

Hypothesis paper

Can we determine a threshold level for allergenic foods by statistical analysis of published data in the literature?

Background: The aim of this paper was to investigate whether a statistical model could be developed to estimate a “threshold” dose for foods eliciting allergic reactions in susceptible patients. The threshold dose is defined to be one that elicits allergic reactions in a given (small) proportion of susceptible patients, using data from published studies.

Methods: Based on data available from the literature, we developed a statistical model using the actual allergen content in the four foods, where data for allergen content are available (peanut, soy, egg, milk).

Results: The model demonstrated that the threshold doses giving a reaction of one in a million in susceptible patients were within the same order of magnitude for egg, milk and soy, but were an order of magnitude lower for peanut flour: 0.005 mg of cow’s milk, 0.002 mg of fresh hen’s egg, 0.0007 mg of peanut, or 0.0013 mg of soy flour.

Conclusions: Although several assumptions were made in creating this statistical model, we demonstrated that the previously published differences in threshold doses for various foods can be largely eliminated by comparing actual allergen content; this may therefore serve as a model for further studies.

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Patients with food allergies (1) always face the risk of allergic reactions by inadvertent intake of the offending food(s). In very sensitive patients, minute amounts may elicit severe reactions – systemic reactions to micrograms of food have been reported (2, 3). Furthermore, food production is becoming increasingly complex, with several different food products being made in the same facility, which carries the risk of cross-contamination of allergenic compounds (4). Labelling of foods is therefore a central measure to avoid allergic reactions in susceptible individuals. However, no hard data exist on a possible threshold dose of contamination below which no allergic patient is likely to experience symptoms, and therefore a zero level is currently used. Industry has responded with a proliferation of precautionary labelling (“may contain peanut”)-an approach which must be used with caution, only when appropriate, and not for legislative reasons.

Without available data on a threshold dose for a specific food, it is neither possible to conduct risk assessments nor to focus quality control on efforts which bring the greatest benefit to the allergic consumer. If different foods really do demonstrate different thresholds, then this problem is further increased for example, the lowest eliciting dose of milk is different (higher) than that of peanut.

At a conference held in South Carolina, USA in 1999, scientists focused on the lowest eliciting dose of an allergenic food. This data demonstrated that the lowest doses are probably in the microgram range, with large differences between various foods (5).

Presently there is no agreement on a common procedure for performing food challenges. Differences in such procedures add to the difficulties in comparing studies. In addition, it is often difficult to obtain a sufficiently large number of patients to base risk assessment calculations on, as many studies use very low numbers of subjects.

The aim of this paper was to investigate, using data from published studies, whether a statistical model could be developed to estimate a “threshold” dose that is, a dose which elicits allergic reactions in a given proportion of susceptible patients.

Material and methods

A MEDLINE survey on clinical trials using open food challenges (OFC), single-blind challenges (SBFC) or double-blind, placebo-controlled food challenges (DBPCFC) was first conducted and yielded 414 publications. After reading the publications, 25 papers were selected based on the availability of dose-response data and a suitable

number of patients. These data were compiled and used for the subsequent analyses. Data on children and adults were included, as were all types of immediate allergic reactions.

A cumulative distribution function (plotting cumulative number of reacting patients against log dose) was used to model this type of dose-response data. A survival analysis approach was then taken to fit a mathematical equation to the log-transformed data, with a normal distribution function being used to model the shape of the curve. Confidence intervals were then calculated and subsequently added to this curve (which can be interpreted as a dose-response curve). The threshold, as defined in this paper, is the lower confidence limit on the dose that results in an allergic response in a given proportion of the studied population. This idea is very close in concept to the “benchmark dose” approach to calculating toxicity “thresholds” as developed by Crump (6). In this paper, results are presented for a one-per-million response rate, as well as a one-per-hundred response rate. Taking the lower confidence limit partly accounts for the experimental and protocol variability inherent in this type of analysis.

The allergen content of different foods varies for example, the protein fraction of cow’s milk only constitutes 3.6 %, which corresponds to 36 mg of allergen per gram of milk. However, de-fatted soy flour contains mostly allergenic proteins (7, 8).

