), the first grade was considered as mild, the second grade was considered as moderate and the last two grades were considered as severe; for the systems that defined 5 grades (Ewan and Clark, 2001; Sampson, 2003), the first two grades were considered as mild, the third was considered as moderate, the fourth was either moderate or severe depending on the specific symptoms and the grading of those symptoms in the other published systems, and the fifth grade was considered as severe. Third, symptoms or reactions that were reported in the literature but not specifically listed in any of the schemes analyzed (e.g., asthenia, colic) were grouped with similar symptoms or assessed individually for severity by the authors and included in the final integrated scoring model. Other inconsistencies among the published systems were resolved by considering the most frequently used classifications in the eight published grading systems. Because most of the published grading systems and oral challenges studies lack detailed information on individual symptoms, we used broad descriptions in our integrated system.

2.2. Selection of allergic reaction data

Published OFC studies that included data on reaction severities were identified by searching the PubMed database using key words including specific allergen names (peanut, milk, egg or soy), and “oral food challenge”, “threshold”, or “double-blind

Table 1
Published allergic reaction grading systems.

System	No. of grades	Purpose of grading system	Consideration of the number of organ systems involved?	Subjective symptoms included?
Sampson, 2003	5	Grading of food-induced anaphylaxis	Yes	Yes (oral ‘tingling’)
Ewan and Clark, 2001	5	Food allergic reaction severity	Yes	No
Cox et al., 2010 ^a	5 (5 = death)	Systemic reaction grading system for subcutaneous immunotherapy reactions	Yes	No
Rank et al., 2008 ^b	5 (0 = no symptoms or nonspecific symptoms)	Grading of severity for systemic side effects	No	No
Mueller, 1966	4	Grading severity for systemic reactions to insect stings	Yes	Yes (malaise, anxiety)
Brockow and Ring, 2009	4	Grading of anaphylactic/anaphylactoid reactions	Yes	No
Brown, 2004 ^c	3	Criteria for the establishing of new priority food allergens	Yes	No
Flinterman et al., 2007	3	Food challenge severity	Yes	Yes (oral cavity)

PF, peak flow; PEF, peak expiratory flow; PEV, forced expiratory volume; AP, abdominal pain.

^a World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System.

^b 2006 EAACI (European Academy of Allergy and Clinical Immunology) Grading of Severity for Systemic Side Effects.

^c Used by Health Canada as part of their Criteria for the Establishing of New Priority Food Allergens.

Table 2
Integrated grading system for scoring reaction severity of oral food challenge (OFC) reactions.

Grade	Symptoms/organ systems	Classification of combined organ systems
Mild	- OAS (Oral allergy syndrome) - Skin: Eczema, erythema, flushing, pruritis, urticaria (not generalized), conjunctivitis, nonlaryngeal angioedema (e.g., lip swelling) - Upper respiratory: rhinitis, rhinoconjunctivitis, nasal congestion, sneeze - Gastrointestinal (GI) (mild): nausea alone, colic	All symptoms in any one system category (OAS, skin, upper respiratory, or nausea) alone
Moderate	- Skin (severe): generalized urticaria, facial swelling - GI: abdominal pain, diarrhea, vomiting, cramps - Mild lower respiratory: dyspnea, cough, chest or throat tightness - Neurological: tiredness, agitated, asthenia, lethargy	All symptoms in GI or mild lower respiratory symptom alone; or combination of symptoms in any two systems of skin, GI, upper respiratory or mild lower respiratory
Severe	- Lower respiratory: asthma, bronchoconstriction (drop in peak flow), wheeze, stridor, hoarseness, laryngeal edema (or throat closing) - Cardiovascular: tachycardia, shock, fall in blood pressure, hypotension, cyanosis - Anaphylaxis, collapse - Reaction requiring epinephrine treatment	Any symptom in lower respiratory and/or cardiovascular systems; or any combination of symptoms in three or more systems of mild/moderate grade (except OAS)

placebo-controlled food challenge". Additional studies were identified through the citations in these publications. The papers retrieved through these searches were screened to identify those that reported MEDs for individual patients (or where the MEDs could be determined) and the symptoms (both subjective and objective) elicited at the MEDs for each patient. When information on reactions at multiple doses was given for an individual patient, only the lowest dose and the reaction at that dose were used (Ballmer-Weber et al., 2007; Mackie et al., 2012; Peeters et al., 2007). Only immediate reactions, as defined in each paper were considered in our analysis. In those papers where both immediate and delayed reactions were reported, only those reactions that were reported to occur within 2 hours of challenge or that caused the challenge to stop were considered (Fiocchi et al., 2003; Hill et al., 1984). This time frame was chosen so as to be consistent with the recommended protocols in Bindslev-Jensen et al. (2004), Taylor et al. (2004), and Sampson et al. (2012).

Most of the published studies reported that some patients did not react to any of the food challenges. It was not possible to determine whether any of these patients would have reacted at higher doses, or how severe their possible reactions would have been. Therefore, these patients were not included in our analysis. Thus, our analysis used only a fraction of the data reported in most publications (Table 3A–D).

