Standardization, validation and outcome of double-blind, placebo-controlled food challenges in children

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About the cover
Incrementing amounts of challenged foods as sequentially administered in test food material in the double-blind, placebo-controlled food challenge (DBPCFC) and subsequent open challenge or home introduction in children at the Food Challenge Unit of the University Medical Center Groningen

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Aan mijn echtgenoot Henk
mijn kinderen Marenne en Miriam

Our little systems have their day;
They have their day and cease to be:
They are but broken in lights of thee
And thou, O Lord, art more than they.

Alfred Tennyson (1809 – 1892)
1849, In Memoriam A.H.H

Paranimfen: Henk Vlieg
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Contents

Chapter I General Introduction

1.1. Accumulating knowledge of food allergy during the last few decades

The understanding of allergic disease by the medical profession is a relatively young phenomenon. Although the technique of skin prick testing originated around 1880, the immunologic mechanism of this test and the diseases with which it was associated was unknown at that point[1]. This lack of understanding was due to the fact that the causative immunoglobulin IgE was discovered only recently[2]. The discovery of IgE had a significant impact on the understanding of the disease, because this greatly improved our understanding of allergic inflammation and diagnoses as well as treatment of allergic diseases[3]. From that moment onwards, it was possible to quantify and measure sensitization in serum[4]. With the discovery of IgE, it became obvious that certain symptoms could be caused by IgE directed towards specific foods in patients with food allergy. Along with the increasing knowledge about food allergy and the mechanisms behind the disease, different nomenclatures emerged over the years[5,6]. Currently, according to an EAACI position paper, the main encompassing term of adverse reactions to food is "food hypersensitivity" (Figure 1). When immunologic mechanisms have been demonstrated, the appropriate term is "food allergy", which can either be "IgE-mediated food allergy" or "non-IgE-mediated food allergy".

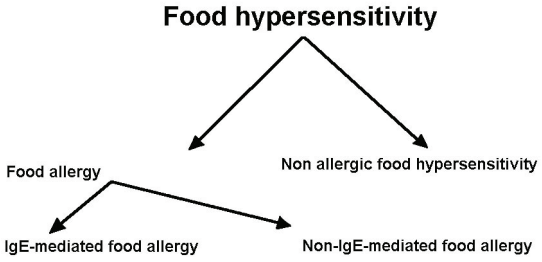

Figure 1. Nomenclature for food hypersensitivity[6]

All other reactions previously referred to as "food intolerance" should be referred to as "non-allergic food hypersensitivity"[6], but in practice, the term "food intolerance" is still being utilized. This thesis deals exclusively with IgE-mediated food allergy, which will be referred to as "food allergy". Food allergy is an atopic disease. Atopy can be defined as a personal or familial tendency to produce IgE antibodies in response to low doses of allergens, usually proteins, and to develop typical symptoms such as asthma, rhino conjunctivitis, or eczema/dermatitis[6]. IgE-mediated food allergic patients, or simply food allergic patients, demonstrate IgE-mediated adverse reactions to the food in question[7], however, IgE may not always be demonstrable using current in vivo and in vitro assays.

Although generally, the use of the internet has increased enormously during the

last decade, the increasing interest for and available data on food allergy may be reflected by the number of articles in Pub med, which has steadily been increasing over the years, and is still increasing. When searching for "food allergy" OR "cow's milk allergy" OR "food hypersensitivity" OR "adverse reactions and food", a number of 94 publications can be found published in the year 1970, 134 articles in 1980, through 268, and 409 articles published in the years 1990 and 2000 respectively. In 2006, a total number of 640 papers were published.

Since more than half a century, the need for objective and unequivocal investigation techniques to study food allergy have been addressed and stressed by several authors. Before then, only articles and books on food allergy were published containing anecdotal, non-controlled reports of symptoms attributed to food allergy[8]. At a food symposium during the 6th Annual Meeting of the American Academy of Allergy in 1950, Dr. F.C. Lowell opened an editorial with the following statement: "There is perhaps no field in medicine in which more divergent views are held than in that of allergy to food. In order to demonstrate a cause-and-effect relationship between food ingestion and symptoms, foods administered should be completely disguised, perhaps best in capsules or by stomach tube"[9,10]. In another editorial by Dr. C.D. May, a few decades later[11], entitled: "Are confusion and controversy about food hypersensitivity really necessary?", he explained that controversy about food allergy can only be removed by unbiased observations, which may only be obtained by the use of the double-blind, placebo-controlled food challenge (DBPCFC) (Box 1).

Initially, C.D. May introduced the DBPCFC in the mid-1970's and, along with S.A. Bock, pioneered the use of the DBPCFC[12,13]. This early work paved the way for several other investigators[14]. A manual and methodological aspects of food challenge procedures were published in 1988 and 1990 respectively[15,16]. In these documents, the practical basis for designing DBPCFCs is described. Many of the

Box 1.

The Double-blind, placebo-controlled food challenge test (DBPCFC)

In a DBPCFC, the patient is challenged with sequentially incrementing amounts of an active suspected allergenic food (or "verum") and with a placebo food. The active and placebo challenges are conducted in random order and preferably on separate days. In earlier days, capsules were used to disguise the food. Currently, the active food is disguised in a test food matrix with similar sensory properties to the placebo test food. Both the patient and the physician are blinded for the sequence of the challenges, until the code is broken at the end of the test. In this test, the patient serves as his/her own control. The purpose of the DBPCFC is to document or refute a causative relation between the suspected food and allergic symptoms. Severity of symptoms should not be reproduced during DBPCFCs.

recommendations given in these publications are still being applied today in performing DBPCFCs.
The DBPCFC has been regarded as the gold standard for diagnosing food allergy for over 20 years[15,17,18]. The statements cited above by F.C. Lowell and C.D. May, as well as the following statement published in 1990 still hold today: "The DBPCFC is currently the only completely objective method for determining the validity of a history of an adverse reaction to a food"[16].
Over the years, lessons learned from the outcome of the DBPCFCs have been playing a crucial role in gaining evidence-based knowledge on food allergy[19]. Reliable and unequivocal information and knowledge on all clinical aspects of food allergy can only be gained by well-conducted DBPCFCs. To date, indications for and purposes of food challenges tests may be 1. to establish or refute the diagnosis of food allergy, 2. to determine resolution or persistence of food allergy, 3. to determine thresholds in food allergic patients, and 4. to gain scientific knowledge and data on food allergy, such as documentation of novel reaction patterns to allergenic foods, and reactions to new allergens.

1.2. Diagnosing food allergy in the Netherlands: From expert opinion towards evidence-based diagnostic procedures

In the Netherlands, it was only in the 1ate 1980's that awareness of food allergy began to increase, and that physicians and dieticians accepted the concept, that common, normally healthy foods, could cause disease. The institution of the consumer's association for food allergic patients, "The Nederlandse Voedselallergie Stichting" (NVAS; currently called: "Stichting Voedselallergie"), initiated by Mrs. Nardi Nieborg, mother of two food allergic children, brought together physicians and dieticians interested and somehow experienced in the field of food allergy. This resulted in a consensus report on food allergy and food intolerance[20] a few years later. However, evidence based publications regarding DBPCFCs were scarce. In a workshop focusing on the methodology for clinical studies of adverse reactions to food[16], a total number of only 21 well-conducted clinical trials on food allergy could be retrieved from Pub med (1983 – 1988). This low number of studies illustrates that very little scientific knowledge was available at that time. In the Netherlands, the diagnosis and management of food allergy were based on expert opinion of physicians and dieticians. It was assumed, for example, that atopic infants were at risk for allergic reactions to "any" food or food components[21]. To date, it has become clear that the majority of food allergic reactions in children is caused by a small number of foods[7]. Also, a delayed introduction of common allergenic foods was generally regarded as effective in preventing food allergy in high risk infants, not only in the Netherlands but world wide[22]. The latter concept is being challenged, as is discussed in Chapter VIII. DBPCFCs were performed only occasionally and in small numbers in a few of the University Medical Centres, such as Groningen,

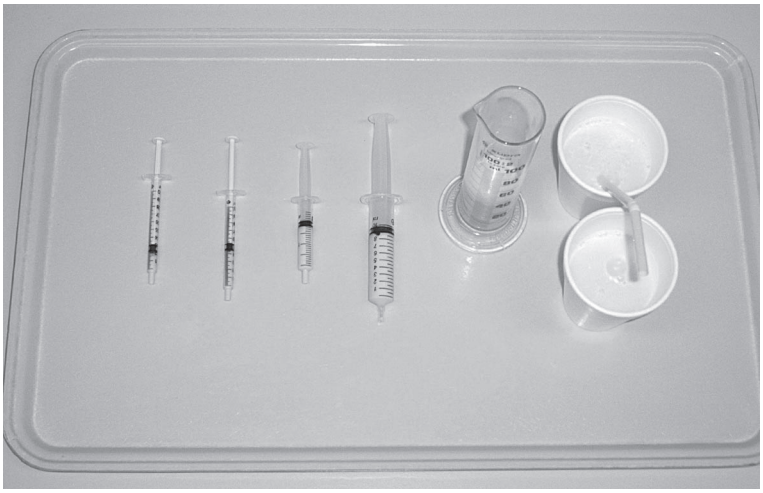

Figure 2. Incrementing challenge doses to be administered during a DBPCFC with soy (Recipe: Soy disguised in cow's milk)

Utrecht and Rotterdam. To date, in the Netherlands DBPCFCs are performed on a regular basis as a routine diagnostic measure and for scientific purposes in an increasing number of centres[23,24]. In 2001, at the University Medical Centre Groningen (UMCG), the Food Challenge Unit (FCU) was established by Prof. Dr. Anthony Dubois, allergist, and Dr. Charles Bijleveld, paediatric gastroenterologist, to diagnose food allergy in children.

The results as described in this thesis are obtained from approximately 500 DBPCFCs performed from 2002 until 2007 at the FCU of the UMCG .

1.3. Limited standardization and validation of the DBPCFC to date

Although the DBPCFC has been the diagnostic procedure of choice over the years, only few attempts have resulted in standardizing and validating (parameters of) the test procedure[18, 25,26] for clinical and scientific purposes. Despite guidelines for the administration of the test procedure[15,16,18,27], to date, no universal protocol for the performance of the DBPCFC has been established. Standardization and validation of DBPCFC procedures would clarify test procedures, and would facilitate comparing scientific results between different centres, would provide the highest diagnostic accuracy, greatest safety, optimal clinical and scientific information, and maximal convenience and patient acceptance. In 2001, Bindslev-Jensen[28] stressed the fact that such standardization was much needed, and described several patient-related and procedure-related parameters of the DBPCFC, which should be agreed upon in a standardization procedure (Table 2).
In the position paper on oral food challenge procedures published a few years later

Table 2. Proposed parameters for standardization of the DBPCFC by Bindslev-Jensen in 2001[28]

Patient-related parameters:
- Selection of patients for challenges
- The use of in vitro and in vivo tests for selection of patients
- The nature of a suspected reaction

Procedure-related parameters:
- The source of food used for challenge
- Starting dose used for challenge
- Dose increment
- Time interval during challenges
- Top dose
- Number of placebo and active challenges

in 2004[18], proposals are made for standardization of the test procedure. However, despite the fact that it is not realistic to have standardized all parameters at all times for every individual patient, several crucial parameters of the DBPCFC procedure remain to be validated. Examples include incremental scales, total and maximum doses, the administration of active and placebo challenges (interspersed or active and placebo challenges administered on separate days), indications for DBPCFC (in contrast to indications for open food challenges), criteria to terminate the test, and assessment of test results. In a recent publication by Niggemann and Beyer[29], pitfalls in DBPCFCs such as a lack of uniform criteria to assess and terminate the challenge, are described, illustrating the fact that much work still remains to be done with regard to standardization of the test procedure.

1.4. Need for practical guidelines

Physicians and dieticians generally consider diagnosing food allergy as difficult, elusive and complicated. Recently, the Health Council of the Netherlands[30] stated that the DBPCFC is the diagnostic procedure of choice for diagnosing food allergy, and that this test should become available for diagnosing food allergy in primary care. Currently, the awareness for the need of objective and unbiased diagnostic procedures in the Netherlands is increasing. Many tertiary and secondary centres, as well as primary health care centres have indicated interest in carrying out DBPCFCs to improve their diagnostic abilities in food allergic patients, and are currently attending workshops and educational sessions on DBPCFCs predominantly provided by the UMCG.

In many publications on food allergy, statements such as "The DBPCFC is the gold standard to establish the diagnosis of food allergy" appear in the text, suggesting that the DBPCFC is an often utilized, well-standardized and validated diagnostic procedure. However, in practice, the DBPCFC is conducted in only a limited number of centres, almost certainly due to several practical factors, such as the labour intensity of the test, lack of available challenge materials and incremental

scales, as well as standardized protocols focusing on assessment of reactions or termination of challenges. Additionally, the perceived risk of severe reactions and lack of compliance in some patients may make physicians hesitate to perform food challenge tests. Therefore, standardized protocols, educational materials, as well as recipes for challenge materials are much needed (Chapter II). This may help physicians and dieticians to set up food challenge tests by providing necessary tools. Finally, not all physicians are convinced of the added diagnostic value of the DBPCFC as compared to open food challenge tests, also due to lack of data in this respect. Especially in case of a convincing history of immediate and objective reactions to food, many health care professionals consider double-blind challenges unnecessary. Thus, convincing data on the necessity of randomized, double-blind, placebo-controlled tests may elucidate the value of unbiased and objective observations (Chapter IV).

1.5. Purpose of this thesis

The aims of this thesis were first, to standardize the procedure of the DBPCFC in children for the FCU of the UMCG, and to validate several parameters of the challenge procedure. Secondly, to examine the outcome of DBPCFC performed from 2002 until 2007 in subgroups of children and to formulate practical guidelines and recommendations for the management of food allergy in children.

1.6. Outline of this thesis

In Chapter II, the development and validation by sensory testing for difference of challenge materials for use in DBPCFCs is described. Recipes with cow's milk, soymilk, egg, peanut, hazelnut, and wheat were first tested by volunteers from the hospital staff and subsequently by a professional panel of food tasters in a food laboratory designed for sensory testing.

In Chapter III, we comment on the method of sensory testing by other authors, using a non-professional panel of food tasters, which may overestimate the validity of recipes.

In Chapter IV, we analyze the occurrence and features of placebo events in DBPCFCs in children sensitized to the challenged food, and assess their diagnostic significance of the DBPCFC.

In Chapter V**,** we describe the development of introduction schedules for major allergenic foods for use at home, to be administered in children with an increased risk of food allergy, but who do not, according to the physician's assessment, warrant food challenge testing. The incrementing amounts of these ready-to-use introduction schedules are based on incremental scales administered in DBPCFCs as a first known exposure, and their feasibility is demonstrated in the paper.

In Chapter VI, dietary assessment is described in children adhering to an allergen avoidance diet from birth, to analyze if elimination was complete and feasible, and

to investigate if dietary assessment can be used in predicting the outcome of the DBPCFC.

In Chapter VII, a study on consecutively performed DBPCFCs in children with a clear-cut history of anaphylaxis to food is described to determine whether the frequency of negative challenge tests in children with anaphylaxis to food is frequent enough to warrant challenge testing, and to document the safety of this procedure.

In Chapter VIII, the main results of this thesis are discussed. Finally, recommendations for future research are made.

References

1 Blackley CH. Hay fever: its causes, treatment and effective prevention: experimental researches. Ed.2. London, 1880, Baillère, Tindall & Cox
2 Stanworth DR, Humphrey JH, Bennich H, Johansson SG. Specific inhibition of the Prausnitz-Kustner reaction by an atypical human myeloma protein. Lancet. 1967; 12;2(7511):330-2.
3 Johansson SaG. The discovery of immunoglobulin E. Allergy Asthma Proc 2006;27(2 Suppl 1):S3-6.
4 Bousquet J. In: 40 Years with IgE. The impact of IgE – past, present and future. Phadia AB, Uppsala, Sweden.
5 Gell PG, Coombs RR. Clinical aspects of immunology. 2ne ed. Oxford: Blackwell, 1968:757-596.
6 Johansson SG, O'B Hourihane J, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy 2001;56:813-24.
7 Sampson HA. Update on food allergy. J Allergy Clin Immunol 2004;113:805-19.
8 Rowe AH. Food allergy. Philadelphia. Lea & Febiger. 1932
9 Lowell FC. Food allergy (editorial). J Allergy 1950;21:563-4.
10 Bock SA. Oral challenge procedures. In: Food allergy. Adverse reactions to foods and food additives. Metcalfe, Sampson and Simon (red). Blackwell Scientific Publications. 1991:81-95.
11 May CD. Are confusion and controversy about food hypersensitivity really necessary? (Editorial). J Allergy Clin Immunol 1985;75:329.
12 May CD. Objective clinical and laboratory studies of immediate hypersensitivity reactions to foods in asthmatic children. J Allergy Clin Immunol 1976:58:500-515.
13 Bock SA, Lee WY, Remigio LK, May CD. Studies of hypersensitivity reactions to foods in infants and children. J Allergy Clin Immunol 1978;62:327-34.
14 Lieberman PL, Kaliner MA, Lockey RF, Simons ER. Pioneers and milestones. J Allergy Clin Immunol 2006;117:478-82.
15 Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: A manual. J. Allergy Clin Immunol 1988;82:986-997.
16 Metcalfe DD, Sampson HA. Workshop on experimental methodology for clinicalstudies of adverse reactions to foods and food additives. J Allergy Clin Immunol 1990;86:421-42.
17 Bruijnzeel-Koomen C, Ortolani C, Aas K, Bindslev-Jensen C, Bjorksten B, Moneret-Vautrin D et al. Adverse reactions to food. Position paper. European Academy of Allergology and Clinical Immunology Subcommittee. Allergy 1995;50:623-35.
18 Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J et al. Standardization of food challenges in patients with immediate reactions to foods – position paper from the European Academy of Allergology and Clinical Immunology. Allergy 2004;59:690-697.
19 Sicherer SH, Bock SA. An expanding evidence base provides food for thought to avoid indigestion in managing difficult dilemmas in food allergy. J Allergy Clin Immunol 2006;117:1419-22.
20 Consensus Food Hypersensitivity. National Organisation for Quality Assurance in Hospitals (CB). 1990. Utrecht. The Netherlands.
21 Monchy JGR de, Martens BPM, Phaff RAS, Nieborg-Eshuis NH (red). Voedselallergie en –intolerantie. Wat doen we ermee. Bohn, Stafleu Van Loghum, Utrecht. 1989.
22 American Academy of Pediatrics, Committee on Nutrition. Hypoallergenic infant formu-

las. Pediatrics 2000;106:346-9.
23 Wensing M, Penninks AH, Hefle SL, Koppelman SJ, Bruijnzeel-Koomen CAFM, Knulst AC. The distribution of individual threshold doses eliciting allergic reactions in a population with peanut allergy. J Allergy Clin Immunol 2002;110:915-20.
24 Wensing M, Penninks AH, Hefle SL, Akkerdaas JH, Ree R van, Koppelman SJ, Bruijnzeel-Koomen CAFM, Knulst AC. The range of minimum provoking doses in hazelnut-allergic patients as determined by double-blind, placebo-controlled food challenges. Clin Exp Allergy 2002;32:1757-62.
25 Taylor SL, Hefle SL, Bindslev-Jensen C, Atkins FM, Andre C,Bruijnzeel-Koomen C et al. A Consensus protocol for the determination of the threshold doses for allergenic foods: how much is too much? Clin Exp Allergy 2004;34:689-695.
26 Vlieg-Boerstra BJ, Bijleveld CMA, van der Heide S, Beusekamp BJ, Wolt-Plompen SAA, Kukler J, Brinkman J, Duiverman EJ, Dubois AEJ. Development and validation of challenge materials for double-blind, placebo-controlled food challenges in children. J Allergy Clin Immunol 2004; 113:341-6.
27 The Food Allergy & Anaphylaxis Network, Mofidi S, Bock SA. A Health professional's guide to food challenges. 2005
28 Bindslev-Jensen C. Standardization of double-blind, placebo-controlled food challenges. Allergy 2001;56: Suppl 67;75-77.
29 Niggemann B, Beyer K. Pitfalls in double-blind, placebo-controlled oral food challenges. Allergy 2007;62:729-32.
30 Gezondheidsraad. Voedselallergie (Health Council of the Netherlands. Food Allergy). 2007.