Furthermore, different sources of food were used for challenge, i.e. freeze-dried food, de-fatted food or whole unprocessed food. Therefore, in order to enable comparisons between the different trials, a conversion from the actual food used in the trial to a calculated content of allergen was subsequently performed. A literature search revealed that for cow’s milk, hen’s egg, peanut and soy, quantitative data for allergen content was available, and so these four foods were chosen for subsequent analysis. These represented 68 published papers on milk, 48 on egg, 33 on peanut, and 33 on soy Table 2.

Results

Table 2 presents the publications generated by the MEDLINE search, together with the number of publications where sufficient dose-response data were available. Both dose-response data and data on the quantitative allergen content were available for only milk, egg, peanut and soy, therefore these four foods were investigated further. Regarding celery and carrot, one well-conducted trial of each has recently been

published (9, 10) but quantitative data on allergen content in celery or carrot are not available and therefore these studies are not included. Regarding hazelnut, one large-scale multicentre trial has been conducted that included dose-response investigations, but the data were published as mean values, so this investigation was not used (11).

An example of the type of model fitted to some dose-response data, for each of the four foods, is presented in Fig. 1(A). The *x*-axis represents the (log of) amount of food in the preparation actually used in the study, while the *y*-axis represents the cumulative proportion of responders. For the studies presented in Fig. 1(A), the lower confidence limit associated with a one-per-million response rate (referred to hereafter as the “threshold dose”) was calculated to be 0.046, 0.001, 0.0023 and 2279 mg for milk, egg, peanut and soy, respectively.

In Fig. 1(B), the amount of food actually used in the separate trials was converted to calculated allergen content, and the studies were pooled within each food “type” (here, therefore, “dose” means the amount of allergen protein). Based on these pooled data, the calculated threshold values for the allergenic protein were 7.1×10^{-5} , 3.3×10^{-6} , 5×10^{-4} , and 0.3 mg, for milk, egg, peanut and soy, respectively. Table 3 shows the predicted dose and the lower confidence limit (threshold) for two different proportions of the susceptible population (one in a hundred and one in a million) giving positive responses for each food. This conversion to calculated allergen content allows for direct comparisons between the different foods. As can be seen, after conversion the threshold values for egg, milk and peanut are within a factor of 100, whereas that obtained for soy is higher (Table 4)

The conversion from allergen intake back to food intake is shown in Table 4. The lower threshold for egg and peanut corresponds well to the clinical finding that allergies to peanut and egg are usually more severe than to milk and soy. Again, the threshold values for egg, milk and peanut are within a factor of 100, whereas that obtained for soy is higher.

Finally, Fig. 2 demonstrates the curve fitted to all data, for all studies included and for all four foods combined. The resulting predicted threshold giving a one-in-a-million response rate is 7×10^{-4} mg with the lower confidence interval being 1.8×10^{-4} mg. By conversion back to the weight of actual food, this would represent 0.005 mg of cow’s milk, 0.002 mg of fresh hen’s egg, 0.0007 mg of peanut or 0.0013 mg of soy (made up weight). Based on these thresholds, it can be seen that the egg, milk and peanut thresholds are within a factor of 10, whilst soy is one order of magnitude higher. If a response rate of one in a hundred is examined, the threshold values are found to be 8.6 mg, 3.4 mg, 1.2 mg and 2.2 mg for milk, egg, peanut and soy flour, respectively.

Table 1 Food type and formulation, total protein content and percentage of allergen within

Food	Formulation	Protein content (% of total protein)	Allergen content number	Reference
Cow’s milk	Skimmed powder	37.5	100	(7, 15, 16)
	Fresh whole	3.6	100	(7, 17, 18)
	Freeze-dried	37.5	100	(7, 19)
Egg	Raw	26	80	(18, 20, 21)
	Dried whole	84	80	(15, 21, 22)
	Egg white	10	80	(21, 23)
Peanut	Whole	25	100	(15, 23-25)
	Flour	45	100	(25, 26)
Soy	Soybean	40	95	(27)
	Isomil	1.8	95	(27, 28)
	Isomil	1.4	95	(27, 29)

Table 2 Analysis of reviewed studies, showing how many give data on dose-responses, patient responses to the first challenge, and allergen contents of foods