2.3. Determination of MEDs (LOAELs) and NOAELs

MEDs (equivalent to the lowest observed adverse effect levels (LOAELs)) were calculated from the discrete non-labial, challenge doses that resulted in the symptoms reported in each publication as described in Table 2. MEDs were expressed as milligrams of protein regardless of the form of test material used (e.g., peanut flour, crushed peanut or peanut butter, cow's milk or dry milk, whole egg or egg white, soy flour or soy formula). For those papers that described the protein content of the test material, the values given in the publication were used directly. For those that did not, protein levels were calculated based on the protein content levels used by Taylor et al. (2002) or on those listed by the USDA National Nutrient Database for Standard Reference (USDA, 2011). For those publications that only presented cumulative eliciting dose values, the dosing scheme provided in the paper was used to derive the discrete MEDs, assuming that all patients followed the published dosing scheme (Astier et al., 2006; Baehler et al., 1996; Grimshaw et al., 2003; Vlieg-Boerstra et al., 2008; Wainstein et al., 2010). If cumulative doses were reported but no dosing scheme was given in the publication, the most-likely dosing scheme was inferred from the pattern of cumulative doses reported and the description in the text, and this inferred scheme was used to calculate the discrete MEDs (Bock et al., 1978; Glaumann et al., 2012; May, 1976). The dose immediately preceding the MED (the highest dose that did not cause any symptoms, equivalent to the no observed adverse effect level or NOAEL) was also determined for each patient for use in interval censored analysis.

2.4. Severity grading

The severity of each reaction was graded independently by three of the authors using the integrated scoring system. Inconsistencies between the individual determinations were resolved by consensus.

Although OAS (mouth itch, lip tingling, etc.) was not included in most of the published scoring systems, it was reported as a reaction in some of the clinical studies that were reviewed. When OAS was the only symptom reported, the reaction was considered to be mild. In addition, the combination of OAS with other symptoms was not considered to be sufficient to upgrade the severity of a reaction. For example, a reaction with OAS, skin and GI symptoms was considered to be the same severity grade as skin and GI symptoms without OAS (moderate).

GI symptoms were generally considered to be of moderate severity. However, based on the severity grades in the published systems, nausea by itself was considered to be mild. Urticaria was considered to be a mild reaction unless it was described as being generalized (moderate).

In general, a reaction was considered mild if only OAS, the skin, or the upper respiratory tract was involved. Any GI symptom (except nausea alone) or mild lower respiratory symptom alone, or a combination of symptoms in any two of skin, GI, upper respiratory or mild lower respiratory was considered moderate. Severe lower respiratory and/or cardiovascular symptoms or any combination of symptoms in three or more organ systems were considered to be severe. For example, if either pruritus (skin) or rhinitis (upper respiratory) was the only symptom elicited, that reaction was graded as mild; however, if both pruritus and rhinitis were elicited, that reaction was graded as moderate because two different organ systems were affected. If in addition to skin and upper respiratory systems, a GI symptom such as abdominal pain (which by itself is moderate) was also reported, then that reaction was graded as severe.

Most of the symptoms seen in food allergic reactions can vary in intensity. For example, asthma can be mild, moderate or severe (Rank et al., 2008). If the intensity of a symptom was indicated in the publication, the severity grade for that reaction was evaluated and adjusted based on the described intensity; otherwise, the symptom was graded as described in Table 2.

2.5. Statistical methods

Statistical analysis was carried out using R software (R Development Core Team, version 2.15.2). Box-plots were plotted on a log scale. The threshold dose distributions

between groups were compared using the Kruskal–Wallis test and the two-sample Kolmogorov–Smirnov test (K–S test) was performed for intergroup comparisons for peanut.

The parametric distribution of the data for all peanut and milk allergic patients and for each sensitivity group of peanut and milk allergic patients was evaluated using interval-censored survival analysis (Taylor et al., 2009). Log-normal, log-logistic and Weibull dose distribution models were fitted to each data set. The Akaike information criterion (AIC) value was calculated for each model, a lower AIC suggesting a relatively better fit between candidate models, for a given dataset. A likelihood ratio test was used for embedded models (e.g., test of one model for all data vs one model per group, for a given distribution). Patients who reacted to the first dose during a challenge were treated as left censored. Patients who did not respond to the highest dose given (right censored data) were not considered because it was not possible to determine the severity of any eventual reactions that might have occurred at a higher dose. Other data were considered as interval censored, bounded by MED and NOAEL data for individuals for each severity group. The fitdistrplus package in R was used for distribution fitting (Pouillot and Delignette-Müller, 2010). Confidence intervals were evaluated using nonparametric bootstrap resampling.

3. Results

3.1. Patients characteristics and severity grading

We were able to collect information on MEDs and reaction severities for 257 peanut allergic subjects from 20 oral challenge studies, 167 milk allergic subjects from 14 studies, 50 egg allergic subjects from 10 studies, and 45 soy allergic subjects from 5 studies (Table 3A–D). Most MEDs were from patients challenged in DBPCFCs while a small fraction of the MEDs were from open challenges in children (no more than 5 years old).

Among the 20 peanut challenge studies, only four (with 26 out of 257 subjects) were carried out in the US. Worldwide, 67% of the subjects in the peanut challenge studies were children (Table 3A). Among the 257 peanut allergic patients there were 68 mild, 122 moderate and 67 severe reactions (Table 4).