Chapter II

Development and validation of challenge materials for double-blind, placebo-controlled food challenges in children

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ABSTRACT

Background: The use of double-blind, placebo-controlled food challenges (DBPCFCs) is considered the gold standard for the diagnosis of food allergy. Despite this, materials and methods used in DBPCFCs have not been standardized.

Objective: The purpose of this study was to develop and validate recipes for use in DBPCFCs in children by using allergenic foods, preferably in their usual edible form.

Methods: Recipes containing milk, soy, cooked egg, raw whole egg, peanut, hazelnut, and wheat were developed. For each food, placebo and active test food recipes were developed that met the requirements of acceptable taste, allowance of a challenge dose high enough to elicit reactions in an acceptable volume, optimal matrix ingredients, and good matching of sensory properties of placebo and active test food recipes. Validation was conducted on the basis of sensory tests for difference by using the triangle test and the paired comparison test. Recipes were first tested by volunteers from the hospital staff and subsequently by a professional panel of food tasters in a food laboratory designed for sensory testing. Recipes were considered to be validated if no statistically significant differences were found.

Results: Twenty-seven recipes were developed and found to be valid by the volunteer panel. Of these 27 recipes, 17 could be validated by the professional panel.

Conclusion: Sensory testing with appropriate statistical analysis allows for objective validation of challenge materials. We recommend the use of professional tasters in the setting of a food laboratory for best results.

INTRODUCTION

The use of double-blind, placebo-controlled food challenges (DBPCFCs) has been considered the gold standard for the diagnosis of food allergy for more than a decade.[1,2] Despite this, challenge materials and methods vary from center to center and have not been standardized. There is a need for such standardization to facilitate the use of DBPCFCs in daily practice and to allow for comparison of results obtained in various centers from different parts of the world.[3-5] The challenge materials used at different centers are diverse. Some centers use freeze-dried or concentrated foods masked in capsules or in other foods,[1,6-13] whereas in other studies fresh or native foods masked in other foods are used.[1,3,8,14-20]

Using freeze-dried, heated, or concentrated allergenic foods for DBPCFCs has disadvantages, such as the risk of altered allergenicity.[21-24] Capsules have the further disadvantage that oropharyngeal symptoms are not diagnosed, that early signs of severe anaphylactic reactions might be missed,[1,2-4,14,25,26] and that, depending on the food, large quantities of capsules might need to be consumed. These problems are largely overcome by using allergenic foods in their usual edible form masked in other foods, and this most closely approaches the everyday consumption of such allergenic foods. The major drawbacks of using allergenic foods in their usual edible form are that large amounts of allergenic foods are difficult to disguise.

A number of authors have described the materials or recipes used in DBPCFC studies in which allergenic foods are masked in vehicles consisting of other foods.[1,3,8-10,13-20,27-29] In some of these studies, the exact composition of the recipes used is not clearly or fully documented, and such recipes are thus difficult to implement. Moreover, the methods used to test these materials and validate adequate blinding have not been uniformly rigorous. The purpose of this study was to develop and validate recipes for use in DBPCFCs in children by using allergenic foods, preferably in their usual edible form.[3,4,8,14] Here we describe the test procedure used to validate adequate blinding of the materials developed and the results of this procedure. The results of validation by volunteers are compared with results of validation by professional panelists in a food laboratory.

METHODS

Development of challenge materials (recipes)

Common allergenic foods were selected for which recipes were to be developed and validated for DBPCFCs. These were cow's milk, heated egg, raw whole egg, raw egg white, soy, peanut, hazelnut, and wheat.[2,30] For each allergenic food, a placebo test food recipe and an active test food recipe was developed. The recipes met the following requirements: (1) acceptable taste; (2) allowance of a

challenge dose high enough to elicit allergic reactions in an acceptable volume (in our experience most young children are able to consume a maximum challenge dose of about 200 mL of liquid challenge material or 50-100 g of solid food within 15 minutes); (3) good matching of sensory properties of placebo and active test food recipes; (4) optimal matrix ingredients, including the avoidance of highly allergenic ingredients for possible use in children allergic to multiple foods; (5) avoidance of the use of frequently suspected foods, such as chocolate; and (6) use of as few ingredients as possible to make recipes acceptable for most patients and to minimize unknown side effects of the ingredients used.[31] We masked protein equivalent amounts of allergenic foods, which would allow us to compare dose-response reactivities to different foods.

For young children, we created recipes that preferably used the regular daily food consumed by the child because of greater acceptance. For older children, we developed a variety of recipes that would be acceptable to even very fussy eaters.[32]

Allergenic food ingredients

The allergenic foods were used, where possible, in their usual edible form: pasteurized skimmed or semiskimmed milk (1.5%) or Protifar Plus (protein enriched cow's milk powder) for milk recipes; soy milk for soy recipes; heated egg, irradiated raw whole egg, and raw egg white (irradiated with 15 kGy; Gammaster, Ede, the Netherlands) for egg recipes; roasted and ground peanuts for the peanut recipe (The Nut Company, Doetinchem, The Netherlands); raw (unroasted) and locally bought ground blanched hazelnuts for the hazelnut recipe; and both plain flour and whole meal plain flour for the wheat recipe.

Vehicles and placebos

For the kind of recipes in which the placebo test food or vehicle consisted of foods used frequently or daily by children (ie, hydrolyzed formulas, soy-based formulas, or milk), exact matching of the placebo test food and the active test food was sought because sensory differences between the placebo and active test foods would be easily recognized by the child. Thus for these recipes, test foods with no perceivable sensory differences of any kind were developed. However, for most recipes, it is extremely difficult to develop placebo and active test foods that are exactly identical. Organoleptic properties of foods might change slightly, and there might be slight differences in conditions when preparing, storing, and transporting the test foods from one occasion to the next. Thus for the remaining recipes, placebo and active test foods were developed that were as similar as possible but in which small differences were acceptable as long as the presence of the allergenic food could not be detected.

Choice of sensory tests for difference

Sensory tests for difference were used to validate the newly developed recipes. It is often stated that placebo and active recipes should be comparable with regard to taste, aspect, odor, and consistency.[13,33] For recipes based on vehicles consisting of foods used frequently or daily by the child and for which no perceivable sensory differences of any kind between placebo and active test foods could be tolerated, we used the triangle test for validation.[34,35] The triangle test belongs to the overall difference tests. The objective of the triangle test is to discover whether a perceivable difference exists between 2 samples, no matter which attribute differs between samples. By using the triangle test, panelists were asked to test 3 samples of a recipe of an allergenic food. Two of these samples were either the placebo or active test food, and the remaining sample was the active or placebo test food, respectively. Samples were coded by using 3-digit random numbers derived from a random table.[34] The 6 possible sample combinations were offered with equal frequency in random order to the panelists. Subjects were asked to identify the odd sample. The triangle test has a forced-choice procedure, requiring panelists to guess the odd sample when the odd sample is not detectable.

For recipes developed in foods not consumed daily by young children, the paired comparison test (or directional difference test) was used.[34,35] The paired comparison test belongs to the attribute difference tests. The objective of this test is to determine in which way a particular sensory characteristic, which in our study was the taste of the allergenic food,[34] differs between 2 samples. For the paired comparison test, more food tasters are needed because of random correct responses of 0.5, compared with the triangle test, in which this chance is 0.33. Using the paired comparison test, panelists were asked to test 1 placebo sample and 1 active sample of a recipe. Samples were coded by using 3-digit random numbers derived from a random table.[34] Panelists were asked to identify the sample containing the allergenic food. The paired comparison test also has a forced-choice procedure, requiring panelists to guess the right answer when the presence of the allergenic food is not detectable.

Study population and validation of challenge materials (recipes)

The difference tests were first conducted by volunteer panelists from the hospital staff. There were no exclusion criteria, except food allergy to any one of the ingredients of the recipes. For the triangle test, 15 to 20 volunteers took part, and for the paired comparison test, 30 to 40 volunteers participated. Liquid foods were offered in an opaque closed cup with a straw inserted through the lid to hide differences in smell and appearance. Solid foods were visible, and not only taste but also appearance and smell were compared. The latter aspects were also included to allow for development of materials not subject to inadvertent unblinding by persons other than the patient involved in the challenge. The volunteers were

allowed to compare samples as often as desired. Samples were tested at either room temperature or cold directly out of the refrigerator.

If the volunteer panel did not detect statistically significant differences between placebo and active samples, the recipes were subsequently tested by professional sensory panelists in a food laboratory (Department of Food and Business, University of Professional Education Groningen). The test room environment in the food laboratory is designed to minimize the subjects' biases, to maximize their sensitivities, and to eliminate variables unrelated to the products themselves. The area is free of crowding and confusion, as well as being comfortable, quiet, temperature controlled, and, above all, free of odors and noise.[34] For liquid foods, artificial light is used to exclude the influence of differences in color on the response of the subjects. The food laboratory accommodates several sensory evaluation booths in which the panelists conduct difference testing individually. There is no contact between the panelists during the testing. Panelists can neutralize their taste by drinking water or eating neutral crackers between tests (Fig 1). On the day of the sensory testing, panelists are not allowed to wear perfumes, wear cosmetics, or use alcohol. During the hours preceding the test, they were asked to abstain from coffee and smoking. This is verified by the panel attendant through questioning of the panelists. The professional panelists are paid for their work (10 Euros per test) and are experienced in conducting sensory difference testing. Panelists are excluded from the sessions if they are allergic to one of the ingredients of the recipes tested or if they are ill or convalescing in any way, as

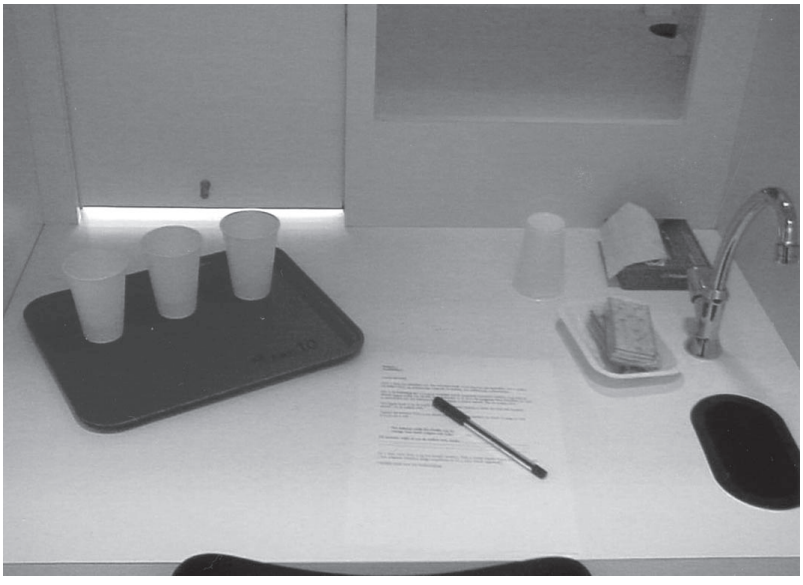

FIG 1. A sensory evaluation booth for sensory testing in the food laboratory

reported by themselves. The panelists have no contact with the investigators and no vested interest in this study or study outcome. In the food laboratory the triangle test was conducted by at least 33 panelists, and the paired comparison test was performed by at least 54 panelists. For the recipe of raw egg white in pudding, only 30 panelists were used because this recipe was developed and validated in an early phase of this study. If there were no statistically significant sensory differences between placebo and active test foods (triangle test) or if the active test food could not be identified statistically significantly more frequently in the active test samples than in the placebo test samples (paired comparison test), the recipe was considered valid.

Statistics

In SPSS software, 10th edition, the binomial exact test was used to test the differences, with a significance level of .05 (1-tailed probability). One-sided testing was used because the outcome of interest was whether the odd or active sample could be detected with significant frequency.

RESULTS

Validated recipes

Recipes validated by the professional sensory panel of the food laboratory ($P > .05$) are shown in Table I. The ingredients in the recipes are presented in Appendix 1, which can be viewed at the Journal's Online Repository. Five cow's milk recipes, 2 soy recipes, 5 cooked egg recipes, and 1 recipe each for raw whole egg, raw whole egg white, peanut, hazelnut, and wheat were validated. For 6 recipes, the triangle test was used, and for 11 recipes, the paired comparison test was used. Altogether, 27 recipes previously validated by the volunteer hospital panelists were tested in the food laboratory. Of these 27 recipes, only the 17 recipes shown could be validated by the professional sensory panel. The panel was able to detect a difference between active and placebo materials in 7 recipes by using the triangle test and to detect the allergenic food in question in 3 recipes by using the paired comparison test, which included skimmed milk in hydrolyzed cow's or soy milk formulas and previous recipes for rice milk, higher concentrations of Protifar Plus in hydrolyzed cow's milk formulas, egg in mashed potatoes, and a previous recipe of peanut in cookies.

To analyze the contribution of the larger number of panelists used in the food laboratory to the increased ability to detect the allergen-containing or odd samples, the results were recalculated with the same number of panelists in the food laboratory as had been used in the initial tests with hospital volunteers. Even when panels of the same size were compared, 7 of the 10 recipes that had been decoded by the professional panel remained decoded.

Table I. Recipes validated by the professional panelists in the food laboratory

Recipe	No. of panelists	No. of correct responses (odd sample or active sample)	Critical no. for significance	*P* values	Type of sensory test for difference used
Milk in Neocate Advance	39	11	19	.688	Triangle test
Milk in Nutramigen	39	18	19	.065	Triangle test
Milk in Rice Dream	58	24	36	.881	Paired comparison test
Protifar in N. Pepti 2	60	23	27	.243	Triangle test
Protifar in N. Pepti 2, higher concentration	60	23	27	.243	Triangle test
Soy milk in cow's milk	33	16	17	.051	Triangle test
Soy milk in Neocate Advance	33	16	17	.051	Triangle test
Egg in pancake	59	26	36	.783	Paired comparison test
Egg in pancake (free of cow's milk, soy based)	58	24	36	.881	Paired comparison test
Egg in minced meat	60	20	37	.994	Paired comparison test
Egg in minced meat (free of cow's milk, rice milk based)	54	32	34	.110	Paired comparison test
Egg in soy custard	56	17	35	.975	Paired comparison test
Raw whole egg in fruit puree	56	5	35	1.000	Paired comparison test
Raw whole egg white in pudding	30	16	20	.428	Paired comparison test
Peanut in cookies	56	27	35	.553	Paired comparison test
Hazelnut in cookies	56	21	35	.959	Paired comparison test
Wheat in minced meat	56	13	35	1.000	Paired comparison test

Amount of allergenic food and volume of highest challenge dose

In Table II the amount of allergenic food that could be masked in the highest challenge dose is shown. The volume of the highest challenge dose is determined by what we thought to be an acceptable volume for one challenge dose and the amount of vehicle necessary to mask the allergenic food. By varying the challenge volume, the challenge dose can be increased or decreased.

For all recipes, except for peanut and hazelnut, we could mask protein equivalent amounts of allergenic food protein (1.75 g) in the maximum challenge dose. The highest challenge doses of peanut and hazelnut were 1.2 g of peanut (approximately 0.35 g of protein) and 2.5 g of hazelnut (approximately 0.35 g of protein), which

Table II. Amount of allergenic food and volume or weight of highest challenge dose of test food recipes

Recipe	Amount of allergenic food in highest challenge dose	Volume or weight of highest challenge dose	Protein equivalent
Milk recipes	50 mL	200 mL drink	1.75 g
Protifar recipes	2 g	200 mL drink	1.75 g
	2.9 g	200 mL drink	2.5 g
Soy milk recipes	50 mL	220 to 230 mL drink	1.75 g
Cooked egg recipes	13.5 g (1/3 egg)	1 small pancake	1.75 g
	13.5 g	1 small meat ball	1.75 g
	13.5 g	280 mL of custard	1.75 g
Raw egg recipes	13.5 g (whole egg)	445 mL fruit puree	1.75 g
	30 g (egg white)	230 g of pudding	3.3 g
Peanut recipe	1.2 g (3 peanuts)	55 g of cookies	0.35 g
Hazelnut recipe	2.5 g (21/2 hazelnuts)	55 g of cookies	0.35 g
Wheat recipe	17.5 g	55 g of minced meat	1.75 g

were less than the protein equivalent amounts of the other allergenic foods. For Protifar and raw whole egg white, we could mask even greater amounts of allergic food protein (2.5 g of milk protein and 3.3 g of raw whole egg white protein, respectively).