Food	Number of studies			Dose-response data available	References	Patients reacting to first dose	Allergen content known
	OFC	SBFC	DBPCFC				
Cow milk	16	1	51	5 of 68	(15-19)	13 of 111	Yes
Egg	7	1	40	5 of 48	(15, 18, 20, 22, 23, 30)	2 of 41	Yes
Peanut	14	1	18	4 of 33	(15, 23, 24, 26)	6 of 40	Yes
Soy	6	1	23	2 of 33	(28, 29)	0 of 18	Yes
Cod	0	1	6	2 of 7	(31, 32)	5 of 12	No
Celery	4	0	6	1 of 6	(10)	10 of 21	No
Wheat	6	1	30	No		–	No
Hazelnut	3	1	3	No		–	No
Mustard	0	2	0	Yes		2 of 15	No
Shrimp	0	1	6	No		–	No
Others	78	15	75	6 of 168	(15, 32–36)	17 of 76	No

OFC: open food challenge] SBFC: single-blind food challenge] DBPCFC: double-blind, placebo-controlled food challenge.

Discussion

In this paper a threshold dose has been defined as that which elicits an allergic reaction in a given proportion of the patients included in the analysis. Based on published data, we were able to create a dose-response curve which enabled determination of such a threshold dose. Determination of a common threshold dose for the four foods studied was enabled by conversion from the amount of food used for the actual challenges to an estimation of the actual allergen content in the preparation, and then using this value as the dose administered to the patients. This conversion revealed that the allergen thresholds of egg, milk and peanut are of the same magnitude (higher for soy). It also shows that the previously published differences found in threshold doses of different foods can be largely eliminated by comparing actual allergen content, which varies from 3.6 % in milk to 95 % per cent in de-fatted soy flour. This model may serve as the basis for further studies.

There are however, several limitations to our approach. Firstly, the true threshold dose may be lower than calculated for two reasons:

1) the most sensitive patients presenting case histories of severe reactions to very minute amounts of the offending food are excluded from most clinical studies

2) in all trials included in this study, a number of patients reacted to the first administered dose (Table 1),

implying that the lowest dose eliciting a response for these subjects would be lower than the lowest dose tested.

Both the exclusion of the most sensitive patients from challenge and the inclusion of patients reacting to the first dose will result in overestimation, and therefore too high a threshold. The true thresholds for this sensitive subpopulation may thus be lower than the values presented here, although by taking the lower confidence limit some conservatism is built into the approach. A no observed effect level (NOEL), below which no patients will experience a reaction, also cannot be determined, due to the inclusion of patients who react to the first administered dose.

Furthermore, there are other limitations in our approach, requiring precaution when interpreting the findings:

1) We compared different trials using different protocols. To make this approach feasible we had to assume that children and adults react identically. We also had to assume that there were no significant differences in certain parameters, such as the inclusion criteria for different patients, the symptoms, the food source, the dose used for the first challenge, dose-increment protocol, time between doses, top dose, and interpretation of a positive result (12), together with the fate of patients reacting to placebo challenges in the blinded protocols (13). Such an assumption must be

Table 3 Predicted doses and lower confidence limits for the four foods investigated expressed as mg allergen

Food type	One per million of population*		One per hundred of population*	
	Lower confidence limit	Predicted	Lower confidence limit	Predicted
Cow's milk (mg)	7.1×10^{-5}	5.9×10^{-4}	0.28	0.87
Egg (mg)	3.3×10^{-6}	9.7×10^{-5}	0.024	0.15
Peanut (mg)	5.0×10^{-4}	4.9×10^{-3}	0.19	0.66
Soy (mg)	0.30	2.4	12.9	41
All allergens (mg)	1.8×10^{-4}	7.0×10^{-4}	0.31	0.66

*Proportion of population giving positive responses.