The majority of the milk allergic patients analyzed were children (93%). Only one out of the 14 studies was done in the US (Table 3B). Among the 167 milk-allergic patients there were 74 mild, 57 moderate, and 36 severe reactions (Table 4).

Among the egg allergic patients (82% children) there were 22 mild, 14 moderate and 14 severe reactions; among soy allergic patients there were 20 mild, 15 moderate and 10 severe reactions (Table 4). It was not possible to determine the age of all of the soy allergic patients from the information in the publications.

3.2. MEDs and reaction severities

Using the data on MEDs and reaction severities described above, we examined the empirical distributions of MEDs among patients who had severe reactions as compared to those who had mild or moderate reactions for each food (Fig. 1). For peanut allergic patients, the median MEDs were 25 mg (range: 0.05–2500 mg), 50 mg (range: 0.1–5000 mg) and 250 mg (range: 0.3–5000 mg) of protein for mild, moderate and severe reactions respectively (Fig. 1). Patients with severe reactions to peanut had statistically significantly higher MEDs than did patients with mild or moderate reactions ($p < 10^{-4}$ and $p = 0.0008$ by K–S test), while there was no significant difference in MEDs between those with mild or moderate reactions ($p = 0.1$) (Fig. 1).

For milk allergic patients, the median MEDs were 396 mg (range: 3.3–7920 mg), 534 mg (range: 9.9–6600 mg) and 396 mg (range: 9.9–9000 mg) protein respectively, for mild, moderate or severe reactions (Fig. 1). Statistical analysis revealed that the MEDs of patients who had mild, moderate or severe reactions were not significantly different from each other ($p = 0.4$ by the Kruskal–Wallis test). There was also no significant difference ($p = 0.18$) among milk allergic children with mild, moderate, or severe reactions. Similarly, there were no significant differences in the MEDs for any of the severity groups for egg and soy allergic patients ($p = 0.28$ and 0.73

Table 3

(A) Peanut oral challenge studies; (B) Milk oral challenge studies; (C). Egg oral challenge studies; (D) Soy oral challenge studies.

(A)						
Studies	Design	Total no. of patients (challenged with peanut)	No. of patients included in this analysis	Country	Population	Test material
(May, 1976)	DBPCFC	38 (27)	8	US	C	Ground unroasted peanut
(Atkins et al., 1985)	DBFC/Open	25 (3)	2	US	A	Crushed peanut
(Bock et al., 1978)	DBPCFC	68 (24 ^a)	12	US	C	Ground unroasted peanut
(Oppenheimer et al., 1992)	DBPCFC	11	4	US	A	Peanut flour
(Hourihane et al., 1997)	DBPCFC	14	8	UK	A	Peanut flour
(Wensing et al., 2002a)	DBPCFC	26	26	NL	A	Defatted peanut meal
(Grimshaw et al., 2003) ^b	DBPCFC	4	4	UK	A	Peanut flour
(Lewis et al., 2005) ^c	DBPCFC	40	40	UK	23C/17A	Peanut flour
(Astier et al., 2006)	DBPCFC	30	22	FR	C	Peanut powder
(Flinterman et al., 2006b)	DBPCFC (6 open ^d)	27	22	NL	C	Peanut flour
(Patriarca et al., 2006)	DBPCFC	1	1	IT	A	Roasted peanut
(Peeters et al., 2007)	DBPCFC	25	22	NL	A	Peanut flour
(Vlieg-Boerstra et al., 2008)	DBPCFC	21 (6)	4	NL	C	Peanut
(Clark et al., 2009)	DBPCFC	4	4	UK	C	Peanut flour
(Nicolaou et al., 2010)	DBPCFC (1 open)	34	11	UK	C	Peanut flour
(Wainstein et al., 2010)	Open	55	3	AU	C	Finely chopped peanut
(Anagnostou et al., 2011)	DBPCFC (3 open)	22	21	UK	C	Peanut flour
(Johannsen et al., 2011)	Open	49	16	AU	C	Peanut butter
(Glaumann et al., 2012)	DBPCFC	38	25	SE	C	Peanut meal
(Mackie et al., 2012) ^b	DBPCFC	2	2	UK, NL	A	Peanut (in dessert)
(B)						
Studies	Design	Total no. of patients (challenged with milk)	No. of patients used in this analysis	Country	Population	Test material
(Bock et al., 1978)	DBPCFC	68 (37 ^a)	7	US	C	CM
(Hill et al., 1984)	Open	100	53	AU	C	CM
(Host and Samuelsson, 1988) ^e	DBPCFC	5	2	DK	C	CM
(Norgaard and Bindslev-Jensen, 1992)	DBPCFC	19 (10)	3	NK	A	CM
(Baehler et al., 1996)	DBPCFC	69	10	CA	C	NFDM
(Patriarca et al., 2002)	DBPCFC	8	8	IT	C	CM
(Fiocchi et al., 2003)	DBPCFC	18	12	IT	C	CM
(Flinterman et al., 2006a)	DBPCFC	11	11	NL	C	NFDM
(Morisset et al., 2007)	SBPCFC	60	11	FR	C	CM
(Lam et al., 2008)	DBPCFC	10	8	NL	A	NFDM
(Vlieg-Boerstra et al., 2008)	DBPCFC	21 (9)	5	NL	C	CM
(Caminiti et al., 2009)	DBPCFC	11	11	IT	C	CM
(Orhan et al., 2009)	DBPCFC	156	4	TR	C	CM
(Dambacher et al., 2013)	DBPCFC	116	21	NL	C	Formula with CMP
(C)						
Studies	Design	Total no. of patients (challenged with egg)	No. of patients used in this analysis	Country	Population	Test material
(May, 1976)	DBPC	38 (15)	4	US	C	Whole egg or dried egg
(Bock et al., 1978)	DBPCFC	68 (20 ^a)	10	US	C	Whole egg
(Atkins et al., 1985)	OFC	25 (2)	1	US	A	Whole egg
(Norgaard and Bindslev-Jensen, 1992)	DBPCFC	19 (13)	7	DK	A	Fresh hen's egg
(Caffarelli et al., 1995)	DBPCFC	33	13	IT	C	Dried egg then one boiled egg
(Eggesbo et al., 2001)	3DBPCFC/4open	41	7	NO	C	Cooked egg
(Knight et al., 2006) ^f	DBPCFC	74	3	US	C	Lyophilized egg white
(Unsel et al., 2007)	DBPCFC	1	1	TR	A	Cooked egg white
(Vlieg-Boerstra et al., 2008)	DBPCFC	21 (2)	1	NL	C	Cooked egg
(Orhan et al., 2009)	DBPCFC	48	3	TR	C	Cooked egg
(D)						
Studies	Design	Total No. of patients (challenged with soy)	No. of patients used in this analysis	Country	Population	Test material
(Bock et al., 1978)	DBPCFC	68 (7 ^a)	2	US	C	Soybean protein
(Magnolfi et al., 1996)	DBPCFC	262	5	IT	C	Soy formula
(Zeiger et al., 1999)	DBPCFC	93	9	US	C	Soy formula
(Fiocchi et al., 2003)	DBPCFC	18	7	IT	C	Soy formula
(Ballmer-Weber et al., 2007)	DBPCFC (1open)	30	22	CH/DK/IT	U	Soy flour