DISCUSSION

A number of recipes have been developed over the years for carrying out DBPCFCs.[1,3,8-10,13-20,27-29] However, the adequacy of the blinding achieved with the use of these challenge materials has not been formally studied. In fact, many authors do not describe the validation procedures or describe a procedure similar to the first step in our protocol by using hospital volunteers.[15,19,27,36] Even though this was done by us in a rigorous manner, including coding of samples, offering all possible sample combinations with equal frequency in random order, and statistical analysis, a significant number of the recipes validated by the hospital volunteers could be decoded by the professional panel in subsequent testing in the food laboratory (10/27 recipes). Furthermore, many of the published recipes contain concentrations of allergenic foods that are higher than the concentrations we were able to validate[3,9,10,13,15,16,19,20,28,29]. We tried to validate recipes containing higher concentrations of milk, soy milk, raw egg, peanut, and hazelnut, but they were decoded by the hospital volunteer panel. Taken together, these observations suggest that the validity of challenge materials used for DBPCFCs in some centers might be overestimated, as has also been suggested by others.[15]

It is generally stated that if up to 10 g of freeze-dried food or 60 to 100 mL or grams of wet or native food is tolerated in children[1,11,12,30] or up to 15 g in adults,[6,25] the allergenic food should be tolerated. However, the conversion factor from dried to wet foods and the exact nature of the food source used has not been clearly documented, and the conversion requires some important assumptions.[5] In the literature the challenge materials used might be expressed in total amounts of allergenic food or in the amount of protein contained in allergenic foods, whereas in other cases assumptions must be made in expressing the challenge dose in total amount of the challenged food or the amount of protein. Again these differences stress the importance of standardization of challenge materials to allow for better comparison of results of DBPCFCs.[5]

The difficulty in developing recipes is masking amounts of allergenic food in an acceptable volume that are high enough to elicit reactions.[25,36] In our study the highest challenge dose was determined on the basis of the maximum amount of allergenic food that could be hidden in what we thought to be an acceptable volume of a single challenge dose for a child (Table II). The challenge procedure in which these materials were used included a 4- to 7-step incremental design in which progressively greater quantities of the same recipe were administered. Our experience in using these recipes for DBPCFCs is that most children are able to consume the highest challenge dose. To date, there are little published data on dose-response relationships in food challenges and the highest dose necessary to avoid false-negative results of DBPCFCs in children.[12,13] Sicherer et al[12] administered a cumulative dose of 10 g dry weight of dehydrated food or an equivalent amount of liquid food to patients, with a final dose of 2.5 g of dehydrated milk, egg, soy, wheat, fish, or peanut, and found that 4% of these negative double-blind challenge results was followed by positive open challenge results. One might assume that the dry weight of the final dose is equivalent to about 25 mL of fresh milk or 25 g of whole egg. Thus our highest dose for egg and milk are in the same order of magnitude as that used by Sicherer et al. Schade et al[13] used Protifar in amounts equivalent to about 45 mL of fresh milk as the highest dose, which is quite close to our maximum dose for milk and soy. Schade et al reported no false-negative immediate reactions. We have also encountered no false-negative immediate allergic reactions in the more than 90 DBPCFCs we have carried out to date.

In the literature different kinds of challenge materials are used in DBPCFCs.[5] Sometimes freeze-dried foods are used, and sometimes native or fresh foods in their usual edible form are used. For egg, most investigators used egg white, and some used whole egg, usually raw but sometimes cooked. For milk, many different foods are used: fresh nonfat or semiskimmed milk, nonfat milk powder, or infant formulas are used. For peanut, ground peanuts are usually used, but peanut butter or peanut flour has also been used.[5] For hazelnuts, some investigators use

raw hazelnuts, whereas in other studies it remains unclear whether raw or roasted hazelnuts are used.[16,20] The use of these different challenge materials complicates the comparison of recipes used and the results obtained.[5] We decided to use allergenic foods in their usual edible form because this would best mimic real-life exposure. Fresh foods are easily available and relatively easy to prepare. This was possible for all recipes, except for the milk recipes in whey hydrolysates, in which Protifar Plus was used. Details about the allergenicity of Protifar Plus are not available. Schade et al[13] used Protifar for DBPCFCs in children and did not observe false-negative reactions, suggesting that the allergenicity of this product has not been reduced in comparison with that of pasteurized milk.

In general, the power of sensory tests for difference is poor. Unattainably large numbers of panelists are needed for high power, depending on the true proportion of panelists able to detect a difference that is considered acceptable.[34,35] For example, for a triangle test with a power of 80%, an α value of .05, a β value of .2, and a true proportion of panelists able to detect a difference of 10%, 325 panelists are required. For the paired comparison test, the panel size is even larger. Thus it is important to increase power by performing sensory testing under optimal conditions by using a professional panel in a food laboratory designed for sensory testing to maximize the panelists' sensory sensitivity. Our results show that sensory testing by professional panelists in a food laboratory has greater power than sensory testing by volunteers. In conclusion, we recommend the use of standardized challenge materials in DBPCFCs. Sensory testing with appropriate statistical analysis allows for objective validation of challenge materials. We recommend the use of professional tasters in the setting of a food laboratory for best results. More work needs to be done on the maximum dose to be used considering the age of the patients and the preparation of the allergenic food used (eg, raw, heated, and freeze-dried).[12,37] To eliminate possible false-negative test results caused by these or other factors, DBPCFCs should always be followed by an open challenge or introduction until the allergenic food is consumed in a meal size portion or in amounts in which it is normally used by the child.

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References

1. Bock SA, Sampson HA, Atkins FM, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. J Allergy Clin Immunol 1988;82:986-97.
2. Bruynzeel-Koomen C, Ortolani C, Aas K, et al. Adverse reactions to food. Allergy 1995;50:623-35.
3. Niggemann B, Wahn U, Sampson HA. Proposals for standardization of oral food challenge tests in infants and children. Pediatr Allergy Immunol 1994;5:11-3.
4. Bindslev-Jensen C. Standardisation of double-blind, placebo-controlled food challenges. Allergy 2001;56(suppl 67):75-7.
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. Pastorello EA, Stocchi LS, Pravettoni V, et al. Role of the elimination diet in adults with food allergy. J Allergy Clin Immunol 1989;84:475-83.
7. Burks AW, Mallory SB, Williams LW, Shirrell MA. Atopic dermatitis: clinical relevance of food hypersensitivity reactions. J Pediatr 1988;113:447-51.
8. 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;95:1215-20.
9. Baehler P, Chad Z, Gurbindo C, Bonin AP, Bouthillier L, Siedman 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;26:254-61.
10. Isolauri E, Turjanmaa K. Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis. J Allergy Clin Immunol 1996;97:9-15.
11. Bock SA. Evaluation of IgE-mediated food hypersensitivities. J Pediatr Gastroenterol Nutr 2000;30(suppl):S20-7.
12. Sicherer SH, Morrow EH, Sampson HA. Dose-response in double-blind placebo-controlled oral food challenges in children with atopic dermatitis. J Allergy Clin Immunol 2000;105:582-6.
13. Schade RP, Meyer J, Pasmans SGMA, Knulst AC, Kimpen JLL, Bruynzeel-Koomen CAFM. Dubbelblinde placebogecontroleerde koemelkprovocatie voor de diagnostiek van koemelkallergie bij zuigelingen en kinderen. Ned Tijdschr Geneeskd 2002;146(37):1739-42.
14. Atkins FM, Steinberg SS, Metcalfe DD. Evaluation of immediate adverse reactions to foods in adult patients. II. A detailed analysis of reaction patterns during oral food challenge. J Allergy Clin Immunol 1985;75:356-63.
15. Norgaard A, Bindslev-Jensen C. Egg and milk allergy in adults. Allergy 1992;47:503-9.
16. Ortolani C, Ballmer-Weber BK, Skamstrup Hansen K, et al. Hazelnut allergy: A double-blind placebo-controlled food challenge multicenter study. J Allergy Clin Immunol 2000;105:577-81.
17. Ballmer-Weber B, Vieths S, Lüttkopf D, Heuschmann P, Wüthrich 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 2000;106:373-8.
18. Ballmer-Weber B, Wüthrich B,Wangorsch A, Fötisch K, Altman F,Vieths S. Carrot allergy: Double-blinded, placebo-controlled food challenge and identification of allergens. J Allergy Clin Immunol 2001;108:301-7.
19. Wensing M, Penninks AH, Hefle SL, Koppelman SJ, Bruijnzeel-Koomen CAFM, Knulst AC. The distribution of individual threshold doses eliciting allergic reactions in a popula-

tion with peanut allergy. J Allergy Clin Immunol 2002;110:915-20.
20. Wensing M, Penninks AH, Hefle SL, et al. The range of minimum provoking doses in hazelnut-allergic patients as determined by double-blind, placebo-controlled food challenges. Clin Exp Allergy 2002;32:1757-62.
21. Eigenmann PA. Anaphylactic reactions to raw eggs after negative challenges with cooked eggs. J Allergy Clin Immunol 2000;105:587-8.
22. Urisu A, Ando H, Morita Y, et al. Allergenic activity of heated and ovomucoid-depleted egg white. J Allergy Clin Immunol 1997;100:171-6.
23. Beyer K, Morrow E, Li XM, et al. Effect of cooking methods on peanut allergenicity. J Allergy Clin Immunol 2001;107:1077-81.
24. Hansen KS, Ballmer-Weber BK, Lüttkopf D, et al. Roasted hazelnuts allergenic activity evaluated by double-blind, placebo-controlled food challenge. Allergy 2003;58:132-8.
25. Pastorello EA, Pravettoni V, Stocchi L, Bigi A, Schilke ML, Zanussi C. Are double-blind food challenges necessary before starting an elimination diet? Allergy Proc 1991;12:319-25.
26. Hourihane JO'B. An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: a randomised, double-blind, placebo-controlled food challenge study. J Allergy Clin Immunol 1997;100:596-600.
27. Huijbers GB, Colen AAM, Niestijl Jansen JJ, Kardinaal AFM, Vlieg- Boerstra BJ, Martens BPM. Masking foods for food challenge: practical aspects of masking foods for a double-blind placebo-controlled food challenge. J Am Diet Assoc 1994;94:645-9.
28. Noe D, Bartemucci L, Mariani N, Cantari D. Practical aspects of baking of foods for double-blind, placebo-controlled food challenge. Allergy 1998;53(suppl 46):75-7.
29. Hill DJ, Cameron DJS, Francis DEM, Gonzalez-Andaya AM, Hosking CS. Challenge confirmation of late-onset reactions to extensively hydrolysed formulas in infants with multiple food protein intolerance. J Allergy Clin Immunol 1995;96:386-94.
30. Sampson HA, Scanlon MS. Natural history of food hypersensitivity in children with atopic dermatitis. J Pediatr 1989;115:23-7.
31. Grimshaw KEC, GHS Vance, RA Briggs, JO Warner. The effect of maternal egg avoidance on atopy at eighteen months. Abstract. J Allergy Clin Immunol 2003;111(suppl):S311.
32. Sicherer SH. Food allergy: when and how to perform oral food challenges. Pediatr Allergy Immunol 1999;10:226-34.
33. Muraro MA. Diagnosis of food allergy: the oral provocation test. Pediatr Allergy Immunol 2001;12(suppl 14):31-6.
34. Meilgaard MC, Civille GV, Carr BT. Sensory evaluation techniques. Boca Raton (FL): CRC Press LLC; 1999.
35. Lawless HT, Heymann H. Sensory evaluation of food. Principles and practices. New York: Kluwer Academic/Plenum Publishers; 1998.
36. Carter C. Double-blind food challenges in children: a dietitian's perspective. Current Medical Literature (Allergy). The Royal Society of Medicine 1995;3:95-9.
37. Caffarelli C, Petroccione T. False-negative food challenges in children with suspected food allergy. Lancet 2001;358:1871-2.

APPENDIX: INGREDIENTS OF VALIDATED RECIPES

Ingredients are listed in the order in which they are incorporated in the recipe.

1. MILK IN NEOCATE ADVANCE [E1-6]

Active test food	**Placebo test food**
150 ml of Neocate Advance [E8, E12]	200 ml of Neocate Advance [E8, E12]
(50 g of powder + 128 ml water)	(50 g of powder + 170 ml water)
50 ml pasteurised skimmed milk	--

2. MILK IN NUTRAMIGEN[E1-6]

Active test food	**Placebo test food**
150 ml of Nutramigen [E9, E13]	200 ml of Nutramigen[E9,E13]
(27 g of powder + 135 ml water)	(27 g of powder + 180 ml water)
50 ml of pasteurised skimmed milk	--

3. MILK IN RICE DREAM [E1-5]

Active test food	**Placebo test food**
130 ml of rice milk with added calcium (Rice Dream) [E14]	180 ml of rice milk with added calcium (Rice Dream) [E14]
50 ml of pasteurised skimmed milk	3 g of dairy free margarine
6 g of ready-to-eat rice flour (Nutrix)[E15]	6 g of ready-to-eat rice flour (Nutrix) [E15]
20 ml of fruit syrup (grenadine)	20 ml of fruit syrup (grenadine)

4. PROTIFAR PLUS IN NUTRILON PEPTI 2[E1-6]

Active test food	**Placebo test food**
200 ml of Nutrilon Pepti 2 [E10, E15]	200 ml of Nutrilon Pepti 2[E10, E15]
(27 g of powder + 180 ml water)	(27 g of powder + 180 ml water)
2 g of Protifar Plus [E11,E15]	--

5. PROTIFAR PLUS IN NUTRILON PEPTI 2[E1-6] (HIGHER CONCENTRATION)

Active test food	**Placebo test food**
200 ml of Nutrilon Pepti 2 [E10, E15]	200 ml of Nutrilon Pepti 2[E10, E15]
(27 g of powder + 180 ml water)	(27 g of powder + 180 ml water)
2,9 g of Protifar Plus [E11,E15]	--

6. SOY MILK IN NEOCATE ADVANCE [E1, E3-7]

Active test food	**Placebo test food**
150 ml of Neocate Advance [E8,E12]	200 ml of Neocate Advance [E8,E12]
(50 g of powder + 128 ml water)	(50 g of powder + 170 ml water)
50 ml of soy milk with no sugar or salt (Alpro)[E16]	3 g of dairy-free margarine
6 g of ready-to-eat rice flour (Nutrix)[E15]	6 g of ready-to-eat rice flour (Nutrix) [E15]
15 ml of fruit syrup (grenadine)	15 ml of fruit syrup (grenadine)

7. SOY MILK IN MILK [E1, E3-6]

Active test food	**Placebo test food**
145 ml of semi-skimmed milk (1,5 %)	185 ml of semi-skimmed milk (1,5%)
50 ml of soy milk with no sugar or salt (Alpro)[E16]	--
20 ml of unwhipped cream (35 %)	30 ml of unwhipped cream (35 %)
15 ml of fruit syrup (grenadine)	15 ml of fruit syrup (grenadine)

8. EGG IN PANCAKE [E2-4]

Active test food	**Placebo test food**
26 g of wheat flour	26 g of wheat flour
53 ml of semi-skimmed milk (1,5 %)	66 ml of semi-skimmed milk (1,5 %)
0,8 g of dried yeast	0,8 g of dried yeast
13,5 g of lightly beaten egg	-
0,2 g of salt	0,2 g of salt
12 g of grated apple	12 g of grated apple
8 g of dairy-free margarine (for baking)	8 g of dairy-free margarine (for baking)
4,5 g of castor sugar	4,5 g of castor sugar

9. EGG IN PANCAKE -SOY BASED [E3, E4, E7]

Active test food	**Placebo test food**
26 g of wheat flour	26 g wheat flour
53 ml of soy milk with no sugar or salt (Alpro)[E16]	66 ml soy milk with no sugar or salt (Alpro) [E16]
1,5 g of soy cream (Soy Cuisine, Alpro) [E16]	4 ml of soy cream (Soy Cuisine, Alpro) [E16]
0,8 g of dried yeast	0,8 g of dried yeast
13,5 g of lightly beaten egg	-
0,2 g of salt	0,2 g of salt
16 g of grated apple	16 g of grated apple
8 g of dairy-free margarine (for baking)	8 g of dairy-free margarine (for baking)
4,5 g of castor sugar	4,5 of g castor sugar

10. EGG IN MINCED MEAT [E2-4]

Active test food	**Placebo test food**
40 g of minced beef	40 g of minced beef
13,5 g of lightly beaten egg	14 ml of semi-skimmed milk (1,5 %)
6,5 g of wheat flour	6,5 g of wheat flour
6,5 g of bread crumbs (egg free)	6,5 g of bread crumbs (egg free)
0,2 g of salt	0,2 g of salt
0,2 g of pepper	0,2 g of pepper
8 g of dairy-free margarine (for baking)	8 g of dairy-free margarine (for baking)

11. EGG IN MINCED MEAT – RICE MILK BASED [E2-4, E7]

Active test food	**Placebo test food**
40 g of minced beef	40 g of minced beef
13,5 g of lightly beaten egg	14 ml of rice milk with added calcium (Rice Dream) [E14]
6,5 g of wheat flour	6,5 g of wheat flour
6,5 g of bread crumbs (egg and dairy free)	6,5 g of bread crumbs (egg and dairy free)
0,2 g of salt	0,2 g of salt
0,2 g of pepper	0,2 g of pepper
8 g of dairy-free margarine (for baking)	8 g of dairy-free margarine (for baking)

12. EGG IN SOY CUSTARD [E3-7]

Active test food	**Placebo test food**
200 ml of soy custard (vanilla, Alpro) [E16]	200 ml of soy custard (vanilla, Alpro) [E16]
67 ml of red grape juice	57 ml of red grape juice
13,5 g of hard boiled and mashed egg	5 g of soy cream (Soy Cuisine, Alpro) [E16]

13. RAW WHOLE EGG IN FRUIT PUREE [E3, E4, E7]

Active test food	**Placebo test food**
300 g of fruit puree (Olvarit, nr. 307) [E15]	300 g of fruit puree (Olvarit, nr. 307) [E15]
120 ml of orange juice	36 ml of orange juice
12 g of soy cream (Soy Cuisine, Alpro) [E16]	24 g of soy cream (Soy Cuisine, Alpro) [E16]
13,5 g of raw lightly beaten egg	--

14. RAW WHOLE EGG WHITE IN PUDDING [E3-6]

Active test food	**Placebo test food**
200 g of prepared vanilla pudding (Saroma) [E17]	230 g of prepared vanilla pudding (Saroma) [E17]
(semi-skimmed milk (1,5 %) and blancmange powder)	(semi-skimmed milk (1,5%) and blancmange powder)
30 g of raw lightly beaten egg white	--

15. PEANUT IN COOKIES [E1, E2, E4, E7]

Active test food	**Placebo test food**
8,5 g of whole wheat flour	8,5 g of whole wheat flour
8,5 g of wheat flour	8,5 g of wheat flour
3 g of wheat germ	3 g of wheat germ
15 g of cane sugar	15 g of cane sugar
15 g of dairy-free margarine	15 g of dairy-free margarine
0,3 of salt	0,3 g of salt
6 g of desiccated coconut	6 g g of desiccated coconut
1,2 g of roasted, ground peanuts	0,03 ml of peanut flavour QL 35189 [E18]
0,03 ml of hazelnut flavour QL 13849 [E18]	0,03 ml of hazelnut flavour QL 13849 [E18]

16. HAZELNUT IN COOKIES [E1-3, E7]

Active test food	**Placebo test food**
8,5 g of whole wheat flour	8,5 g of whole wheat flour
8,5 g of wheat flour	8,5 g of wheat flour
3 g of wheat germ	3 g of wheat germ
15 g of cane sugar	15 g of cane sugar
15 g of dairy-free margarine	15 g of dairy-free margarine
0,3 of salt	0,3 g of salt
6 g of desiccated coconut	6 g of desiccated coconut
2,5 of g ground blanched unroasted hazelnuts	0,045 ml of hazelnut flavour QL 13849 [E18]

17. WHEAT IN MINCED MEAT [E1-4, E7]

Active test food	**Placebo test food**
60 g of minced beef	60 g of minced beef
16 ml of rice milk with added calcium (Rice Dream) [E14]	24 ml of rice milk with added calcium (Rice dream) [E14]
5,5 g of whole wheat flour	5,5 g of whole buckwheat flour
12 g of wheat flour	12 g of whole rice flour
0,3 g of salt	0,3 g of salt
0,3 g of pepper	0,3 g of pepper
16 g of dairy-free margarine for baking	16 g of dairy-free margarine for baking

Characteristics of recipes:

E1	Recipe contains no egg
E2	Recipe contains no soy or soy lecithin
E3	Recipe contains no peanut or peanut oil
E4	Recipe contains no nuts or nut oil
E5	Recipe contains no wheat
E6	Recipe contains no gluten
E7	Recipe contains no cow's milk

Product type:

E8	Amino acid-based infant formula
E9	Intensively hydrolysed casein formula
E10	Intensively hydrolysed whey formula
E11	Protein-enriched cow's milk powder (comparable to Resource Instant protein, Novartis)

Manufacturers:

E12	SHS International Ltd,UK
E13	Mead Johnson, Division of Bristol-Myers Squibb, Avansville USA
E14	Imagine foods Ltd, London, UK
E15	Nutricia/Numico, Zoetermeer, The Netherlands
E16	N.V. Vandemoortele, Roosendaal, The Netherlands
E17	Honig, Koog a/d Zaan, The Netherlands.
E18	Internatio Möller, Mechelen, Belgium

Chapter III Published recipes for double-blind, placebo-controlled food challenges

Letter to the Editor

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Allergy 2005: 60: 1212

With great interest we read the publication by van Odijk et al[1], entitled: "Double-blind, placebo-controlled challenges for peanut allergy, the efficiency of blinding procedures and the allergenic activity of peanut availability in the recipes". We have a few comments on this well-conducted study.