Table 4 Predicted doses and lower confidence limits for the four foods investigated expressed as mg food consumed. Data are expressed as whole milk (allergenic protein content 3.6%), raw egg (allergenic protein content 9%), whole peanut (allergenic protein content 25%) and isomil soy flour (allergenic protein content 13.8%)

Food type	One per million of population*		One per hundred of population*	
	Lower confidence limit	Predicted	Lower confidence limit	Predicted
Cow's milk (mg)	2.0×10^{-3}	1.6×10^{-2}	7.76	24.62
Egg (mg)	3.6×10^{-5}	1.1×10^{-3}	0.26	1.69
Peanut (mg)	2.0×10^{-3}	2.0×10^{-2}	0.76	2.65
Soy (mg)	2.2	17.7	93.6	295

*Proportion of population giving positive responses.

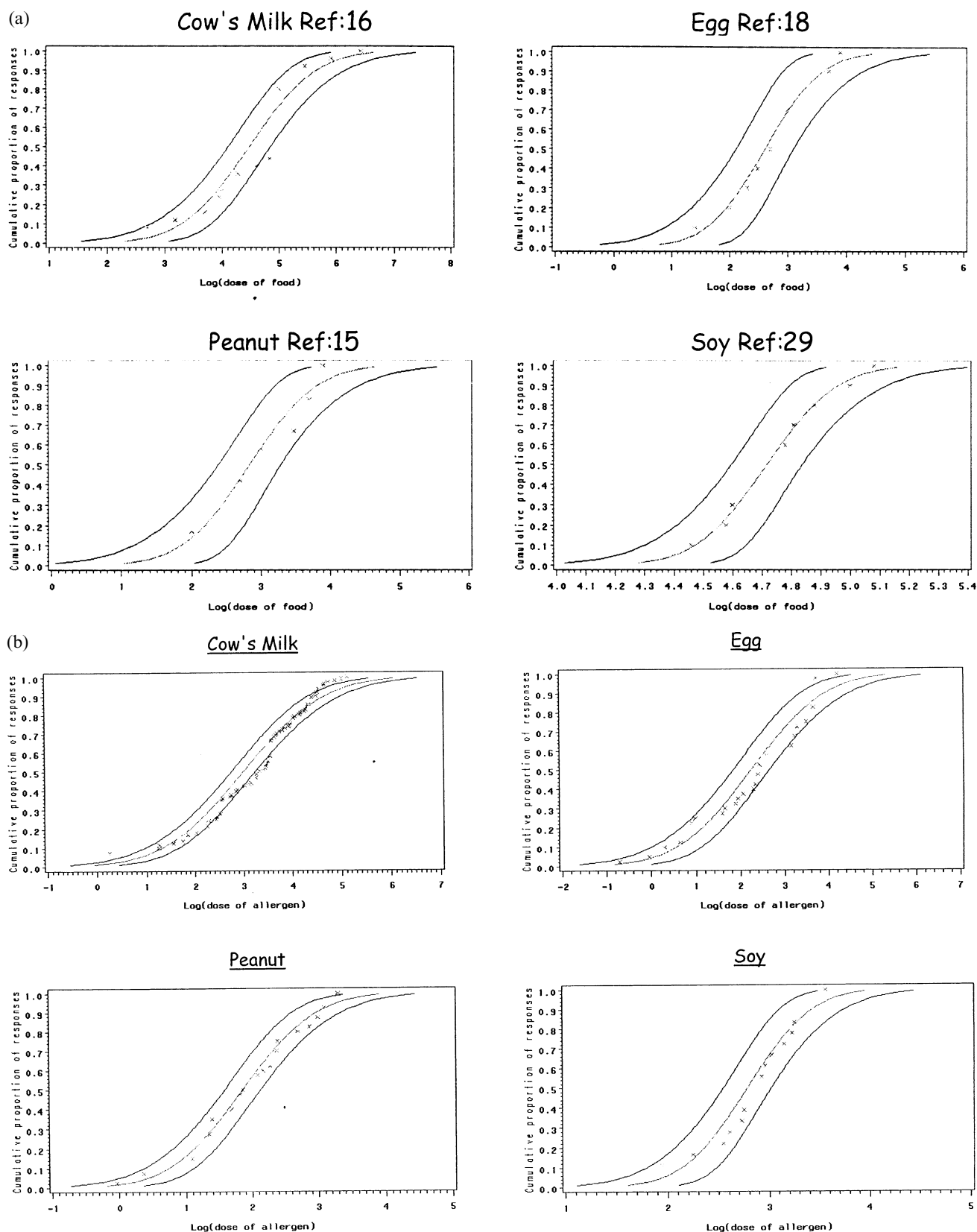


Figure 1

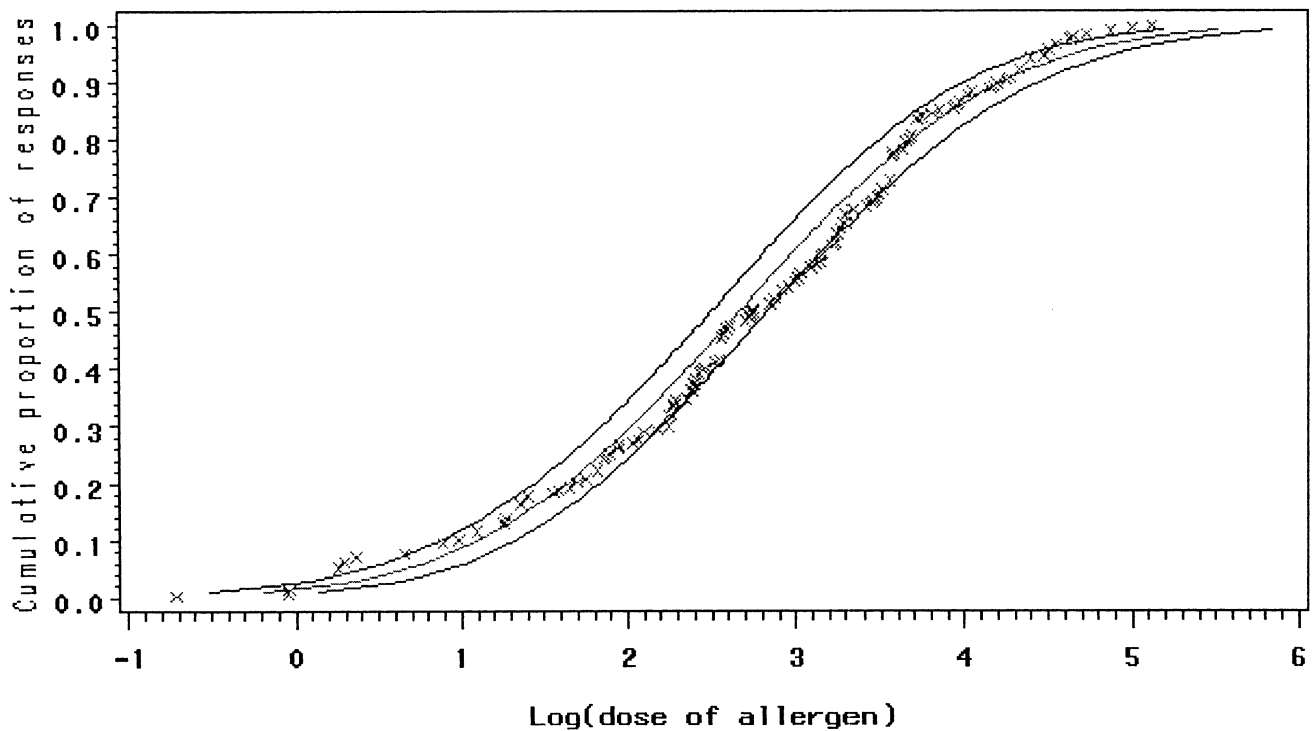


Figure 2

used with caution. Furthermore, we also assumed that the presence of the expected amounts of allergens in the preparations and that all the known allergens in a particular food were of equal importance to all patients but this may not always be true.

4) In the calculations we assumed that the natural variability of the protein content was zero. This is especially not true for soy, where the allergen content may vary by up to two-fold depending on where it is grown (14), and it may account for the different results obtained for soy compared to the other foods.

Despite these limitations, we have demonstrated for the first time an approach which may be helpful both to manufacturers and to government bodies who responsible for producing guidelines for risk assessment, and for subsequent legislation in this field.

In order to verify and substantiate the model, particularly to determine the thresholds with increased accuracy, the following approach is recommended:

1) Compiling raw data from various well-conducted clinical trials in collaboration with the original authors of these studies. Contact with the relevant individuals is in progress.