^a Number of subjects with history of reaction to each allergen, not clear how many were challenged with each allergen.^b MEDs determined using the low fat recipes in these two studies were used in our analysis.^c Scores 1–4, 6, 16, 18 were re-graded as mild, scores 5, 7–13 as moderate, and scores 14, 15, 17, 19–25 as severe (Hourihane et al., 2005).^d The six patients did not react to the DBPCFC but reacted to the one dose (10 g peanut) of open challenge.^e Data from challenges with homogenized and pasteurized milk were used because that is the form usually consumed in the US.^f Only the three subjects who were treated with epinephrine and had MEDs provided were included.

DBPCFC: double blind placebo controlled food challenge; Open: open food challenge; C: children; A: adults; U: undefined; CM: cow's milk; NFDM: nonfat dried milk; CMP: cow's milk protein.

Table 4
Number of patients in each severity grade for the four allergens.

Allergen	Mild (%)	Moderate (%)	Severe (%)	Total
Peanut	68 (26.5)	122 (47.5)	67 (26.0)	257
Milk	74 (44.3)	57 (34.1)	36 (21.6)	167
Egg	22 (44)	14 (28)	14 (28)	50
Soy	20 (44.4)	15 (33.3)	10 (22.2)	45

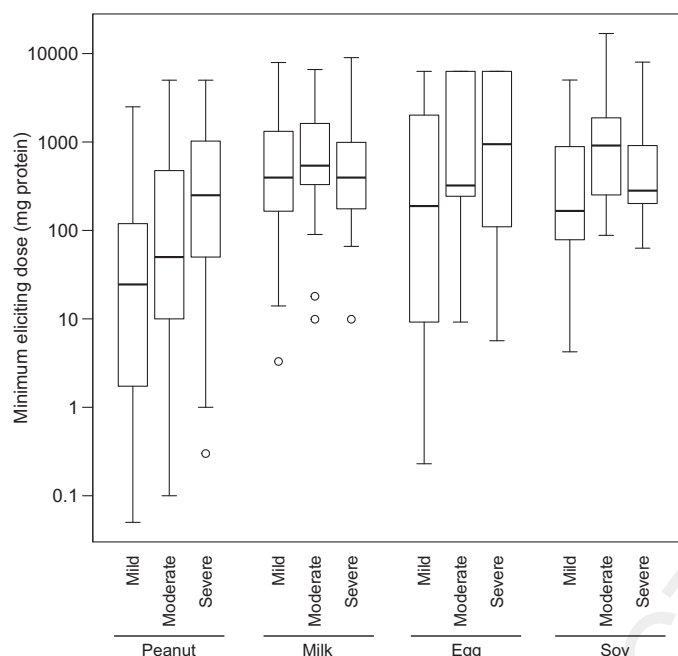


Fig. 1. Box plot with whiskers for minimum eliciting doses (mg protein) of each severity grade for the four allergens (median: —, the first and third quartile: □, 1.5 interquartile range: —, outliers: ○).

by the Kruskal–Wallis test). As for milk, there were no significant differences in the MEDs among the three severity groups for egg allergic children ($p = 0.24$). Among the milk allergic patients, 11 were adults with 2 mild, 5 moderate and 4 severe reactions and among the egg allergic patients, 8 were adults with 1 mild, 2 moderate and 4 severe reactions. Some studies did not provide age information for each patient; therefore, we could not analyze data specifically from peanut and soy-allergic children (Ballmer-Weber et al., 2007; Lewis et al., 2005).