First, the authors state that, although difficulty in masking suspected ingredients has been elucidated by Vlieg-Boerstra et al[2], the recipes have not been published. This statement is incorrect: recipes for double-blind, placebo-controlled food challenges (DBPCFCs) containing milk, egg, peanut, nuts, soy, and wheat belonging to this paper can be viewed on the Online Repository of the Journal of Allergy and Clinical Immunology, as is mentioned in the text of the article. Furthermore, we can provide the recipes by mail on request.

Secondly, van Odijk et al describe how they validated two of the recipes they developed by sensory testing. We think they succeeded in developing good tasting recipes in a small volume. However, sensory testing was conducted in a non-professional setting by volunteers (students). We have shown that it is important to perform sensory testing in a professional food laboratory, using professional panelists for optimal test results[2]. We compared the results of validation of recipes in a non-professional setting using hospital volunteers (as van Odijk did) to validation in a food laboratory by professional panelists. In our study, only 17 of the 27 recipes validated by the volunteer panelists could subsequently be validated in the professional food laboratory. Thus, 10 of the 27 recipes "validated" by the hospital volunteers were decoded by the professional panelists. Since the achievable power of validation studies is relatively limited, using a food laboratory designed for sensory testing aimed at minimizing panelist's biases and to maximize panelist's sensory abilities yields the best possible results. A food laboratory is free of crowding, odors, noise and is quiet, temperature controlled and comfortable. There is no contact between the panelists during the testing. Furthermore, panelists are prepared for their job. The panelists are experienced in participating in sensory testing and are paid for it. They are not allowed to wear perfumes or cosmetics on the day of the testing, they should be in a good physical condition and should refrain from smoking and consuming alcohol, coffee, and spicy food before they participate in sensory testing.

Thus, validating recipes in a non-professional environment is likely to overestimate the validity of recipes. We would stress the use of food laboratories and professional panelists in validating recipes for DBPCFC.

References

1 Van Odijk J, Ahlstedt S, Bengtsson U, Borres MP, Hulthén L. Double-blind placebo-controlled challenges for peanut allergy. The efficiency of blinding procedures and the allergenic activity of peanut availability in the recipes. Allergy 2005:60;602-605.

2 Vlieg-Boerstra BJ, Bijleveld CMA, van der Heide S, Beusekamp BJ, Wolt-Plompen SAA, Kukler et al. Development and validation of challenge materials for double-blind, placebo-controlled food challenges in children. J Allergy Clin Immunol 2004;113:341-6.

Chapter IV

Placebo reactions in double-blind, placebo-controlled food challenges in children

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ABSTRACT

Background: A cardinal feature of the double-blind, placebo-controlled food challenge (DBPCFC) is that placebo administration is included as a control. To date, the occurrence and diagnostic significance of placebo events have not extensively been documented.
Objective: To analyze the occurrence and features of placebo events in DBPCFCs, and to assess their contribution to the diagnostic accuracy of the DBPCFC in children.
Methods: The study population consisted of 132 challenges in 105 sensitized children (age range 0,7 -16,6 years, median 5,3 years), who underwent DBPCFCs with cow's milk, egg, peanut, hazelnut, and soy. Placebo and active food challenges were performed on different days.
Results: A total number of 17 (12.9%) positive placebo events occurred, which could be classified as immediate (9/17), late-onset (8/17), objective (11/17) or subjective (6/17). Four out of 74 (5.4%) positive active food challenges were revealed to be false positive by administration of a placebo challenge. This is 3% (4/132) of all challenges. When computed by a statistical model, the false positive rate was 0.129 (12.9% of all challenges).
Conclusion: Placebo events with diverse clinical characteristics occur in DBPCFCs in a significant number of children. The diagnostic significance of the administration of a placebo challenge is first, to identify false positive diagnoses in DBPCFCs by refuting false positive tests in individual patients. Secondly, to allow for blinding of the active food challenge. Thirdly, applying a statistical model demonstrates that some positive challenges may be false positive, and that the test may need to be repeated in selected cases.

INTRODUCTION

A cardinal feature of the double-blind, placebo-controlled food challenge (DBPCFC) is that placebo administration is included as a control. Despite the importance of placebos, very little has been published on the occurrence and features of placebo "reactions" or events following placebo administration, which we will here refer to as "placebo events". In many studies on DBPCFCs, the occurrence of placebo events are not described[1-8]. In other studies, placebo events are not described in detail[9-13], and it thus remains unclear how DBPCFCs were assessed when placebo events occurred.

To date, attempts to standardize the DBPCFC have not resulted in universally accepted procedures for this test[14-17]. Assumptions have been made in designing test procedures derived mainly from clinical practice, including the timing of the administration of active and placebo challenges, criteria to terminate the test (subjective or objective symptoms) and interpretation of test results (only immediate or also late onset symptoms). All these features may influence the number of reactions seen to active challenges as well as the number of placebo events, and hence the outcome of DBPCFCs.

The purpose of this study was to document the prevalence and features of placebo events in a large population of children suspected of IgE-mediated food allergy. Placebo events were assessed according to a standardized algorithm for the assessment of each challenge session, and according to a protocol for the assessment of the results of the complete DBPCFC. We also estimated the diagnostic significance of placebo administration in DBPCFCs by comparing outcomes of the entire test to outcomes of challenge sessions with the active food only, as well as by analysis applying a statistical model.

METHODS

Study population

The study population consisted of consecutive sensitized children in whom DBPCFCs with cow's milk, egg, peanut, hazelnut or soy were performed in our centre between January 2004 and September 2005. Non-sensitized children, and children suspected of having non-IgE mediated allergic disorders were excluded. This study was exempt from medical ethical approval, as DBPCFCs in children were performed as a routine diagnostic test. Information on gender, age, allergic symptoms at the time of challenge, dietary history with regard to the challenged food, and sensitization was obtained. Medical assessment of allergic symptoms was performed just before the DBPCFC was performed. Clinical symptoms and overall condition had to be stable, and children were instructed to discontinue antihistamines 72 hours prior to DBPCFC if possible.

Sensitization to the allergenic food in question was determined by ImmunoCap RAST (Phadia AB, Uppsala, Sweden) and skin prick test (SPT) with commercially available extracts (ALK-Abelló Hørsholm, Denmark) within 6 months prior to the DBPCFC. RAST results of ≥ 0.35 kU/l and SPTs of ≥ 3 mm were considered positive. Children showing either or both positive SPT or specific IgE to the food tested were considered as sensitized to the food in question.

Challenge procedure

Prior to the DBPCFC, elimination of the food in question for at least 6 weeks was confirmed by a dietician. Placebo and active challenges were administered in a random order, and were administered on separate days with at least two weeks interval in between. Randomisation was determined by computer. Recipes for the test foods were prepared for each challenge session individually, and recipe and randomisation code were verified by a second individual. For the active challenge, the suspected allergenic food was disguised in a food matrix to which the patient was tolerant. Unequivocal tolerance to the food matrix was ascertained by dietary history by the dietician. Placebo and active foods were as similar as possible in sensory properties. Validation of adequate blinding of the test materials was achieved by sensory testing in a dedicated food laboratory[18].

Total challenge dose and incremental scale

The challenge procedure included a 4- to 6-step incremental design in which progressively greater quantities of the same allergenic food were administered. Pasteurised cow's or soy milk, baked egg, roasted peanuts, and unroasted hazelnuts were used. The incremental scale and total challenge dose used are shown in Table 1. The incremental scale was achieved by varying the volume of the test food. Time interval between two challenge doses was 30 minutes in most cases. The total amount of allergenic food administered was limited by: 1. the total

Table 1. Incremental scale and challenge doses used in DBPCFCs

	Cow's milk (ml)	Soy milk (ml)	Egg (mg)	**Protein equivalent (mg)**	Peanut (mg)	Hazelnut (mg)	**Protein equivalent (mg)**
Dose 1	0.05	0.05	13	**1.75**	6	12	**1.75**
Dose 2	0.1	0.1	27	**3.50**	12	25	**3.5**
Dose 3	0.4	0.4	108	**14**	48	100	**14**
Dose 4	2.0	2.0	538	**70**	241	500	**70**
Dose 5	10.0	10.0	2690	**350**	480	860	**130**
Dose 6	50.0	50.0	13460	**1750**	1206	2500	**350**
Total	63.0	63.0	16830 (~1/3 egg)	**2190**	2000 (~5-7 peanut kernels)	4000 (~ 4 small hazelnuts)	**570**

amount of allergenic food that could be masked in the food matrices as the highest challenge dose in an acceptable volume, 2. the starting dose, and 3. an acceptable duration of the challenge session (4 – 6 hours), taking into consideration that the challenge had to be performed in an out-patient clinical setting.

Assessment protocol of challenge sessions and total DBPCFCs

Challenge sessions in which children consumed less than 75% of the maximum challenge dose in absence of symptoms, were considered invalid. The challenge was discontinued when objective allergic symptoms occurred, or subjective allergic symptoms occurred twice on two successive administrations of the challenge material. Objective symptoms and signs were defined as (angio) oedema, urticaria, exacerbation of atopic eczema, rash, vomiting, diarrhoea, lip or tongue swelling, rhinoconjunctivitis, stridor, coughing, wheezing, hoarseness, collapse, tachycardia, and hypotension. Subjective symptoms were defined as exacerbation of generalized itch (in case of atopic eczema), abdominal pain, nausea and/or cramp, oral allergy symptoms, itchy throat or sensation of throat swelling, difficulty in swallowing, and "other" symptoms such as drowsiness and irritability. Immediate symptoms were defined as symptoms occurring during the challenge or within 2 hours after the last challenge dose. Two days after each

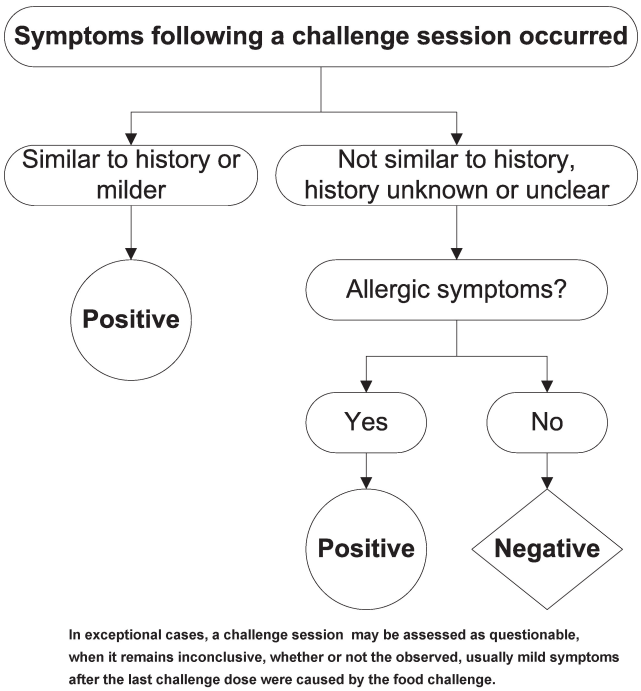

Figure. 1. Algorithm for assessment of allergic symptoms following a challenge session in DBPCFCs (with the exception of non-IgE mediated allergic disorders)

challenge session late onset reactions were recorded by telephone questionnaire. Late onset symptoms were defined as symptoms occurring between 2 and 48 hours after the last challenge dose.

For the optimal consistency of assessment of challenge results, we devised a standardized algorithm to assess immediate and late onset events following each challenge session (Fig 1). Events following test food administration were classified as (strongly) positive or negative.

Forty-eight hours after the second challenge session, the code was broken and the outcome of the DBPCFC was assessed according to a protocol, shown in Table 2. Negative DBPCFCs were followed by introduction of the challenged food into the diet. Patients received written instructions explaining how to introduce the food at home, using incrementing amounts of allergenic food ranging from the maximum challenge dose to normal daily food servings. Results of introduction were evaluated by telephone 1 month after the DBPCFC.

Table 2. Assessment protocol for the outcome of DBPCFCs

Active food challenge	Placebo challenge	Assessment of DBPCFC
positive	negative	positive
positive (clearly more positive than placebo)	positive	positive
negative	negative	negative
negative (or positive, but clearly less positive than placebo)	positive	negative

In exceptional cases, a challenge session or total DBPCFC may be assessed as questionable. Questionable DBPCFCs are repeated.

Documentation of placebo events and statistics

The prevalence and features of placebo events were recorded in the whole study population, and were classified according to whether symptoms were immediate or late onset symptoms, and according to whether symptoms were objective or subjective.

The clinical relevance of placebo administration in DBPCFCs was estimated by comparing outcomes of the entire test to outcomes of challenge sessions with the active food only. Furthermore, according to a statistical model of Brigs et al[19] and Hansen et al[20], the false positive rate was calculated as the number of subjects who responded with a positive reaction to the placebo challenge, divided by the total number of challenges.

The Chi-square test (SPSS Software, 12th edition) was used to analyze differences between immediate and late onset placebo events with regard to type of symptoms following challenges, type of food, and challenge order. For statistical analysis, symptoms following challenges were categorized to a nominal scale as 1. dermal

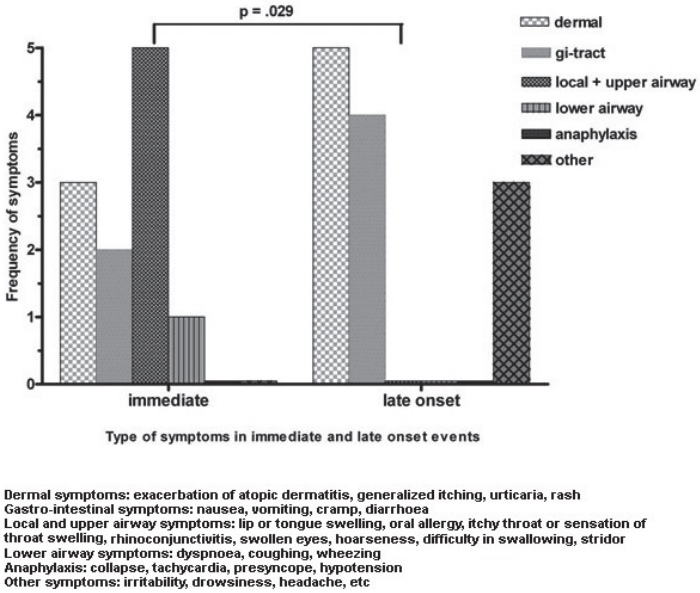

Figure 2. Comparison of type of symptoms which occurred in immediate and late onset placebo events

symptoms, 2. gastro-intestinal symptoms, 3. local and upper airway symptoms, 4. lower airway symptoms, 5. anaphylaxis, and 6. other symptoms. (Fig. 2).
Differences in age between immediate and late onset placebo events were assessed by Mann-Whitney test for not normally distributed values (two sided).

RESULTS

Study population

A total number of 105 children (median 5.3 years, range 0.7 – 16.6 years; 68 males, 37 females), were included in the study. Three of these children had their first known exposure to these foods by these food challenges. At the time of challenge, 93 children reported symptoms of atopic eczema (89%), 39 rhinitis (37%), and 58 asthma (55%). The median SPT (HEP) to the food in question was .90 (range 0 – 2,9) (124 cases), the median RAST score (kU/l) was 3.54 (<0.35 - >100) (131 cases).

Outcome of DBPCFCs

132 challenges were included in this study. These DBPCFCs were performed with cow's milk (n = 43), hen's egg (n = 31), peanut (n = 35), hazelnut (n = 17), and soy (n = 6).
70 DBPCFCs (53%) were assessed as positive and 62 (47%) DBPCFCs were

Table 3. Characteristics of placebo events

Pt. No.	Food on active challenge day	Symptoms on placebo day	Time of onset	Objective/subjective symptoms
1	egg	diarrhoea generalized itching	immediate	objective (and subjective)
2	peanut	diarrhoea cramp nausea	"	objective (and subjective)
3	egg	lip swelling	"	objective
4	soy	coughing stridor hoarseness	"	objective
5	peanut	rash urticaria	"	objective
6	cow's milk	tight and itchy throat, generalized itching	"	subjective
7	hazelnut	tight and itchy throat itchy tongue	"	subjective
	peanut	abdominal pain cramp	"	subjective
9	hazelnut	itchy mouth and tongue	"	subjective
10	egg	generalized itching exacerbation of atopic eczema	late onset	objective (and subjective)
11	cow's milk	generalized itching exacerbation of atopic eczema	"	objective (and subjective)
12	cow's milk	generalized itching exacerbation of atopic eczema irritability	"	objective (and subjective)
13	hazelnut	exacerbation of atopic eczema nausea vomiting drowsiness	"	objective (and subjective)
14	cow's milk	diarrhoea	"	objective
15	cow's milk	diarrhoea	"	objective
16	egg	generalized itching irritability	"	subjective
17	egg	nausea cramp	"	subjective

negative. No reactions were reported following a negative DBPCFC when introducing the challenged food at home, except in 3 cases (one each of egg, milk and peanut) (4.8%). These patients reported mild recurrent symptoms of skin rash, oral allergy symptoms or abdominal pain when consuming a normal food serving. They were offered a repeated DBPCFC, but they declined. For the purpose of this analysis, these DBPCFCs were considered as negative.

Placebo events

A total of 132 placebo challenges was analyzed, and 17 (12.9%) positive placebo events occurred in 17 different children (Table 3). All categories of symptoms occurred (dermal, gastro-intestinal, local and upper airway, lower airway and "other" symptoms), with the exception of anaphylaxis.