2) Development of a standardized challenge protocol to be used by all investigators in future trials. Such a protocol must ensure a sufficiently low starting dose for the challenge to avoid patients who react to the first dose.

3) Subsequently, prospective multicentre trials, that fulfil the above criteria, should be conducted using different foods in order to verify or discard the model.

This work is currently being finalized under the auspices of the European Academy of Allergy and Clinical Immunology.

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References

1. JOHANSSON SGO, HOURIHANE JO'B et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;**56**:813–824.
2. WUTHRICH B. Lethal or life-threatening allergic reactions to food. *J Invest Allergol Clin Immunol* 2000;**10**:59–65.
3. WUTHRICH B, BALLMER-WEBER BK. Food-induced anaphylaxis. *Allergy* 2001;**56**:102–104.
4. TAYLOR SL, HEFLE SL. Ingredient and labelling issues associated with allergenic foods. *Allergy* 2001;**56**:64–69.

5. TAYLOR SL, HEFLE SL, BINDSLEV-JENSEN C et al. Factors affecting the determination of threshold doses for allergenic foods: how much is too much? *J Allergy Clin Immunol* 2002;**109**:24–30.
6. CRUMP KS. A new method for determining allowable daily intakes. *Fundam Appl Toxicol* 1984;**4**:854–871.
7. SWAISGOOD HE. Food chemistry. Characteristics of Edible Fluids of Animal Origin: Milk, 2nd edition. New York: Marcel Dekker, 1985:791–827.
8. DAUSANT J, MOSSE J, VAUGHAN J. Breeding for protein quantity and protein quality in seed crops. *Seed Proteins*. London: Academic Press 1983:223–253.
9. BALLMER-WEBER BK, WUTHRICH B, WANGORSCH A, FOTISCH K, ALTMANN F, VIETHS S. Carrot allergy: double-blind, placebo-controlled food challenge and identification of allergens. *J Allergy Clin Immunol* 2001;**108**:301–307.
10. BALLMER-WEBER BK, VIETHS S, LUTTKOPF D, HEUSCHMANN P, WUTHRICH B. Celery allergy confirmed by double-blind, placebo-controlled food challenge: a clinical study in 32 subjects with a history of adverse reactions to celery root. *J Allergy Clin Immunol* Aug 2000;**106**:373–378.
11. ORTOLANI C, BALLMER-WEBER BK, HANSEN KS et al. Hazelnut allergy: a double-blind, placebo-controlled food challenge multicenter study. *J Allergy Clin Immunol* Mar 2000;**105**:577–581.
12. BINDSLEV-JENSEN C. Standardization of double-blind, placebo-controlled food challenges. *Allergy* 2001;**56**(Suppl.):75–77.
13. BRIGGS D, ASPINALL L, DICKENS A, BINDSLEV-JENSEN C. Statistical model for assessing the proportion of subjects with subjective sensitisations in adverse reactions to foods. *Allergy* 2001;**56**:83–85.
14. STEN EP, STAHL SKOV SB, ANDERSEN AM, et al. Comparative study of the allergenic potency of wild type and glyphosate tolerant gene modified soybean cultivars. *Intern Arch Allergy Immunol* 2002 (in press).
15. BOCK SA, LEE WY, REMIGIO LK, MAY CD. Studies of hypersensitivity reactions to foods in infants and children. *J Allergy Clin Immunol* 1978;**62**:327–334.
16. BAEHLER P, CHAD Z, GURBINDO C, BONIN AP, BOUTHILLIER L, SEIDMAN EG. Distinct patterns of cow's milk allergy in infancy defined by prolonged, two-stage double-blind, placebo-controlled food challenges. *Clin Exp Allergy* 1996;**26**:254–261.
17. HILL DJ, DUKE AM, HOSKING CS, HUDSON IL. Clinical manifestations of cows' milk allergy in childhood. II. The diagnostic value of skin tests and RAST. *Clin Allergy* 1988;**18**:481–490.