3.3. Threshold distributions for peanut and milk

Among the three distribution models tested (Weibull, log-normal, log-logistic), the Weibull distribution had the lowest AIC values for all three severity groups for peanut, two of the three severity groups for milk, and for the total populations of all the four allergens. The log-normal distribution model had the lowest AIC value for milk patients who had severe reactions. Because several

other studies have used log-normal distribution models to estimate the ED_{01} , ED_{05} , or ED_{10} (Allen et al., 2014; Blumchen et al., 2014; Taylor et al., 2010, 2013), we applied both Weibull and log-normal models to estimate the ED_{10} values for the total peanut and milk allergic population as well as the ED_{10} 's for each severity group of peanut and milk allergic patients (Table 5 and Fig. 2). We did not estimate the ED_{05} 's or ED_{01} 's due to the limitation on sample sizes for most population groups. Weibull distribution models were also used to estimate the ED_{10} 's for the total soy and egg data, but too few data points were available to model each severity grade for the egg or soy allergic populations.

The ED_{10} estimated using the Weibull distribution model for all peanut allergic patients was 0.4 mg peanut protein (95% confidence interval of 0.2 to 0.8 mg) and was 0.7 mg (95% confidence interval of 0.4 to 1.3 mg) using the log-normal distribution (Table 5). Separating the analysis by severity groups provided a significantly better fit than the analysis on all groups (likelihood ratio test: $p < 10^{-4}$ for Weibull). The ED_{10} 's for each severity group estimated by the Weibull models ranged from 0.05 mg of peanut protein (95% confidence interval of 0.01 to 0.2 mg) for patients who had mild reactions to 3.6 mg (95% confidence interval of 1.2 to 10.5 mg) for patients who had severe reactions. The values estimated by the log-normal models were slightly higher (Table 5). The 95% confidence interval (CI) for the severe group does not overlap with the 95% CI for the mild group for either model. There was a slight overlap in the 95% CIs between the moderate and severe group for the Weibull model and between the moderate group and both other groups for the log-normal model. Similarly, while the population threshold distributions for the three severity grades were visually distinct from each other, the mild and moderate distributions were not significantly different from each other, and the distribution for severe reactions was significantly different from both of the other two. Patients who had severe reactions were comparatively less sensitive (i.e., the threshold distribution was shifted to the right) (Fig. 2A).

For milk allergic patients, the ED_{10} for the total population estimated by the Weibull model was 25.2 mg of protein (95% CI of 15.2 to 42.0 mg) and by the log-normal model was 32.9 mg protein (95% CI of 19.0 to 52.8 mg). Separating the analysis by severity groups did not provide a significant better fit than a single analysis on all data (likelihood ratio test: $p = 0.29$ for Weibull, $p = 0.13$ for log-normal). The ED_{10} 's estimated by the Weibull distribution for those who experienced severe reactions were 21.4 mg of milk protein (95% CI of 7.6 to 58.2 mg), 51.4 mg protein for those who had moderate reactions (95% CI of 23.2 to 111.7 mg), and 15.1 mg for those with mild reactions (95% CI of 5.7 to 39.6 mg). The ED_{10} 's estimated by the log-normal distribution were slightly higher for the mild and moderate groups and almost double for the severe group (Table 5). As the log-normal distribution was the best fit model for the severe group, the ED_{10} estimated by the log-normal model may be more reliable for this group. While the three Weibull distribution models for milk allergic patients were not significantly different from each other, the distributions for patients who had mild or severe reactions were indistinguishable (Fig. 2B).

The ED_{10} (95% CI) estimated by the Weibull distribution model for soy allergic patients was 10.6 mg (3.4 to 30.9 mg) and for egg allergic patients was 25.2 mg (14.0 to 42.5 mg).

Table 5
 ED_{10} doses (mg protein) and the 95% confidence interval for peanut and milk allergic patients assessed by Weibull and log-normal probability distribution models.

Grade	Peanut (mg protein)		Milk (mg protein)	
	Weibull	Log-normal	Weibull	Log-normal
Mild	0.05 (0.01,0.2)	0.1 (0.04,0.5)	15.1 (5.7,39.6)	18.8 (8.0,43.8)
Moderate	0.5 (0.2,1.2)	0.9 (0.4,2.0)	51.4 (23.2,111.7)	63.9 (31.0,132.1)
Severe	3.6 (1.2,10.5)	4.3 (1.7,11.5)	21.4 (7.6,58.2)	38.2 (16.9,94.0)
Combined	0.4 (0.2,0.8)	0.7 (0.4,1.3)	25.2 (15.2,42.0)	32.9 (19.0,52.8)