Further 9/17 (53%) placebo events were classified as immediate events, and 8/17 (47%) as late onset events. In 11/17 (65%) placebo events objective symptoms were observed, were as in 6/17 (35%) placebo events only subjective symptoms were reported (Table 3 and Fig. 3).

Immediate symptoms consisted of generalized itching, rash and urticaria in 3 challenges; diarrhoea, cramp, nausea and abdominal pain in 3 challenges; lip swelling, stridor, tight and itchy throat, mouth or tongue in 5 challenges; and coughing and hoarseness in 1 challenge.

Late-onset symptoms consisted of generalized itching and exacerbation of atopic dermatitis in 5 challenges; nausea, vomiting, diarrhoea, and cramp in 4 challenges; and irritability or drowsiness in 3 patients.

Objective symptoms consisted of rash, urticaria and exacerbation of atopic eczema in 5 challenges; diarrhoea and vomiting in 5 challenges; lip swelling and stridor in

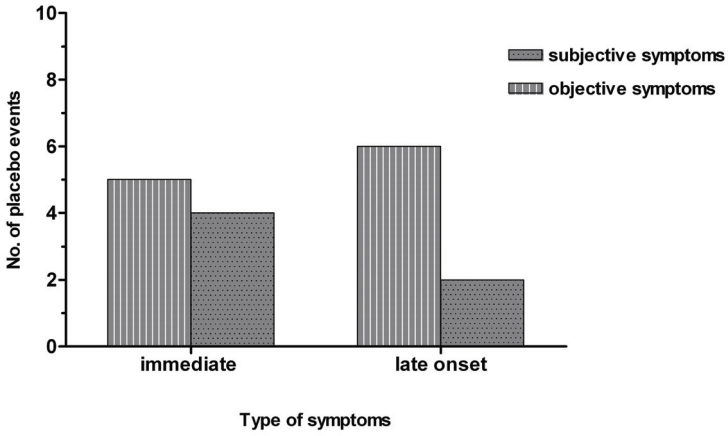

Figure 3. Occurrence of placebo events classified according to time of onset and objective or subjective symptoms

2 challenges; and lower airway symptoms in 1 challenge.
Subjective symptoms consisted of generalized itching in 6 challenges; cramp, nausea, and abdominal pain in 4 challenges; tight and itchy throat, mouth or tongue in 3 challenges; and irritability and drowsiness in 3 challenges.
There were no significant differences between the frequency of placebo events during the first and the second challenge session.

Comparison between immediate and late onset placebo events
A comparison of the type of symptoms of immediate and late onset placebo symptoms is shown in Fig. 2. "Local and upper airway symptoms" occurred significantly more often in immediate than late placebo events ($p = .029$). Late onset placebo events tended to consist of (mild) exacerbations of atopic dermatitis/ generalized itching and/or mild gastro-intestinal symptoms, such as abdominal pain or diarrhoea, but these differences were not statistically significant. The comparison of foods involved in immediate and late onset placebo events shows, that late onset placebo events tended to be observed more often for cow's milk and hen's egg than for other foods, but these differences were not statistically significant. There were no significant differences in age between children showing placebo events during DBPCFCs with cow's milk or hen's egg, and those showing placebo events during DBPCFCs to other foods.
There were no significant differences with regard to the age of the patients and challenge order of the placebo between immediate and late onset placebo events.

Diagnostic significance of placebo events
A total of 70 DBPCFCs (53%) were assessed as positive. When considering only the active food challenge sessions, 74/132 of such challenge sessions were assessed as positive. When comparing the results of the positive DBPCFCs to the results of the positive active food challenge sessions alone, 4 of 74 (5.4%) positive active food challenges were thus revealed to be false positive by administration of a placebo challenge (Table 3, patients no 1, 3, 12, and 17). This is 3% (4/132) of all challenges. In these 4 DBPCFCs, both active food and placebo challenges were positive. Two of these placebo events occurred immediately, 2 occurred late-onset. One (of four) events was objective only, 1 subjective only, and the remaining 2 events were classified as both objective and subjective. These 4 children introduced the challenged food successfully according to the protocol for negative DBPCFCs. The other 13 DBPCFCs in which placebo events were observed were assessed as negative (10 cases), because the active food challenges were negative. In the remaining 3 DBPCFCs in which a placebo event was observed, the active food challenge sessions were clearly more positive than the placebo challenges. One of these DBPCFCs was repeated, and was assessed as positive.

The other 2 children declined from repeated challenge. For the purpose of this analysis, these two challenges were assessed as positive.
According to the statistical model[19,20], the false positive rate was 0.129 (17/132), which is 12.9% of all challenges.

DISCUSSION

In this study, we found a rate of placebo events of 17/132 (12.9%), when all symptoms (objective, subjective, immediate and late onset) were considered valid. Approximately 50% of all placebo events consisted of either immediate or late onset events. Approximately 65% of all placebo events consisted of objective symptoms, and 35% of the symptoms were subjective. Thus, clinicians should be aware that all these types of placebo events may occur in a significant number of sensitized children. Furthermore, placebo events may present with a variety of symptoms, such as dermal, gastro-intestinal, local- and upper airway symptoms, lower airway symptoms, but we observed no anaphylaxis (cardio-vascular symptoms).
Rates of placebo events similar to ours were reported in other studies on food allergy. Rates of 0.2% to 3.6% were reported[9-13,21,22], but also somewhat higher prevalences were reported by Ballmer-Weber in studies of carrot allergy (10%), and of celery allergy (6%) respectively[23,24], and by Ortolani in hazelnut allergy (10%)[25]. In threshold studies, rates of 4 to 7% placebo events were found[26-28]. These events were all immediate placebo events, and these rates are comparable to the number of immediate placebo events in our study.
In some studies placebo events were not observed[29-32]. In some cases, this may be due to short observation periods following placebo administration, such as when active food and placebo are administered on the same day[1,6,10,29], or interspersed with each other[12,13,16,23,32,33]. Because we performed active and placebo challenges on separate days at an interval of at least two weeks, we were able to clearly distinguish between immediate and late onset reactions to active challenges and events following placebo challenges.
Monitoring and assessment of symptoms during challenge tests represent a key problem in the assessment of the outcome of DBPCFCs[16]. For this reason, we standardized the assessment procedures of each challenge session and the complete DBPCFC, which facilitates comparison to similar studies and allows for a consistent assessment of each challenge (session).
Depending on the criteria used for the assessment of symptoms and termination of a challenge session, the rate of placebo events differs considerably. To date, validation and clinical relevance of immediate versus late onset, and objective versus subjective symptoms have not been established, and there is no universal consensus which symptoms are necessary and sufficient to terminate the challenge.

If subjective symptoms are observed, repeated challenges are generally thought to be required[34]. In our protocol, the challenge session was considered positive when objective or repeated subjective allergic symptoms occurred.

For reliable results, it is important not to exclude placebo events from statistical analysis[14,15,19,20]. Only a few studies report on the assessment of placebo events in the context of the total challenge assessment[20,21,26-28]. In many other studies no details are provided. To date, little has been published on the interpretation and clinical significance of placebo events[14,15,19,20]. We calculated a false positive rate of 4/74 (5.4%) positive food challenges for all positive test results, because in 4 DBPCFCs, the active food challenge was refuted as "positive" because of administration of a placebo challenge. This is 3% (4/132) of all test results. However, when applying the statistical model of Briggs et al[19] and Hansen et al[20], the false positive rate calculated for all test results was higher (17/132 = 12.9%). In this model, all subjects with the tendency to give false positive responses (all placebo events) are estimated and incorporated in the calculation. Thus, clinicians should be aware that, statistically there is a chance that some double-blind, placebo-controlled positive test results will be false positive, and that some tests may need to be repeated in selected cases.

DBPCFCs with a negative active food challenge session and a positive placebo challenge session were assessed as being negative. DBPCFCs in which both placebo and active food challenge sessions were positive could theoretically be assessed as either negative or as inconclusive. In our protocol, the latter DBPCFCs were assessed as negative (4 DBPCFCs). These 4 children introduced the challenged foods successfully, according to the protocol for negative DBPCFCs, suggesting that such results are indeed negative. This protocol provides for the active and careful monitoring of successful introduction of the food in question at home, and thus excludes the possibility of false negative test results. DBPCFCs in which active food challenges are clearly more positive than placebo challenges could either be assessed as inconclusive or positive. In our protocol, these DBPCFCs were assessed as positive, but may be repeated (3 DBPCFCs).

It is generally accepted that the DBPCFC is the gold standard for the diagnosis of food allergy, whereas open food challenges (OFCs) may render false positive results because of lack of blinding and a lack of administration of placebos[34]. However, little data have been published comparing the results of these two types of challenges[35,36]. Brouwer et al[35] found that in 14 infants with atopic eczema with a positive OFC who were recruited from a primary care setting the diagnosis cow's milk allergy could be confirmed in only 4 infants by DBPCFC, resulting in a false positive rate of 71% (10/14) of all positive test results. In a prevalence study by Venter and et al[36], a false positive rate of 20.5% (8/39) for OFCs in twelve-months' -old children was found. These differences in positive test results suggest that the diagnostic contribution of the administration of a placebo challenge is not

only to identify placebo events, but quantitatively more importantly, to allow for blinding of the active food challenge. Blinded administration of placebos is thus important to minimize false positive food challenges.

In conclusion, placebo events with diverse clinical characteristics occur in DBPCFCs in a significant number of children. Placebo events may be immediate or late onset, and objective or subjective. The diagnostic significance of the administration of a placebo challenge is first, to identify false positive diagnoses in DBPCFCs by refuting false positive test results in individual patients. Secondly, to allow for blinding of the active food challenges. Thirdly, clinicians should also be aware that single challenge tests may be false positive, and that the test may need to be repeated in selected cases.

Conflict of interest: none

Funding: University Medical Centre Groningen, University of Groningen, The Netherlands

REFERENCES

1. Bernhisel-Broadbent J, Scanlon SM, Sampson HA. Fish hypersensitivity. I: In vitro and oral challenge results in fish-allergic patients. J Allergy Clin Immunol 1992;89:730-7.
2. Hill DJ, Cameron DJS, Francis DEM, Gonzalez-Andaya AM, Hosking CS. Challenge confirmation of late-onset reactions to extensively hydrolyzed formulas in infants with multiple food protein intolerance. J Allergy Clin Immunol 1995;96:386-94.
3. Norgaard A, Bindslev-Jensen C, Stahl Skov P, Poulsen LK. Specific serum IgE in the diagnosis of egg and milk allergy in adults. Allergy 1995;50:636-647.
4. 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.
5. Hourihane JO'B, Bedwani SJ, Dean TP, Warner JO. Randomised, double blind, crossover challenge study of allergenicity of peanut oils in subjects allergic to peanuts. BMJ 1997;314:1084-88.
6. Sicherer SH, Morrow EH, Sampson HA. Dose-response in double-blind, placebo-controlled oral food challenges in children with atopic dermatitis. J Allergy Clin Immunol 2000;105:582-6.
7. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol 2001;107:891-6
8. Mehl A, Verstege A, Staden U, Kulig M, Nocon M, Beyer K et al. Utility of the ratio of food-specific IgE/total IgE in predicting symptomatic food allergy in children. Allergy 2005; 60:1034-1039.
9. Sampson HA, McCaskill CC. Food hypersensitivity and atopic dermatitis: Evaluation of 113 patients. J Pediatr 1985;107:669-675.
10. Burks AW, Mallory SB, Williams LW, Shirrell MA. Atopic dermatitis: Clinical relevance of food hypersensitivity reactions. J Pediatr 1988;113:447-51.
11. Bock SA, Atkins FM. Patterns of food hypersensitivity during sixteen years of double-blind, placebo-controlled food challenges. J Pediatr 1990;117:561-7.
12. Niggeman B, Sielaff B, Beyer K, Binder C and Wahn U. Outcome of double-blind, placebo-controlled food challenge tests in 107 children with atopic dermatitis. Clin Exp Allergy 1999;29:91-96.
13. Verstege A, Mehl A, Rolinck-Werninghaus C, Staden U, Nocon M, Beyer K et al. The predictive value of the skin prick test weal size for the outcome of oral food challenges. Clin Exp Allergy 2005;35:1220-26.
14. Bindslev-Jensen C. Standardisation of double-blind, placebo-controlled food challenges. Allergy 2001:56; Suppl. 67:75-7.
15. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J et al. Standardization of food challenges in patients with immediate reactions to foods – position paper from the European Academy of Allergology and Clinical Immunology. Allergy 2004;59:690-697.
16. Niggemann B, Wahn U, Sampson HA. Proposals for standardization of oral food challenge tests in infants and children. Pediatr Allergy Immunol 1994;5:11-3.
17. Taylor SL, Hefle SL, Bindslev-Jensen C, Atkins FM, Andre C,Bruijnzeel-Koomen C et al. A Consensus protocol for the determination of the threshold doses for allergenic foods: how much is too much? Clin Exp Allergy 2004;34:689-695.
18. Vlieg-Boerstra BJ, Bijleveld CMA, van der Heide S, Beusekamp BJ, Wolt-Plompen

SAA, Kukler J, Brinkman J, Duiverman EJ, Dubois AEJ. Development and validation of challenge materials for double-blind, placebo-controlled food challenges in children. J Allergy Clin Immunol 2004; 113:341-6.

19. 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:Suppl.67;83-85.
20. Hansen TK, Poulsen LK, Skov PS, Hefle SL, Hlywka JJ, Taylor SL et al. A randomized, double-blined, placebo-controlled oral challenge study to evaluate the allergenicitiy of commercial, food-grade fish gelatine. Food and Chemical Toxicology 2004;42:2037-
21. Norgaard A, Bindslev-Jensen C. Egg and milk allergy in adults. Allergy 1992;47:503-9.
22. Knight AK, Schreffler WG, Sampson HA, Sicherer SH, Noone S, Mofidi S et al. Skin prick test to egg white provides additional diagnostic utitlity to serum egg white-specific IgE antibody concentration in children. J Allergy Clin Immunol 2006;117:842-7.
23. Ballmer-Weber BK, Vieths S, Lüttkopf D, Heuschmann P, Wüthrich 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 2000;106:373-8.
24. Ballmer-Weber BK, Wüthrich B, Wangorsch A, Fötisch K, Altman F, Vieths S. Carrot allergy: Double-blinded, placebo-controlled food challenge and identification of allergens. J Allergy Clin Immunol 2001;108:301-7.
25. Ortolani C, Ballmer-Weber BK, Skamstrup Hansen K, Ispano M, Wüthrich B, Bindslev-Jensen C et al. Hazelnut allergy: A double-blind placebo-controlled food challenge multicenter study. J Allergy Clin Immunol 2000;105:577-581
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 randomised, double-blind, placebo-controlled food challenge study. J Allergy Clin Immunol 1997;100:596-600.
27. Wensing M, Penninks AH, Hefle SL, Koppelman SJ, Bruijnzeel-Koomen CAFM, Knulst AC. The distribution of individual threshold doses eliciting allergic reactions in a population with peanut allergy. J Allergy Clin Immunol 2002;110:915-20.
28. Wensing M, Penninks AH, Hefle SL, Akkerdaas JH, Ree R van, Koppelman SJ, Bruijnzeel-Koomen CAFM, Knulst AC. The range of minimum provoking doses in hazelnut-allergic patients as determined by double-blind, placebo-controlled food challenges. Clin Exp Allergy 2002;32:1757-62.
29. Sampson HA. Role of immeditate food hypersensitivity in the pathogenesis of atopic dermatitis. J Allergy Clin Immunol 1983;71:473-480.
30. Hansen TK, Bindslev-Jensen C. Codfish allergy in adults. Allergy 1992;47:610-617.
31. Isolauri E, Turjanmaa K. Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis. J Allergy Clin Immunol 1996;97:9-15.
32. Niggemann B, Reibel S, Roehr CC, Felger D, Ziegert M, Sommerfelt C et al. Predictors of positive food challenge outcome in non-IgE mediated reactions to food in children with atopic dermatitis. J Allergy Clin Immunol 2001;108:1053-8
33. Hourihane JO'B, Grimshaw KEC, Lewis SA, Briggs RA, Trewin JB, King RM et al. Does severity of low-dose, double-blind, placebo-controlled food challenges reflect severity of allergic reactions to peanut in the community? Clin Exp Allergy 2005;35:1227-1233.
34. Sampson HA. Immunologically mediated food allergy: the importance of food challenge

procedures. Ann Allergy 1988;60:262-9.

35. Brouwer M.L., Wolt-Plompen SAA, Dubois AEJ, Heide S van der, Jansen DF, Hoijer MA et al. No effects of probiotics on atopic dermatitis in infancy: a randomized placebo-controlled trial. Clin Exp Allergy 2006;36:899-906.
36. Venter C, Pereira B, Grundy J, Clayton CB, Roberts G, Higgins B et al. Incidence of parentally reported and clinically diagnosed food hypersensitivity in the first year of life. J Allergy Clin Immunol 2006;117:1118-24.

Chapter V

Ready-to-use introduction schedules for first exposure to allergenic foods in children at home

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ABSTRACT

Background: The vast majority of children will undergo their first exposure to common allergenic foods at home. However, first exposure may lead to clinical reactions. It has been proposed to introduce allergenic foods gradually into the diets of children at risk for food allergy, but no practical dietary advice has been devised.
Objective: The aim of this study was to devise safe introduction schedules for common allergenic foods for use at home, based on the challenge doses as administered in DBPCFCs in children never previously exposed to these foods.
Methods: Seventy two double-blind, placebo-controlled food challenges (DBPCFCs) were performed in 63 children as a first known exposure. The incrementing challenge doses were converted into equivalent portions of these foods in their usual household form and incorporated in introduction schedules. The feasibility of the introduction schedules was tested in parents of children attending our clinic.
Results: Based on the results of the positive challenges (37) in which severe reactions did not occur, detailed introduction schedules and a reference photograph of the required increasing amounts of food were devised for use at home. Feasibility testing showed that, when using these introduction schedules, parents portioned initial doses significantly lower than without detailed instructions.
Conclusions: The introduction schedules and reference photograph provide information for parents to introduce the required amounts of allergenic foods in initial low doses at home. This may be expected to improve the safety of this procedure.

INTRODUCTION

The vast majority of children, including those who are at risk for food allergy, will undergo their first exposure to common allergenic foods, such as cow's milk, egg, peanut and tree nuts at home. However, it is known that the first exposure to foods may lead to clinical reactions, both in food challenge studies and uncontrolled conditions[1-5]. Severe and potentially life-threatening reactions following such first exposures in uncontrolled conditions have been reported[1]. In a study by Sicherer et al[1] it was shown, that the majority of the initial reactions occurred at home (72%). Similar figures were found in the UK in children developing peanut allergy[6]. While ideally all such children should undergo their first exposure to allergenic foods under medical supervision, or undergo skin testing and/or specific IgE determination for further risk stratification, it is clear that such a recommendation is unachievable. The physician's assessment, taking account of the patient's age, co-existing asthma, and the food to be introduced (peanuts/nuts *vs* other foods)[7-9], may lead to the recommendation that introduction should either occur under medical supervision, or at home. Current guidelines recommend the introduction of these common allergenic foods gradually and individually into the diets of infants at risk for atopic disease[10], but no practical advice as to how this may be done safely has been developed so far. Furthermore, little is known about the eliciting dose at first known exposure upon which safe dietary advice may be based. Thus, while medical supervision may be needed for children with the highest risk, providing guidelines with improved safety for home introduction of allergenic foods is equally important.