18. NORGGAARD A, BINDSLEV-JENSEN C. Egg and milk allergy in adults. Diagnosis characterization. *Allergy* 1992;**47**:503–509.
19. PASTORELLO EA, STOCCHI L, PRAVETTONI V et al. Role of the elimination diet in adults with food allergy. *J Allergy Clin Immunol* 1989;**84**:475–483.
20. EGGESBO M, BOTTEN G, HALVORSEN R, MAGNUS P. The prevalence of allergy to egg: a population-based study in young children. *Allergy* 2001;**56**:403–411.
21. POWRIE WD, NAKAI S. Characteristics of edible fluids of animal origin: eggs. *Food chemistry*, 2nd edition. New York: Marcel Dekker 1985:829–855.
22. CAFFARELLI C, CAVAGNI G, GIORDANO S, STAPANE I, ROSSI C. Relationship between oral challenges with previously uningested egg and egg-specific IgE antibodies and skin prick tests in infants with food allergy. *J Allergy Clin Immunol* 1995;**95**:1215–1220.
23. MAY CD. Objective clinical and laboratory studies of immediate hypersensitivity reactions to foods in asthmatic children. *J Allergy Clin Immunol* 1976;**58**:500–515.
24. MONERET-VAUTRIN DA, RANCE F, KANNY G et al. Food allergy to peanuts in France – evaluation of 142 observations. *Clin Exp Allergy* 1998;**28**:1113–1119.
25. KOPPELMAN SJ, VLOOSWIJK RA, KNIPPELS LM et al., Quantification of major peanut allergens Ara h 1 and Ara h 2 in the peanut varieties Runner, Spanish, Virginia, and Valencia, bred in different parts of the world. *Allergy* 2001;**56**:132–137.
26. HOURIHANE JO'B, KILBURN SA, NORDLEE JA, HEFLE SL, TAYLOR SL, WARNER JO. An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: a randomized, double-blind, placebo-controlled food challenge study. *J Allergy Clin Immunol* 1997;**100**:596–600.
27. SHIBASAKI M, SUZUKI S, TAJIMA S, NEMOTO H, KUROUME T. Allergenicity of major component proteins of soybean. *Int Arch Allergy Appl Immunol* 1980;**61**:441–448.
28. MAGNOLFI CF, ZANI G, LACAVA L, PATRIA MF, BARDARE M. Soy allergy in atopic children. *Ann Allergy Asthma Immunol* 1996;**77**:197–201.
29. ZEIGER RS, SAMPSON HA, BOCK SA et al. Soy allergy in infants and children with IgE-associated cow's milk allergy. *J Pediatr* 1999;**134**:614–622.
30. BOCK SA, MUNOZ-FURLONG A, SAMPSON HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001;**107**:191193.
31. HANSEN TK, BINDSLEV-JENSEN C. Codfish allergy in adults. Identification Diagnosis *Allergy* 1992;**47**:610–617.
32. HELBLING A, HAYDEL RJ, McCANTS ML, MUSMAND JJ, EL-DAHR J, LEHRER SB. Fish allergy: is cross-reactivity among fish species relevant? Double-blind placebo-controlled food challenge studies of fish allergic adults. *Ann Allergy Asthma Immunol* 1999;**83**:517–523.
33. PASTORELLO EA, PRAVETTONI V, FARIOLI L et al. Clinical role of a lipid transfer protein that acts as a new apple-specific allergen. *J Allergy Clin Immunol* 1999;**104**:1099–1106.
34. TANAKA LG, EL-DAHR JM, LEHRER SB. Double-blind, placebo-controlled corn challenge resulting in anaphylaxis. *J Allergy Clin Immunol* 2001;**107**:744.
35. BELLIONI-BUSINCO B, PAGANELLI R, LUCENTI P, GIAMPIETRO PG, PERBORN H, BUSINCO L. Allergenicity of goat's milk in children with cow's milk allergy. *J Allergy Clin Immunol* 1999;**103**:1191–1194.
36. REINDL J, ANLIKER MD, KARAMLOO F, VIETHS S, WUTHRICH B. Allergy caused by ingestion of zucchini (*Cucurbita pepo*): characterization of allergens and cross-reactivity to pollen and other foods. *J Allergy Clin Immunol* 2000;**106**:379–385.