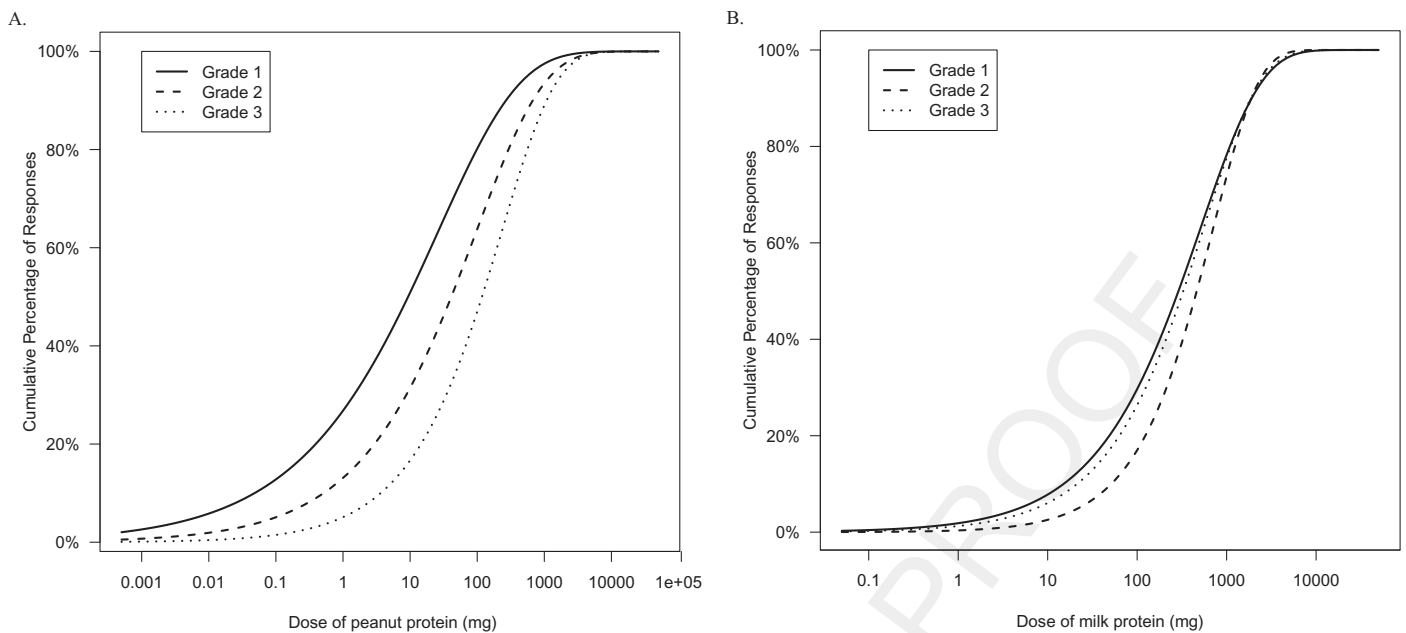


Fig. 2. Weibull probability distribution models of peanut and milk threshold doses (in milligrams of protein) for each severity grade of peanut or milk allergic patients: (A) peanut; (B) milk.

4. Discussion

The main objective of this study was to carry out a retrospective analysis of published data from oral food challenge studies to evaluate the potential relationship between MEDs and reaction severities at those MEDs in the food allergic population. Using an integrated scoring system, we found that peanut allergic patients who experienced severe reactions had significantly higher MEDs and threshold doses than those who experienced mild or moderate reactions. In contrast, no significant differences were seen for milk, egg and soy allergic patients.

The result for peanut allergic patients is consistent with anecdotal clinical observations that many reactions occurring at low MEDs during peanut challenges are relatively mild. However, this does not mean that low doses of peanut are safe for peanut allergic patients, as doses as low as 0.3 mg of peanut protein have been observed to cause severe reactions (Wensing et al., 2002b). In addition it is possible that severe reactions occurred in those patients who reacted at low doses but whose symptoms were not reported. That there were no significant differences in MEDs between the severity grades for milk, egg and soy allergic patients also suggests that those patients could experience a severe reaction when exposed to a small amount of allergen.

The ED₁₀ for peanut allergic patients was 0.4 mg protein when determined by the Weibull or 0.7 mg protein by the log-normal distribution model. These correspond to 1.4 or 2.8 mg whole peanut respectively, which are lower than the ED₁₀'s reported in some other published studies (Blumchen et al., 2014; Eller et al., 2012; Taylor et al., 2009, 2010), but were similar to the value (0.52 mg protein) reported by Blom et al., 2013. This difference could be due to the fact that, by using data from multiple studies, we modeled a different population and we included data from patients with subjective symptoms such as OAS who have not been included in most of other studies (the 0.52 mg protein from Blom et al., 2013 was derived from patients with both subjective and objective symptoms). The fact that we excluded all the right-censored individuals in our analysis may also affect the ED estimates and skew them to be more sensitive. Allen et al. (2014) and Taylor et al. (2013) were able to use a large dataset that included both published and unpublished clinical records. Many, but not all, of the studies included in their analysis were also

included ours; thus the differences in EDs could also be due to modeling the different subsets of patients.