In general, the lower the dose, the less severe the symptoms[11]. Therefore, the incremental scales of food challenges, provided that no severe reactions occurred, may serve as the basis for these introduction schedules for children eligible to introduce at home.

The aim of this study was to devise safe introduction schedules for common allergenic foods for use at home, based on the challenge doses as administered in DBPCFCs in children never previously exposed to these foods.

METHODS

Study population

All children at high risk for food allergy, who consecutively attended our paediatric allergy clinic between January 2003 and June 2006 for the assessment of possible food allergy, and who had never knowingly ingested the food in question before, were included in this study.

Children at high risk for food allergy were defined as children with manifestations of atopic disease (asthma, allergic rhinitis, eczema, food allergy to foods other than the food in question), and/or with at least one first-degree relative with atopic disease, and/or having sensitization to food as assessed by skin prick tests (SPT) or specific IgE.

Because of dietary preventive measures, the children had been adhering to a diet from birth in which certain known allergenic foods (cow's milk, egg, peanut, hazelnut, soy or walnut) were avoided prior to this study. In some individuals, exposure to allergenic foods was even delayed until teenage. In all children, the dietary restrictions were imposed by other health care professionals or were initiated by the parents themselves in the past. Because of uncertainty as to whether the avoided foods would be tolerated, or because of positive SPTs or positive RAST results for the avoided foods, introduction was postponed.
Elimination of the food in question was confirmed by an experienced dietician, as has been described previously[12]. Information on sex, age, allergic symptoms, family history (number of first degree family members with atopic dermatitis, asthma, rhinitis or food allergy) was obtained. This study was exempt from medical ethical approval, as DBPCFCs in these children were performed as a routine diagnostic test.

Determination of sensitization
Sensitization to the allergenic food in question was determined by SPT with commercially available extracts (ALK-Abelló, Hørsholm, Denmark) and CAP-RAST (Phadia AB, Uppsala, Sweden) within 3 months prior to the DBPCFC. SPTs were expressed both in mm and as Histamine Equivalent Prick (HEP)[13]. The HEP-index is computed by dividing the size of the wheal of the SPT of the food tested (mm) by the wheal of histamine of the SPT (mm). RAST-results of ≥0.35 kU/l and SPT-scores ≥ 3mm were considered positive. Children showing either positive SPT or specific IgE or both to the food tested were considered to be sensitized to the food in question.

Food challenges
DBPCFCs with cow's milk, egg, peanut, soy, hazelnut, and walnut were performed. Foods chosen for DBPCFC were those foods which had been eliminated from birth. DBPCFCs were performed and assessed as described previously[14]. Briefly, placebo and active challenges were administered in a random order, and were administered on separate days with at least two weeks interval in between. The incremental scale and total challenge doses used are shown in Table 1. At the lowest doses, doses were doubled, whereas the higher doses increased 4 to 5 fold. Time interval between two challenge doses was 30 minutes.
The challenge was discontinued when objective allergic symptoms occurred, or subjective allergic symptoms occurred twice on two successive administrations of the challenge material. Immediate symptoms were defined as symptoms occurring during the challenge or within 2 hours after the last challenge dose. Two days after each challenge session late onset reactions were recorded by telephone questionnaire. Late onset symptoms were defined as symptoms occurring between 2 and 48 hours after the last challenge dose. All challenge sessions were assessed according to a standardized algorithm. Forty-eight hours after the second challenge session, the code was broken and the outcome of the DBPCFC

Table 1. Incremental scales and challenge doses used in DBPCFCs

	Cow's milk (ml~g)	Soy Milk (ml~g)	Egg (mg)	**Protein equivalent (mg)**	Peanut (mg)	Hazelnut (mg)	Walnut (mg)	**Protein equivalent (mg)**
Dose 1	0.05	0.05	13	**1.75**	6	12	12	**1.75**
Dose 2	0.1	0.1	27	**3.50**	12	25	23	**3.50**
Dose 3	0.4	0.4	108	**14**	48	100	93	**14**
Dose 4	2.0	2.0	538	**70**	241	500	470	**70**
Dose 5	10.0	10.0	2690	**350**	480	860	870	**130**
Dose 6	50.0	50.0	13460	**1750**	1206	2500	2330	**350**
Total	63.0	63.0	16830 (~1/3 egg)*	**2190**	2000 (~5-7 peanut kernels)**	4000 (~4 small hazelnuts)	3800 (~1walnut)	**570**

Protein equivalent amounts of allergenic food were administered, except for the highest amounts of peanut, where the high amounts of peanut and nuts could not be validated. The total and maximum challenge doses were determined by the limitations of adequate blinding in sensory testing
* 1 medium egg = 50 gram
** 1 peanut kernel = 290– 400 gram

was assessed according to a standardized protocol[14].

Design of introduction schedules and feasibility testing

All challenge doses of the incremental scales used in DBPCFCs, as well as two additional higher challenge doses, were converted into equivalent portions of these foods in their usual household form. We examined foods likely to be frequently used by parents of young children (except for walnut), which are universally available, have standardized recipes, can easily be prepared in a household setting, and are acceptable for young children. We examined cow's milk, soy milk, egg, peanut and nuts, as well as manufactured foods, such as sponge fingers, home made muffins, peanut butter[15], and Nutella© (Ferrero). Foods in their most appropriate and practical household forms were incorporated in the home introduction schedules, taking into account the young age of most children in whom the introduction schedules are to be applied and the small amounts in which the portions have to be administered. The increasing portions of the introduction schedules were photographed (except for cow's milk or soy milk) to visualise the required amounts (Figure 1).

Without providing detailed instructions other than to introduce these foods gradually in increasing amounts, we then asked 10 parents of children who were attending our clinic for DBPCFC, how much and in which form they would administer cow's or soy milk, egg, peanut, hazelnut or walnut as a first known exposure.

Figure 1. Incremental portions of egg, peanut, hazelnut and walnut of the introduction schedules for use at home

Finally, in these 10 parents, we tested the feasibility of the introduction schedules for the lowest portions (doses 1 and 2 for cow's milk, doses 1 to 3 for egg, doses 1 to 4 for peanut, doses 1 and 2 for hazelnut, and doses 1 to 3 for walnut) (Figure 1 and Appendix A). We examined whether these parents were able to portion these foods with sufficient accuracy to ensure the safety of the procedure. The feasibility testing was done in the Food Challenge Unit of our clinic under supervision of one of the staff members. The staff member explained the purpose of the introduction schedules to the parents. Subsequently, she asked the parents to portion the required doses using the written instructions from Appendix A and, for the solid foods, the photograph as shown in Figure 1. She did not demonstrate

the portioning, nor did she train the parents in using the introduction schedules or intervene during the feasibility testing by the parents. The parents were not allowed to practice first. For the portioning of cow's milk and soy milk, we asked the parents to use a medicinal dropper. As indicated in Appendix A, a sharp knife was provided to portion the lowest doses of egg and peanut. For the portioning of Nutella, a type of knife as shown in figure 1 was provided for the testing. We ascertained the accuracy of the portioning by weighing.

Statistics

The Chi-square test (SPSS Software, 14th edition, SPSS Inc, Chicago III, USA) was used to analyse differences between the rate of positive results of DBPCFCs performed in sensitized and non-sensitized children.

The Wilcoxon Signed Rank test was used to analyse differences between portions of allergenic foods administered by parents with and without instructions. Differences in mean age between the age of children having positive and negative challenges was determined by the two sample t test (two sided) (normally distributed).

RESULTS

Patients' characteristics

A total number of 72 DBPCFCs were performed in 63 children: nine children underwent more than one DBPCFC with different foods. The characteristics of the patients who underwent the 72 DBPCFCs are shown in Table 2.

Table 2: Characteristics of 72 DBPCFCs

Patient characteristics	
Gender, n (%)	
males	46 (64%)
females	26 (36%)
Age (yrs), median (range)	5.7 (0.7-15.9)
Atopic disease at time of challenge, n (%)	
Atopic dermatitis	48 (67%)
Asthma	45 (63%)
Allergic rhinitis	28 (39%)
None	-
1st degree family member with atopy, n (%)	
0	14 (19%)
≥1	58 (81%)
Degree of sensitisation to the food in question, median (range)	
SPT (mm) (n =67)	4 (0 - 19)
SPT (HEP) (n = 67)	0.8 (0 - 2.5)
Specific IgE (kU/l (n = 65)	1.71 (0 - > 100)
Sensitization rate, n (%)	
sensitized	57 (79%)
non-sensitized	15 (21%)

Food challenges

A total number of 37/72 challenges (51%) were positive. Positive DBPCFCs were found for cow's milk (n = 6 out of 10), egg (n = 8 out of 19), peanut (n = 18 out of 26), soy (n = 1 out of 7), hazelnut (n = 3 out of 9), and walnut (n = 1 out of 1). There were no significant differences in age between children having a positive and negative DBPCFC. Positive DBPCFCs occurred in 33/57 sensitized cases (58%), and in 4/15 cases not sensitized to the food in question (27%). These frequencies were significantly different (p = .043).

Type of symptoms

The type of symptoms observed in positive challenges were dermal (54%), gastro-intestinal (49%), local and upper airway (49%), lower airway symptoms (11%), accompanied by other symptoms, such as drowsiness and irritability in 43% of the challenges.

No severe symptoms or signs of difficulty in swallowing, stridor, hoarseness, or anaphylaxis occurred. No epinephrine was administered. In 30/37 positive active food challenge sessions, immediate symptoms occurred (within two hours after the last challenge dose), whereas in 7/37 positive active food challenge sessions, only late onset symptoms were reported by the parents (2 – 48 hours after the last challenge dose).

In cases in which only late onset symptoms were observed, dermal symptoms were reported in 3 cases (exacerbation of eczema, rash), gastro-intestinal symptoms in 4 cases (cramp, diarrhoea), and lower airway symptoms in 1 case (coughing, wheezing).

In two cases, a positive diagnosis could be refuted because of placebo reactions.

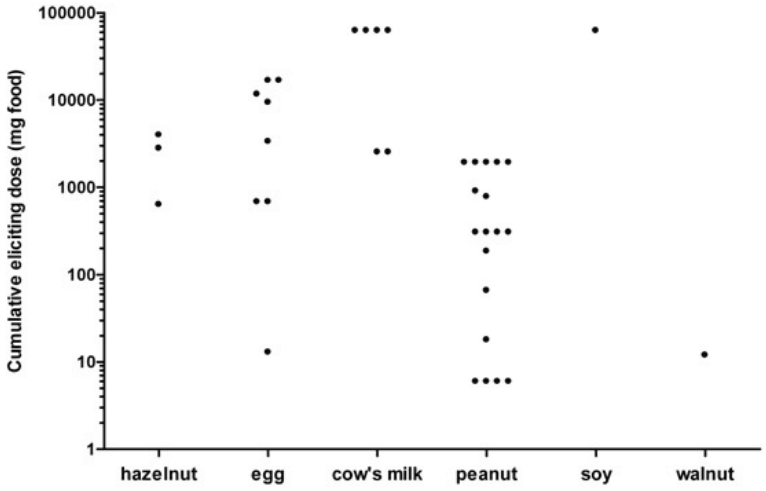

Figure 2: Cumulative eliciting doses (mg) of allergenic foods in positive DBPCFCs

Eliciting doses

In Figure 2, the cumulative eliciting doses as administered in the DBPCFCs are expressed in amounts of food (mg). For egg, peanut and walnut, the lowest eliciting doses were observed (dose 1), while in cow's milk, soy and hazelnut, the most sensitive children reacted to dose 4. In DBPCFCs performed in sensitized children showing positive SPTs and/or positive RAST results to the food in question, the eliciting doses ranged from dose 1 to dose 6, whereas in DBPCFCs performed in non-sensitized children, all reactions occurred to dose 6. All children with late onset symptoms reacted to dose 6.

Introduction schedules and feasibility testing

In Appendix A the home introduction schedule with selected foods is shown. In Figure 1, a reference photograph of the increasing amounts of the solid foods of

Appendix A. Introduction schedules for common allergenic foods for introduction at home (see also Fig 1).

	Day 1			Day 2		Day 3		Day 4
	Dose 1	**Dose 2**	**Dose 3**	**Dose 4**	**Dose 5**	**Dose 6**	**Dose 7**	**Dose 8**
cow's milk or soy milk	1 drop[1]	2 drops[1]	8 drops[1]	½ teaspoon[3]	2 teaspoons[3]	1/3 serving (50 ml)	2/3 serving (100 ml)	1 serving (150 ml)
hard boiled egg	1 very small crumb[2] of egg white	1 small crumb[2] of egg white	1 crumb[2] of egg white	1/8 teaspoon[3] of crumbs of hard boiled egg	1/2 teaspoon[3] of crumbs of hard boiled egg	¼ medium hard boiled egg (12,5 g)	½ medium hard boiled egg (25 g)	1 medium hard boiled egg (50 g)
peanut and peanut butter	1 very small crumb[2] of peanut	1 small crumb[2] of peanut	1 small knife-point[4] of peanut butter	1 knife-point[4] of peanut butter	almost 1/8 teaspoon[3] of peanut butter	almost ¼ teaspoon[3] of peanut butter	almost ½ teaspoon[3] of peanut butter	almost 1 teaspoon[3] of peanut butter
Nutella © and hazelnuts	1 small knife-point[4] of Nutella	1 knife-point[4] of Nutella	1/8 teaspoon[3] of Nutella	½ teaspoon[3] of Nutella	1 teaspoon[3] of Nutella	2 large hazelnuts	3 ½ large hazelnuts	7 large hazelnuts
walnut	1 very small crumb[2] of walnut	1 small crumb[2] of walnut	1 crumb[2] of walnut	1/10 walnut	1/5 walnut	½ walnut	1 walnut	2 walnuts

1 To be administered by a medicinal dropper
2 Crumbs of egg, peanut and walnut can be obtained by using a sharp and pointed knife.
3 To be administered using a measuring spoon set: 1/8 teaspoon = 0.625 ml; ¼ teaspoon = 1.25 ml; ½ teaspoon = 2.5 ml; 1 teaspoon = 5 ml
4 To be administered by a kind of knife as shown on the photograph

For optimal safety, one to 3 doses may be administered on one day, with a time interval of at least one hour in between the doses.
When allergic symptoms occur, the introduction should be terminated, and a physician should be contacted

the incremental scales is shown.
From Appendix A and Figure 1 it becomes clear, that the lowest doses of these introduction schedules consist of very small amounts of allergenic foods. For cow's milk and soy, the lowest doses consist of 1 drop of cow's milk or one drop of soy milk. For solid foods, the lowest doses consist of crumbs of the allergenic food, equivalent to milligrams of food.
The median portions the parents would administer as a first exposure without detailed instructions are shown in Table 3 (not normally distributed). These amounts of foods were ascertained by weighing. For cow's milk or soy milk, median portions were similar to the reference (i.e. doses 1 of the introduction schedule). For egg, hazelnut and walnut these median portions were at least approximately 8 times higher than the references. For peanut, the median portion was 25 times higher. Especially in the form of peanut butter, parents portioned relatively great amounts.

Table 3. Amounts of allergenic food (mg or ml) administered by parents with and without detailed instructions

Food	Administration without detailed instructions dose 1 median (range)	ref	Administration using instructions from Appendix A and figure 1 dose 1 median (range)	ref	dose 2 median (range)	ref	dose 3 median (range)	ref	dose 4 median (range)	ref
cow's/ soy milk	0.08 (0.05 – 30)	0.05	0.05	0.05	0.1	0.1	n.a.	n.a.	n.a.	n.a.
Egg	108* (27-2690)	13	11.5* (2-31)	13	15.5 (2–131)	27	35 (26–440)	108	n.a.	n.a.
Peanut	150** (6–2600)	6	6.5** (1-32)	6	8 (1–76)	12	45 (9–107)	50[3]	175 (3 – 955)	260[4]
hazelnut	100 (12-520)	12	52 (11–90)	100[1]	177 (41–230)	200[2]	n.a.	n.a.	n.a.	n.a.
Walnut	93*** (12-470)	12	11.5*** (2–41)	12	23 (2–51)	23	48 (20-235)	93	n.a.	n.a.

* p = .011
** p = .021
*** p = .038
1. 100 mg Nutella is protein equivalent to 12 mg hazelnut
2. 200 mg Nutella is protein equivalent to 25 mg hazelnut
3. 50 mg peanut butter is protein equivalent to 48 mg peanut
4. 260 mg peanut butter is protein equivalent to 241 mg peanut butter
n.a.= applicable

The amounts of the foods the parents portioned when using the written instructions of the introduction schedules (Appendix A) and the photograph in Figure 1, with use of a medicinal dropper for cow's milk or soy milk, are also shown in Table 3 (not normally distributed). These amounts of foods were also ascertained by weighing. For all 140 portions, the median amounts were similar to or smaller than the reference amounts of foods. The median of doses 1 of egg, peanut and walnut when portioned using the introduction schedules were significantly lower than when portioned without detailed instructions (p = .011, .021, and .038 respectively).
In 105/140 (75%) portions, the absolute weighed amounts were similar to or lower than the reference amounts of food, i.e. the required amounts of foods used in the introduction schedules (data not shown). In the remaining 35/140 (25%) portions, the absolute weights exceeded the reference amounts, but not for cow's milk and soy. These greater amounts exceeded the required amounts of food by no more than one dose of the incremental scale used in DBPCFCs.

DISCUSSION

In our study, we devised ready-to-use home introduction schedules for common allergenic foods in young children designed to avoid severe reactions at first exposure. Although it may be a matter of debate in which children these guidelines are to be used, they could be recommended for any child at risk who does not warrant first exposure under medical supervision. Since there is no consensus about the clinical characteristics of children who should introduce allergenic foods at home, and which children should be challenged under medical supervision because of a significant risk for (severe) allergic reactions, additional studies are needed to answer this question. Although increasingly higher values of food-specific IgE are strongly associated with an increasing probability of clinical reactivity to food[16], they are not associated with the severity of a reaction. In only one study an association was found between specific IgE to peanut and the severity of reaction in DBPCFCs[17]. It is noteworthy that in this study the dose of allergen in DBPCFCs was considered and incorporated into the statistical model. However, several risk factors are known to be associated with severe reactions, such as the coexistence of asthma, adolescence or young adult age, reacting to trace amounts of the offending food, (suspected) allergy to peanuts or tree nuts, and distance to emergency medical care[7-9]. The physician's assessment of the individual child will decide whether the child should be referred for introduction under medical supervision or at home. The latter population will generally consist of children in whom there is an increased risk for food allergy, but in whom there is little suspicion for the specific food in question. These children may be found for example in primary or secondary care centres, or may have older siblings with food allergy or atopic parents. They may have atopic dermatitis, or may be known with allergy to foods unrelated to the foods in question, such as cow's milk.
Little is known about the eliciting dose at first known exposure to allergenic foods.