Our estimated ED₁₀ values for milk allergic patients (25.2 mg protein by the Weibull model and 32.9 mg protein by the log-normal model) lie in between the ED₁₀ values of 4.14 mg protein reported by Blom et al. (2013) and 100.2 mg protein reported by Eller et al. (2012). This may suggest that the threshold doses for different milk allergic populations differ substantially. Our estimated ED₁₀ for egg allergic individuals (23.9 mg protein) was much higher than the values reported by Blom et al. (2013) (5.36 mg protein) or Eller et al. (2012) (5.82 mg protein). This could reflect differences in the number of individuals considered in our analysis because symptoms were not reported for many of the individuals included by Blom et al. (2013) and Eller et al. (2012). We were not able to collect a good amount of data for soy allergic individuals, mainly because there were not much challenge data published and available (Allen et al., 2014).

The chief limitation of our analysis is that it was a retrospective analysis of data from multiple studies. Different studies may have different challenge protocols, different food matrices, different time intervals between doses, and different reporting of symptoms and eliciting doses. For example, some OFC trials excluded patients who had a history of severe reactions, possibly excluding those most likely to develop severe reactions during challenges (Glaumann et al., 2012; Nicolaou et al., 2010). Further, the published studies used different forms of the test material and different blinding vehicles which may affect the apparent MED (Grimshaw et al., 2003; Mackie et al., 2012). However, as discussed in Crevel et al., 2007, this heterogeneity of the population "might have the advantage of "averaging out" these differences so that the final merged dataset better resembles the exposure scenarios which the wider allergic population experiences while increasing the statistical power of the predicted estimates" (Crevel et al., 2007). Ideally, a well-designed prospective study such as that described by Crevel et al. (2008) for the Europevall project would be able to overcome many of these limitations. However, it is appropriate to use all available data (both prospective and retrospective) in a food safety risk assessment.

A second limitation is that the grading of reaction severity was based on our integrated grading system. Though our grading system

was based on integration of previously published schemes, it is clear that there is no single universally accepted system for classifying allergic reaction severity. It is possible that some of the reaction symptoms would have been graded differently if a different grading system had been used. This could also account for the fact that some other studies did not find any appreciable difference in MEDs for different reaction severities for peanut allergic patients (Flinterman et al., 2006b; Glaumann et al., 2012). Ideally, a consensus grading system with detailed descriptions of individual symptoms should be developed through broad discussions among different stakeholders, including patients.

Several of the oral challenge studies used well-designed protocols and established the MEDs for each patient tested but did not report the observed MEDs for each individual (Fleischer et al., 2013; Rance et al., 2002), while other studies reported the MEDs but not the symptoms associated with them (Benhamou et al., 2008; Blom et al., 2013; Blumchen et al., 2010; Knight et al., 2006; Leung et al., 2003; Longo et al., 2008; Morisset et al., 2003; Nelson et al., 1997; Skripak et al., 2008). To evaluate the impact of the missing symptom information, a Bayesian model was developed for the peanut data, where the (unobserved) grade for individuals without recorded symptoms was considered as a latent variable. The prior probability belonging to a given grade was specified using an uninformative Dirichlet distribution. The MED and NOAEL data for all patients were fit to three independent Weibull distributions. Uninformative prior distributions were used for the Weibull parameters. The model was evaluated using JAGS (Plummer, 2003). Incorporating data from individuals without recorded symptoms in the model increased the uncertainty around the estimates (data not shown), but did not change the conclusion that the ED₁₀ of the peanut allergic patients who had severe reactions (ED₁₀ = 3.0 mg protein) was higher than those who had mild (ED₁₀ = 0.09 mg protein) or moderate (ED₁₀ = 0.5 mg protein) reactions.

There were a large number of patients who participated in the challenges in the studies we reviewed but were not included in our analysis, either because of incomplete information (such as missing symptoms and/or MEDs) or they did not react during the challenges. Patients who did not react to the highest dose given in a food challenge might have reacted to a higher dose. However, we could not determine how severe their possible reactions would have been. Therefore, we were not able to include these patients in our analysis, which is a source of uncertainty in our analysis.

To our knowledge, this is the first study to investigate the relationship between MEDs and reaction severities at the MEDs with a large population and for four major food allergens (peanut, milk, egg and soy). Given that no significant difference in the MEDs and threshold doses were found between mild and moderate reaction groups for any of the four allergens, it may be reasonable to compare the severity of food allergic reactions based on only two categories: mild/moderate and severe in the future. The relationship between threshold dose distribution and reaction severity based on these grading criteria differed between peanut and other allergens, and severe reactions occurred in some patients at low MEDs for all of these food allergens.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.fct.2015.02.023.

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Appendix: Supplementary Table. The eight published grading systems we evaluated for developing the integrated grading system.