Our study shows that the eliciting dose may vary from very low doses in the most sensitive individuals, to the maximum challenge dose in less sensitive children. The lowest eliciting doses were found for egg, peanut and walnut (dose 1, which contained 13 mg of egg, 6 mg of peanut or 12 mg of walnut). Although we did not observe reactions to the lowest doses of cow's milk, soy and hazelnut, we cannot exclude that some sensitive children might react to lower doses.

We thus converted all challenge doses of all foods into the home introduction schedules.

Caffarelli et al.[4] also reported low eliciting doses to freeze dried (raw) egg on first exposure in DBPCFC, ranging from 0.5 to 20 mg, presumably equivalent to 2 to 80 mg of fresh egg. Similarly, Lack et al.[5] described eliciting doses ranging from 50 mg to 8g of peanut, but it is not clear in the latter study if 50 mg was the lowest challenge dose used.

Although we observed a large spectrum of symptoms, no severe symptoms occurred. Most symptoms occurred immediately (30/37 positive challenges). Anaphylaxis did not occur, and no patient required epinephrine. The most severe reactions seen in our study consisted of lower airway symptoms seen in a small number of children (4/37). These were easily controlled with bronchodilator therapy. Thus, using these introduction schedules is likely to contribute to avoiding severe reactions at home. Importantly, the parents of the children using the introduction schedules are instructed to terminate the introduction, as soon as allergic symptoms occur. Additionally, sufficiently long time intervals are important to avoid severe reactions. Consequently, we propose that one to a maximum of 3 doses should be administered on one day, with a time interval of at least one hour in between the doses when using the introduction schedules (Appendix A). However, the improved safety of these introduction schedules needs to be validated in further studies.

Without instructions, doses administered at home are likely to be much higher than the low doses we administered in DBPCFCs. From our data it becomes clear, that for egg, hazelnut and walnut, without detailed instructions, parents would administer median doses which are approximately at least 8 times higher than the first doses of the introduction schedules, and for peanut a median of 25 times higher.

The lowest challenge doses of our incremental scales represent very small amounts of foods in their normal household form. Therefore, in order to achieve very low initial doses, the gradual introduction of allergenic foods in the household setting, as has been proposed previously[10], should be accompanied by detailed instructions on administering these very small amounts of allergenic foods, as have been devised in this study.

We tested the feasibility of these introduction schedules in parents of allergic children. Our results showed that for all doses parents seemed capable of portioning the required median amounts of foods with great accuracy. They were all similar to or lower than the reference amounts. Furthermore, the median doses of egg, peanut and walnut when portioned using the introduction schedules were significantly

lower (p = .011, .021, and .038 respectively) than when portioned without detailed instructions. We did not train the parents before using the introduction schedules, because the introduction schedules were meant to be usable for a large majority of parents without specific instructions. The types of knifes we used for the portioning are commonly used in the home. With regard to the absolute required amounts, for cow's milk and soy milk it seemed feasible to portion the smallest amounts using medicinal droppers, which are widely available. For the solid foods, in 75% of the portions it seemed feasible for the parents to estimate the required amounts of the incremental scale quite accurately. In 35/140 (25%) portions, the absolute weights exceeded the reference amounts, but the maximum doses administered by some of the parents were much lower than the maximum amounts administered without use of the introduction schedule, often by a factor of 10 to 100 fold. Altogether, for children introducing common allergenic foods at home, these introduction schedules provide instructions for parents which may be expected to improve the safety of this procedure by allowing the administration of the required amounts of allergenic foods in initially low doses.

Conclusions: Children may react to very small amounts of foods on first exposure. Consequently, the gradual introduction of common allergenic foods at home should start at very low initial doses. In this study, introduction schedules and a reference photograph of the incrementally increasing amounts of allergenic foods for use at home are described. These schedules provide instructions for parents to introduce the required amounts of allergenic foods in low initial doses at home with improved accuracy. This may be expected to improve the safety of this procedure.

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REFERENCES

1. Sicherer SH, Burks AW, Sampson HA. Clinical features of acute allergic reactions to peanut and tree nuts in children. Pediatr 1998;102:e6.
2. Monti G., Muratore MC, Peltran A, Bonfante G, Silvestro R, Oggero R, Mussa GC. High incidence of adverse reactions to egg challenge on first known exposure in young atopic dermatitis children: predictive value of skin prick test and radioallergosorbent test to egg proteins. Clin Exp Allergy 2002;32:1515-19.
3. Kagan R, Hayami D., Joseph L, St. Pierre Y, Clarke AE. The predictive value of a positive prick skin test to peanut in atopic, peanut-naïve children. Ann Allergy Asthma Immunol. 2003;90,640-5.
4. 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; 95:1215-20.
5. Lack G, Fox D, Northstone K, Golding J. Factors associated with the development of peanut allergy in childhood. N Engl J Med 2003;348(11):977-85.
6. Ewan P. Clinial study of peanut and nut allergy in 62 consecutive patients: new features and associations. Br Med J 1996;312:1074-8.
7. Sampson HA, Mendelson L, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. N Engl J Med 1992;327(6):380-4.
8. Bock SA, Muñoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. J Allergy Clin Immunol 2001;107:191-3.
9. Muraro A, Roberts G, Clark A, Eigenmann PA, Halken S, Lack G et al. EAACI task force on Anaphylaxis in children. The management of anaphylaxis in childhood: position paper of the European academy of allergology and clinical immunology.Allergy 2007;62:857-71.
10. Fiocchi A, Assa'ad A, Bahna S. for the adverse reactions to foods committee of the American College of Allergy, Asthma and Immunology. Food allergy and the introduction of solid foods to infants: a consensus document. Ann Allergy Asthma Immunol 2006;97:10-21.
11. Crevel RW, Briggs D, Hefle SL, Knulst AC, Taylor SL. Hazard characterisation in food allergen risk assessment: The application of statistical approach and the use of clinical data. Food and Chemical Toxicology 2007;45:691-701.
12. Vlieg-Boerstra BJ, van der Heide S, Bijleveld CM, Kukler J, Duiverman EJ, Wolt-Plompen SA et al. Dietary assessment in children adhering to a food allergen avoidance diet for allergy prevention. Eur J Clin Nutr 2006;60:1384-1390.
13. Malling HJ. Quantitative skin prick testing. Dose-response of histamine- and allergen-induced wheal reaction. Allergy 1987;42:196-204.
14. Vlieg-Boerstra BJ, van der Heide S, Bijleveld CMA, Kukler J, Duiverman EJ, Dubois AEJ. Placebo reactions in double-blind, placebo-controlled food challenges in children. Allergy 2007;62:905-12.
15. Turke J, Venter C, Deant T. Maternal experiences of peanut avoidance during pregnancy/lactation: An in-depth quality study. Pediatr Allergy Immunol 2005:16;512-18.
16. Sampson HA. Utility of food-specific IgE concentrations in prediciting symptomatic food allergy. J Allergy Clin Immunol 2001;107:891-6.
17. Hourihane JO, Grishaw KEC, Lewis SA, Briggs RA, Trewin JB, Kings RM et al. Does severity of low-dose, double-blind, placebo-controlled food challenges reflect severity of allergic reactions to peanut in the community? Clin Exp Allergy 2005;35:1227-33.

Chapter VI Dietary assessment in children adhering to a food allergen avoidance diet for allergy prevention

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ABSTRACT

Objective: The purpose of this investigation was to verify if avoidance of allergenic foods in children adhering to a food allergen avoidance diet from birth was complete and feasible, and whether dietary assessment can be used as a tool in predicting the outcome of double-blind, placebo-controlled food challenges (DBPCFCs).
Design: Children adhering to an allergen avoidance diet from birth underwent DBPCFCs. The investigator-dietician verified whether the elimination was complete, using food frequency questionnaires for common allergenic foods.
Setting: University Medical Centre Groningen, the Netherlands.
Subjects: 38 children aged 1-13 years, who were consecutively referred to the University Medical Centre Groningen for DBPCFC between January 2002 and February 2004.
Results: Amongst the 38 children undergoing DBPCFCs, there were 15 challenges with egg, 15 with peanut, 5 with hazelnut and 3 with soy. Fifteen food challenges (39%) were positive. Small quantities of allergenic foods were inadvertently present in the diets of 13 patients (34%), were possibly present in the diets of 14 patients (37%) and could not be identified in the diets of 11 patients (29%). 7 patients (54%) who had inadvertently ingested small quantities of allergenic foods without sequelae had a positive DBPCFC.
Conclusion: Dietary avoidance was incomplete and not feasible in most cases. Tolerance of small amounts of allergenic foods does not preclude positive challenge reactions. Dietary assessment does not seem a useful tool in predicting the outcome of DBPCFC in children adhering to an elimination diet.

INTRODUCTION

In several national and international guidelines, parents of infants at high risk for developing food allergy are recommended to delay the introduction of allergenic foods, such as egg, peanut, tree nuts, and fish, until the age of 1 to 3 as an allergy prevention measure (American Academy of Paediatrics, 2000; Commisie Standaard, 2005). WAO and European guidelines have not established recommendations on delayed introduction of potentially allergenic foods (Høst et al, 1999; WAO, 2004). However, the evidence for preventive effects by avoidance diets after the age of 6 months is poor (Muraro et al, 2004). In practice, guidelines on dietary prevention result in long term and sometimes indefinite elimination of these allergenic foods from birth. Parents are hesitant to introduce these foods because of uncertainty that these foods will be tolerated, or only because of positive skin prick tests (SPTs) or RAST-results for the eliminated food in the past. Elimination is thus sometimes continued for many years, because parents are often unable to obtain diagnostic certainty. This was the case with our study population, who attended our clinic to have the food allergy assessed by double-blind, placebo-controlled food challenge tests (DBPCFCs), while still adhering to these elimination diets.

The predictive value for clinical reactivity of positive skin prick tests (SPTs) and specific IgE by RAST is generally poor, and only 50% for some foods in some studies (Sampson, 2001). Therefore, the clinical relevance of positive SPTs and RAST results must ideally be verified by food challenge tests. The DBPCFC is the best available test in diagnosing food allergy (Bruÿnzeel-Koomen et al, 1995). Nevertheless, to date, this test is not widely utilized because it is labour intensive. Only recently some standardization in protocols and challenge materials for this test have been proposed (Bindslev-Jensen et al, 2004; Vlieg-Boerstra et al, 2004).

Complete dietary avoidance of allergenic ingredients in packaged foods is considered difficult by patients and parents partly because of undeclared ingredients and misleading label terminology (Gowland, 2001; Altshul et al, 2001; Vierk et al, 2002). To date, only one study has addressed the (in)adequacy of complete avoidance of allergenic foods by parents of allergic children on elimination diets (Joshi et al, 2002)

The purpose of this investigation was twofold: firstly, we wanted to verify if avoidance of allergenic foods in children adhering to a food allergen avoidance diet from birth was complete and feasible. Secondly, we wished to examine whether tolerance of small amounts of allergenic foods in the context of unintentional ingestion would predict negative challenge reactions, and whether dietary assessment can thus be used to predict the outcome of DBPCFCs.

METHODS

Study population

The study population consisted of children who were consecutively referred to the University Medical Centre Groningen for DBPCFC between January 2002 and February 2004. On the initiative of the parents or health care professionals, these children had eliminated allergenic foods (egg, peanut, hazelnut or soy) from the diet from birth as a dietary preventive measure and they had never knowingly eaten these foods before, as reported by the parents. The children were referred to our clinic because of concern about possible reactions. Therefore, study subjects underwent DBPCFC with these eliminated foods. Children in whom allergic reactions to the food, which was being avoided, were found to have occurred by history were excluded for dietary analysis. Information on atopic symptoms and family history for atopy was obtained. To perform this study medical ethical approval was obtained from the Institutional Review Board of the University Medical Centre Groningen.

Specific IgE and food challenges

Sensitisation to the allergenic food in question was determined by CAP-RAST (kU/l) (Pharmacia Diagnostics Sweden) and SPT (mm) with commercially available extracts (ALK-Abelló, Denmark) within 6 month prior to DBPCFC. RAST results of < 0,35 kU/l and SPTs of < 3 mm were considered negative. When performing DBPCFCs, placebo and active test food challenges were administered in a random order. Both the patient and health care professionals involved in the test were blinded as to the order of the food administration. Active and placebo tests foods were administered on separate days. For the placebo test food challenge, food matrices (recipes) were used which were similar in taste and smell to the matrices used for the active test food challenges. For the active test food challenge, the suspected allergenic food was disguised in a food matrix (recipe) consisting of food components to which the patient was tolerant. Validation of adequate blinding was achieved by sensory testing in a professional food laboratory (Vlieg-Boerstra et al, 2004). The challenge procedure included a 4- to 6-step incremental design, sometimes preceded by labial challenge, in which progressively greater quantities of the same allergenic food were administered, using allergenic foods in their usual edible form. The challenge was discontinued when clear-cut subjective or objective symptoms appeared. The total challenge dose, administered in the absence of a clinical reaction, consisted of 2.2g of egg protein or soy protein, equivalent to 17g of whole egg or 63 ml of soy milk, and 0.57g of peanut or hazelnut protein, equivalent to 5 peanuts (2g) or 5 hazelnuts (4g). Negative food challenges were followed by introduction of the food in question into the diet. If patients were reluctant to introduce foods at home they were encouraged to discontinue the

elimination and results of introduction were evaluated by contacting the patient one month after the negative challenge. In this way unnecessary elimination diets were discontinued and possibly false negative results were excluded.

Dietary questioning

Until up to 6 months before the DBPCFC a dietician (BV-B) with experience in food allergy examined the diets of the children during the previous 6-month period and verified whether the elimination of the allergenic foods to be avoided was complete. The parents were asked whether a dietician had been involved in establishing the dietary recommendations. Mothers were asked whether they had avoided allergenic foods while beast feeding. In order to verify if elimination of the avoided allergenic foods was complete, the dietician-investigator developed food frequency questionnaires (FFQs) for food groups containing either egg, soy, peanut or tree nuts specified for foods and brands frequently used in the Netherlands.

The following foods and food groups were included: breads and bread alternatives; cereals; baked goods, grains and grain products; pastas; starches; dairy products, cheese and desserts; alternative dairy products (soy and rice drinks, other mammalian milks such as goat milk); fats, margarine and oil; fruits, vegetables, meats, fish, poultry, egg, potatoes, beans, peanuts, nuts, seeds and respective products; vegetarian meat alternatives; sweets, chocolates and candy bars; cookies and biscuits; juices, lemonade and beverages; instant sauces, instant gravies, instant soups, instant mixes; herbs and spices; crisps and savoury snack food; spreads; products from health food stores; Asian foods; take-away meals; food supplements.

Specific terms indicative of the presence of the allergenic food in question were incorporated in the FFQs, including ambiguous labelling terms, such as "(natural) flavours" or "hydrolysed vegetable protein". By comprehensive questioning of parents by telephone, including FFQ administration, the dietician verified whether the elimination of the allergenic foods was started from birth, whether the mother ate these foods during breast-feeding, whether avoidance was complete, whether label identification was interpreted accurately concerning indicative terms with respect to the allergenic food in question, and whether the composition of packaged foods was verified by the parents, by obtaining data from the national allergen databank ALBA (TNO Nutrition and Food Research, The Netherlands) or by inquiring with the manufacturers. If the parents had not done the latter with respect to a small number of different foods, this was done by the dietician. However, when commercial foods were used on a regular basis at home or in a food service setting and information on allergenic ingredients was only obtained from the ingredient label without the exact composition being verified by data from ALBA or the manufacturer, the presence of small amounts of allergenic ingredients in these foods was assessed as being possibly present.

According to the data thus obtained, patients were divided into three categories: 1. Allergenic food present in the diet on one or more occasions, 2. Presence of allergenic food in the diet is suspected or possible and 3. No allergenic food identified in the diet. The principal contributing factors for the presence or possible presence of allergenic ingredients were analysed for each allergenic food.

Statistical Analysis

In all three categories, statistical differences between the number of positive DBPCFCs and the number of parents who obtained dietary counselling were tested by the X^2-test, two sided, using SPSS software, 12th edition. Differences in mean age between the three categories were assessed by Student's t-test (two sided) (normally distributed).

RESULTS

Study population

Thirty-eight children were included in this study for dietary assessment. Three children were excluded from dietary assessment, because the parents were reluctant in participating in the study or because of family circumstances. The mean age was 7 years (range 2 – 14 years). At the time food challenges were performed 27 of these 38 children (71%) had symptoms of atopic dermatitis, 33 children (87%) had asthma and 20 children (53%) had symptoms of allergic rhinitis. Family history for atopic disease (atopic dermatitis, asthma, and allergic rhinitis or food allergy) was positive in the majority of the children: 11 of these 38 children (29%) had one and 21 children (55%) had more than one first-degree family member with atopic disease. Six children (16%) were born in a family with no atopic first-degree family members.

Specific IgE and food challenges

Fifteen DBPCFCs were performed with egg, 15 with peanut, 5 with hazelnut and 3 with soy. Fifteen DBPCFCs (39%) were positive and twenty-three (61%) were negative (Table 1). All reactions were mild, except in one child. In mild reactions (n = 14) the following symptoms were observed: gastrointestinal symptoms (10x), itch and/or rash (3x), urticaria (3x), oedema (4x), nasal and ocular symptoms (3x), respiratory symptoms (3x), drowsiness (3x). In the child with the severe reaction, urticaria, swollen eyes and an asthmatic reaction were observed.

Most children (thirty-three children, 87%) were sensitized to the foods in question, showing both positive RASTs and skin prick tests (SPTs) (27 patients) or only positive RAST or SPT (6 patients) (Table 1).