Grade	1 (mild)	2 (mild+)	3 (moderate)	4 (moderate+)	5 (severe)
Sampson 2003	Skin: localized pruritus, flushing, urticaria, angioedema; GI: oral pruritus, oral “tingling,” mild lip swelling	Skin: generalized pruritus, flushing, urticaria, angioedema; GI: any of the above, nausea and/or emesis x’s 1; Respiratory: nasal congestion and/or sneezing; Neurological: change in activity level	Skin: any of the above; GI: any of the above plus repetitive vomiting; Respiratory: rhinorrhea, marked congestion, sensation of throat pruritus or tightness; Cardiovascular: tachycardia (increase 15 beats/min); Neurological: change in activity level plus anxiety	Skin: any of the above; GI: any of the above plus diarrhea; Respiratory: any of the above, hoarseness, “barky” cough, difficulty swallowing, dyspnea, wheezing, cyanosis; Cardiovascular: any of the above, dysrhythmia and/or mild hypotension; Neurological: “Light headedness,” feeling of “pending doom”	Skin: any of the above; GI: Any of the above, loss of bowel control; Respiratory: any of the above, respiratory arrest; Cardiovascular: severe bradycardia and/or hypotension or cardiac arrest; Neurological: loss of consciousness
Evan and Clark, 2001	(mild) localized cutaneous erythema/urticaria/ angioedema/oral pruritis	(mild) generalised erythema/ urticaria/angio-oedema	(mild) at least grade 1 or 2 plus gastrointestinal symptoms/ rhinoconjunctivitis	(moderate) mild laryngeal oedema (voice change/ tightening of throat)/mild asthma	(severe) pronounced dyspnoea/ hypotensive symptoms (light-headedness/ collapse/loss of consciousness)
Cox et al., 2010	Symptom(s)/sign(s) of 1 organ system present <u>Cutaneous</u> : generalized pruritus, urticaria, flushing, or sensation of heat or warmth or Angioedema (not laryngeal, tongue or uvular) or <u>Upper respiratory</u> : Rhinitis - (eg, sneezing, rhinorrhea, nasal pruritus and/or nasal congestion) or Throat-clearing (itchy throat) or Cough perceived to originate in the upper airway, not the lung, larynx, or trachea or <u>Conjunctival</u> : Erythema, pruritus or tearing <u>Other</u> : Nausea, metallic taste, or headache	Symptom(s)/ sign(s) of more than 1 organ system present or <u>Lower respiratory</u> Asthma: cough, wheezing, shortness of breath (eg, less than 40% PEF or FEV1 drop, responding to an inhaled bronchodilator) or <u>Gastrointestinal</u> Abdominal cramps, vomiting, or diarrhea or <u>Other</u> Uterine cramps	<u>Lower respiratory</u> : Asthma (eg, 40% PEF or FEV1 Drop NOT responding to an inhaled bronchodilator) or <u>Upper respiratory</u> Laryngeal, uvula, or tongue edema with or without stridor	<u>Lower or upper respiratory</u> Respiratory failure with or without loss of consciousness or <u>Cardiovascular</u> Hypotension with or without loss of consciousness	Death
Rank et al., 2008	Mild systemic reactions: localized urticaria, rhinitis or mild asthma (PF < 20% decrease from baseline)	Moderate systemic reactions: slow onset (>15 min) of generalized urticaria and/or moderate asthma (PF < 40% decrease from baseline)		Severe (nonlife-threatening) systemic reactions: rapid onset (<15 min) of generalized urticaria, angioedema, or severe asthma (PF > 40% decrease from	Anaphylactic shock: immediate itching, flushing, erythema, generalized urticaria, stridor (angioedema), asthma, hypotension

				baseline)	
Mueller, 1966	Slight general reaction: generalized urticaria, itching, malaise and anxiety	General reaction: any of the above plus two or more of the following: generalized edema; constriction in chest; wheezing; abdominal pain, nausea and vomiting; and dizziness		Severe general reactions: any of the above plus two or more of the following: dyspnea; dysphagia; hoarseness or thickened speech; confusion; and feeling of impending disaster	Shock reaction: any of the above plus two or more of the following: cyanosis; fall in blood pressure; collapse incontinence; and unconsciousness
Brockow and Ring, 2009	Dermal: pruritus, flush, urticaria, angioedema		Dermal: pruritus, flush, urticaria, angioedema (not mandatory); Abdominal: nausea, cramping; Respiratory: rhinorrhea, hoarseness, dyspnea; Cardiovascular: tachycardia (> 20 bpm), hypotension (>20 mmHg systolic), arrhythmia	Dermal: Pruritus, flush, urticaria, angioedema (not mandatory); Abdominal: vomiting, defecation, diarrhea; Respiratory: laryngeal edema, bronchospasm, cyanosis; Cardiovascular: shock	Dermal: pruritus, flush, urticaria, angioedema (not mandatory); Abdominal: vomiting, defecation, diarrhea; Respiratory: respiratory arrest; Cardiovascular: cardiac arrest
Brown 2004	Mild (skin and subcutaneous tissues only): generalized erythema, urticaria, periorbital edema, or angioedema		Moderate (features suggesting respiratory, cardiovascular, or gastrointestinal involvement): dyspnea, stridor, wheeze, nausea, vomiting, dizziness (presyncope), diaphoresis, chest or throat tightness, or abdominal pain		Severe (hypoxia, hypotension, or neurologic compromise): cyanosis or SpO ₂ ≤ 92% at any stage, hypotension (SBP < 90 mm Hg in adults), confusion, collapse, loss of consciousness, or incontinence
Flinterman et al., 2007	oral cavity, the skin and/or mucous membranes (urticaria, angio-dedema, rhinitis, conjunctivitis)	gastrointestinal symptoms (diarrhea, vomiting, nausea, abdominal pain)		respiratory symptoms (bronchoconstriction, stridor, hoarseness) and/or cardiovascular symptoms	