Of the 15 children with a positive food challenge, nearly all (14) were sensitized, showing both positive RASTs and SPTs to the allergenic food in question. One

Table 1 Results of DBPCFC, dietary assessment, RAST scores and SPT values

Patient No	Food	Results of DBPCFC	Presence of allergenic food in the diet	RAST (kU/l)	RAST (class)	SPT (mm)	Age (months)
1	Egg	Positive	Present	21.80	4	4	127
2	Egg	Positive	Present	6.85	3	2	83
3	Egg	Positive	Present	11.20	3	6	83
4	Egg	Positive	Present	61.00	4	5	97
5	Egg	Positive	Not identified	0.40	1	4	91
6	Egg	Positive	Not identified	<0.35	0	0	29
7	Peanut	Positive	Present	19.10	4	10	45
8	Peanut	Positive	Present	56.80	4	3	75
9	Peanut	Positive	Present	2.60	2	8	112
10	Peanut	Positive	Possibly present	4.07	3	7	134
11	Peanut	Positive	Possibly present	24.40	4	7	48
12	Peanut	Positive	Possibly present	>100.00	6	12	58
13	Peanut	Positive	Possibly present	5.35	3	9	90
14	Peanut	Positive	Possibly present	78.00	4	7	72
15	Soy	Positive	Not identified	>100.00	6	3	119
16	Egg	Negative	Present	6.45	3	7	32
17	Egg	Negative	Present	0.38	1	0	52
18	Egg	Negative	Present	0.90	2	3	129
19	Egg	Negative	Present	0.92	2	6	116
20	Egg	Negative	Possibly present	2.24	2	4	145
21	Egg	Negative	Possibly present	3.93	3	5	24
22	Egg	Negative	Not identified	<0.35	0	0	68
23	Egg	Negative	Not identified	1.50	2	4	41
24	Egg	Negative	Not identified	1.42	2	4	135
25	Peanut	Negative	Present	1.46	2	4	50
26	Peanut	Negative	Present	2.27	2	9	48
27	Peanut	Negative	Possibly present	0.47	1	0	102
28	Peanut	Negative	Possibly present	6.80	3	6	153
29	Peanut	Negative	Not identified	0.64	1	0	67
30	Peanut	Negative	Not identified	<0.35	0	0	130
31	Peanut	Negative	Not identified	1.67	2	6	170
32	Soy	Negative	Possibly present	<0.35	0	0	59
33	Soy	Negative	Not identified	0.5	1	0	100
34	Hazelnut	Negative	Possibly present	0.94	2	5	61
35	Hazelnut	Negative	Possibly present	<0.35	0	5	91
36	Hazelnut	Negative	Possibly present	13.8	3	2	67
37	Hazelnut	Negative	Possibly present	<0.35	0	0	62
38	Hazelnut	Negative	Not identified	0.50	1	0	127

patient who reacted to egg was not sensitized to egg by either test. Of the 23 children with a negative food challenge, most children (19) were sensitized of whom 13 had both positive RASTs and SPTs and 6 had either a positive RAST or SPT. Four children with a negative food challenge were not sensitized to the food in question. No reactions were reported following a negative DBPCFC when introducing the challenged food at home.

Food avoidance

All parents had tried to keep the allergenic food in question out of their child's

diet from birth until the DBPCFC was performed. Thirty-five of 38 children were breast-fed for at least 2 weeks. Of these 35 children, only one mother eliminated allergenic foods from her own diet during breast-feeding from the birth of her child as a dietary preventive measure. Four mothers started avoiding allergenic foods on their own initiative while breast feeding when they suspected food allergy in their child. The other 30 mothers did not eliminate allergenic foods when breast-feeding, but all avoided introducing these foods when solid foods were introduced into the diet of their child. 24 (63%) of the mothers obtained dietary counselling from a dietician with regard to the dietary preventive measures taken.

Degree of elimination

No patients avoided vegetable oil when eliminating peanuts or nuts or avoided soy lecithin when eliminating soy. However, when the source of the oil was explicitly stated on the label of a commercial food and was labelled as "peanut oil", "nut oil", or "soybean oil" all patients avoided these foods. The results of the degree of elimination are shown in Table 2. In approximately 1/3 (34%) of the patients, the presence of the allergenic ingredients in question in the diets of the children was revealed by the dietician (category 1). In more than 1/3 (37%) of the children, the presence of the allergenic ingredients remained unclear and was assessed as possible (category 2). In these patients, manufactured foods were frequently used based on ingredient declaration on the label, while the exact and complete composition of these foods was not verified by the parents. None of these unintentionally ingested small amounts of allergenic foods resulted in clinical reactions. In less than 1/3 (29 %) of the children, the presence of allergenic ingredients could be excluded by comprehensive questioning (category 3). These children, allocated to the category of "no allergenic food identified", hardly used any processed foods. Most meals were prepared from basic ingredients and commercial brands were selected carefully by parents after contacting manufacturers and/or having checked the absence of allergenic ingredients by data from the national allergen databank ALBA.

Table 2 Degree of elimination: presence of allergenic food

Presence of allergenic food in the diet	No. of children	No. of positive DBPCFC results	No. of patients who obtained counselling by dietician
Category 1: yes	13 (34%)	7 (54%)	10 (77%)
Category 2: possible	14 (37%)	5 (36%)	8 (57%)
Category 3: nil	11 (29%)	3 (27%)	6 (54%)

In all three categories, a number of DBPCFCs was positive *(*Table 2*)*. 54% of the children who had ingested small amounts of allergenic foods (category 1) without sequelae had a positive DBPCFC. Although there was a trend towards lower frequencies of positive DBPCFCs in the children in whom allergenic foods could not be identified, there were no significant differences between the frequencies of allergic responses (% of positive DBPCFCs) in these 3 categories as analysed by the X^2-test.

In all three categories, a number of parents obtained counselling by a dietician with regard to the dietary preventive measures taken (Table 2). There were no significant differences between the number of parents who obtained dietary counselling in these 3 categories as analysed by the X^2-test. There were no significant differences in mean age between the 3 categories.

Causative factors for presence or suspected presence of allergenic ingredients

In Tables 3 and 4, major causes for the presence and suspected or possible presence of allergenic ingredients are presented. Contributing factors for "no strict avoidance" (Table 3) were general dietary permissiveness and mistakes. Contributing factors for "incorrect label reading" (Table 3) were not identifying or not noticing clear and unambiguous indicative labelling terms, such as "egg white" or "traces of peanut". Contributing factors for "ambiguous label terminology" (Tables 3 and 4) consisted in all patients of misinterpretations of ambiguous or complex label terminology, such as "hydrolysed vegetable protein" or "natural

Table 3 Causes of presence of allergenic food

Allergenic food	No. of patients	No. strict avoidance	Incorrect label reading	Ambiguous labelling or undeclared ingredients	Accidental intake by the child
Egg	8	3	2	2	1
Peanut	5	1	2	2	1

Table 4 Causes of possible or suspected presence of allergenic food

Allergenic food	No of patients	Use of foods of unknown composition	Ambiguous labelling or undeclared ingredients
Egg	2	2	2
Peanut	8	—	8
Hazelnut	3	2	3
Soy	1	1	1

flavour" for peanut, often occurring on labels of meat products, soup and dried mixes for sauce or soup.
Patients who used or possibly used "undeclared ingredients" (Tables 3 and 4) used manufactured compound products without verifying the exact composition with ALBA or the manufacturer or used precautionary labelled foods ("may contain ..").
"Accidental intake by the child" (Table 3) included a child given the wrong sort of potato chips (in this case flavoured with peanut) and a child given a meal prepared with an egg-contaminated knife by other family members or friends.
"Use of foods of unknown composition" (Table 4) usually occurred outdoors or in a food service setting, without the labels being read or the composition of the used foods and meals verified by the parents.
Taken together, the identification of peanut was the most problematic for parents (13 of 15 patients), due mainly to misleading or ambiguous labelling or undeclared. Second was the identification of egg (10 of 16 patients) which was problematic for the parents due to several contributing factors, including incorrect label reading and ambiguous labelling or undeclared ingredients.

DISCUSSION

Dietary avoidance of allergenic foods is the only effective therapeutic measure currently available in the treatment of food allergy. Complete dietary avoidance is known to be troublesome for allergic consumers (Vierk et al, 2002, Joshi et al, 2002). Although in our study unintentional exposure to allergenic ingredients did not provoke clinical symptoms, inadvertent use of foods was found in most patients. Thus, absolute avoidance did not seem feasible for these patients. Most patients were not aware of the mistakes they had made and thought they were avoiding the food successfully. Furthermore, most of the mothers had not avoided allergenic foods when breast-feeding. Studies have shown that peptides of allergenic foods, eaten by the mother, can be found in breast-milk (Fukushima et al, 1997). Thus, most of the children were exposed during breast-feeding.
Our results showed that the identification of peanut was more problematic than other food allergens. Incorrect label reading as a result of ambiguous label terminology most often occurred in patients misinterpreting label terminology such as "natural flavour" or "vegetable protein hydrolyzate", mainly in food stuffs used for the preparation of hot meals, such as instant soup, instant sauce and meat products. The identification of egg was also problematic, caused by several contributing factors such as undeclared ingredients. Although most parents had received dietary counselling from a dietician in the past, the difficulties and mistakes in identifying allergenic ingredients from labels suggest that parental education in correct label reading would be beneficial in improving allergen avoidance, for

example by dieticians having experience in food allergy.
Another study conducted in the USA addressing the adequacy of allergen avoidance also found that most parents were unable to identify common allergenic food ingredients such as milk (92%), egg (7%), soy (78%), peanut (46%) and wheat (12 %) (Joshi et al, 2002).
They found, as we did, that peanut was hard to identify. However, in contrast to our results, egg was relatively easy to identify for parents. This difference is probably due to the "25% rule" by which egg is not declared in many egg-containing European food products.
We agree with Wood that under the 25% rule complete dietary avoidance is quite impossible without first calling the food product's manufacturer (Wood, 2002) or verifying the composition of foods by data from a databank such as ALBA. We found that the 25% rule was one of the 3 contributing factors for (suspected) presence of undeclared allergenic ingredients (Tables 3 and 4). Firstly, by the so-called 25% rule (Taylor & Hefle, 2001; European Parliament, 2003), compound ingredients that make up less than 25% of the final food product are not required to be listed on the ingredient list of processed foods when manufactured before the end of November 2005. A second cause for undeclared ingredients was the fact that ingredients may be exempt from labelling, because they are considered to be processing aids whose presence in the food is due solely to the fact that it was contained in an ingredient of the food and has no specific function in the finished product (Taylor & Hefle, 2001; European Parliament, 2003). Thirdly, cross contamination with dietary allergens during food processing, caused by cross contact, could be a cause for presence of undeclared allergenic ingredients. Cross contact is contamination, usually caused by using shared equipment within the food industry for products with several different formulations (Taylor & Hefle, 2001). However, we could not ascertain for this factor by dietary assessment, but considered it possible in cases where the manufacturer used precautionary labelling. The national allergen databank ALBA does not ascertain cross contact. Other means of detection of dietary allergens were not available in this study.
New food labelling rules in the European Union have replaced the 25% rule from November 2005 onwards (European Parliament and Council, 2003), requiring a limited number of well known allergenic foods, such as gluten, crustaceans, egg, fish, peanut, soy, milk, nuts, celery, mustard, sesame, and sulphite to be clearly and unambiguously labelled on packaged foods. This may help allergic consumers in preventing inadvertent use of these food substances. Our results show that incomplete labelling is an important cause of dietary mistakes and support the need for improved labelling of foods as proposed by European regulatory authorities.
As a result of the poor predictive values of specific IgE by RAST and SPTs, an additional tool in predicting clinical reactivity to DBPCFCs, especially in sensitized children, would be most helpful in managing and diagnosing food allergy. However,

we found that dietary assessment is not useful in predicting the outcome of DBPCFC in children having avoided these foods from birth: in all three categories a number of patients showed positive reactions to DBPCFC (no significant differences between the three categories) and 54% of the children with unintentional previous exposure to allergenic foods (category 1) had a positive DBPCFC. Thus tolerance of small amounts of allergenic foods does not predict the outcome of DBPCFC. A possible explanation for this observation might be that the ingested food was consumed in a quantity below the threshold dose for that patient. Determination of the intake of allergenic foods by dietary history was too imprecise to allow for direct comparison. Furthermore, no databanks for common allergenic foods are available which would allow for calculation of the protein content of allergenic ingredients present in the diets of these children.

In this study, 39% of the children had clinical symptoms on their first known exposure to common allergenic foods (the DBPCFC), as has been described by others (Lack et al, 2003). Thus, physicians and dieticians should carefully consider the circumstances under which potentially allergenic foods are introduced in the diet, especially in sensitized patients.

In conclusion, complete dietary avoidance of allergenic foods is difficult, often incomplete and not feasible in most cases. Our data suggest that complete elimination of allergenic foods as a measure to prevent the development of allergic disease is not feasible, as inadvertent contact with the allergenic food may happen by incomplete label identification, mistakes or possibly by cross contact. Furthermore, ascertainment of previous asymptomatic ingestion of small amounts of allergenic foods does not preclude positive challenge reactions: 54% of the children who had ingested small amounts of allergenic foods without sequelae had a positive DBPCFC.

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REFERENCES

* Altshul AS, Scherrer DL, Muñoz-Furlong A, Sicherer SH (2001): Manufacturing and labelling issues for commercial products: relevance to food allergy. J Allergy Clin Immunol.108, 468.
* American Academy of Pediatrics, Committee on Nutrition (2000): Hypoallergenic infant formulas. Pediatrics, 106, 346-349.
* Bruynzeel-Koomen, C, Ortolani C, Aas K, Bindslev-Jensen C, Bjorksten B, Moneret-Vautrin D et al (1995): Adverse reactions to food. European Academy of Allergology and Clinical Immunology Subcommittee. Allergy. 50, 623-35.
* Commissie "Standaard" (2005): Landelijke standaard voedselallergie bij zuigelingen. Standaard voor de diagnose, behandeling en preventie van voedselallergie bij zuigelingen op het consultatiebureau [Committee on food hypersensitivity. Nation-wide standard for the diagnosis, treatment and prevention of food hypersensitivity in infants at the well-baby clinic]. 5th ed. Den Haag: Voedingscentrum.
* European Parliament and Council (2003): Directive 2003/89/EC amending Directive 2000/13/EC. Official Journal of the European Union L 308, 25.11.03, p.15.
* Fukushima Y, Kawata Y, Onda T, Kitagawa M (1997): Consumption of cow milk and egg by lactating women and the presence of β-lactoglobulin in breast milk. Am J Clin Nutr. 65, 30-5.
* Gowland MH (2001): Food allergen avoidance – the patient's viewpoint. Allergy. 56, Suppl, 67, 117-120.
* Høst A, Koletzko B, Dreborg S, Muraro A, Wahn U, Aggett P et al (1999): Dietary products used in infants for treatment and prevention of food allergy. Joint statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) Committee on hypoallergenic formulas and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on nutrition. Arch Dis Child. 81, 80-4.
* Joshi P, Modifi S, Sicherer SH (2002): Interpretation of commercial food ingredient labels by parents of food-allergic children. J Allergy Clin Immunol. 109, 1019-21.
* Lack G, Fox D, Northstone K, Golding J (2003): Factors associated with the development of peanut allergy in childhood. N Engl J Med. 348 (11), 977-85.
* Muraro A, Dreborg S, Halken S, Høst A, Niggeman B, Aalberse R et al (2004): Dietary prevention of allergic diseases in infants and small children. Part III: Critical review of published peer-reviewed observational and interventional studies and final recommendations. Pediatr Allergy Immunol. 15, 291-307.
* Sampson HA (2001): Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol.107, 891-6.
* Taylor SL, Hefle SL (2001): Ingredient and labelling issues associated with allergenic foods. Allergy. 56, Suppl.67, 64-69.
* Vierk K, Falci K, Wolyniak C, Klontz KC (2002): Recalls of foods containing undeclared allergens reported to the US Food and Drug Administration, fiscal year 1999. J Allergy Clin Immunol. 109, 1022-6.
* Vlieg-Boerstra BJ, Bijleveld CMA, van der Heide S, Beusekamp BJ, Wolt-Plompen SAA, Kukler J et al (2004). Development and validation of challenge materials for double-blind, placebo-controlled food challenges in children. J Allergy Clin Immunol. 113, 341-6.
* World Allergy Organization. Johansson SGO, Haahtela T (eds) (2004): World Allergy

Organization guidelines for prevention of allergy and allergic asthma. Condensed version. Int Arch Allergy Immunol. 135, 83-92.

* Wood RA (2002): Food manufacturing and the allergenic consumer: Accidents waiting to happen. J Allergy Clin Immunol. 109, 920-2.

Chapter VII Should children with a history of anaphylaxis to foods undergo challenge testing?

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ABSTRACT

Background: Data on the frequency of resolution of anaphylaxis to foods are not available, but such resolution is generally assumed to be rare.
Objective: To determine whether the frequency of negative challenge tests in children with a history of anaphylaxis to foods is frequent enough to warrant challenge testing, and to document the safety of this procedure.
Methods: All consecutively referred children with a history of anaphylaxis were enrolled, and underwent double-blind, placebo-controlled food challenges (DBPCFCs) between January 2003 and March 2007. Anaphylaxis was defined as symptoms and signs of cardiovascular instability, occurring within 2 hours after ingestion of the suspected food.
Results: Twenty-one children were enrolled (median age 6.1 years, range 0.8 – 14.4). The median time interval between the most recent anaphylactic reaction and the DBPCFC was 4.25 years, range 0.3 – 12.8. Twenty-one DBPCFCs were performed in 21 children. 18/21 children were sensitized to the food in question. Six DBPCFCs were negative (29%): 3 for cow's milk, 1 for egg, 1 for peanut, 1 for wheat. In the positive DBPCFCs, no severe reactions occurred, epinephrine administration was not required.
Conclusion: In children with a history of anaphylaxis to food and specific IgE levels below established decision points, re-evaluation of clinical reactivity to food by DBPCFC should be considered, even when there are no indications in history that anaphylaxis has resolved. DBPCFCs can be performed safely in these children, allowing a substantial number of these children to return to a normal diet and to relinquish their epinephrine self administration devices.

Clinical implications:
In children with a history of anaphylaxis to food and specific IgE levels below established decision points, re-evaluation of the diagnosis by DBPCFC should be considered.

Capsule summary
This first study in a consecutive series of children with a history of anaphylaxis to foods shows that double-blind food challenges are negative in a substantial number of these patients, and may be performed safely.

INTRODUCTION

Food allergy may present with a variety of symptoms, of which anaphylactic symptoms are the most severe. Anaphylaxis to food may be life-threatening or even fatal.[1,2] Because food allergy may be misdiagnosed and true food allergy may resolve in some patients[3-6], the diagnosis food allergy generally needs to be confirmed by challenge tests.[7] However, challenge testing in patients with a clear-cut history of anaphylaxis to foods has been deemed unnecessary, unsafe and undesirable by several authorities, particularly if there is IgE sensitization to the food in question[8,9], unless the patient is believed to have outgrown the food allergy.[9,10] According to these guidelines, patients with a clear-cut history of anaphylaxis to a clearly identified food are excluded from food challenge testing.[11,12]

To date, little is known about long-term sequelae following food anaphylaxis. Although no data are available on the frequency of non-recurrence or resolution of anaphylaxis to food, it is generally assumed that resolution of anaphylaxis to foods is rare, although this has been reported in some studies in individual patients.[13-16] To date, no studies have been performed in consecutive series of patients with a history of anaphylaxis to food to estimate the prevalence of resolution or persistence of the food allergy. Therefore, the aims of this study were first, to determine whether the non-recurrence or resolution of anaphylaxis in children with histories of clear-cut anaphylaxis to food is sufficiently frequent to warrant challenge testing, and, second, to document the safety of this procedure in these patients.

METHODS

Study population and sensitization

Consecutively referred children (n = 441) were screened for symptoms of anaphylaxis to food by history. Anaphylaxis was defined as cardiovascular symptoms and signs, such as anaphylactic shock with objectified hypotension, collapse, syncope, hypotonic reactions, or decreased level of consciousness, occurring within 2 hours after ingestion of the suspected food. Anaphylactic symptoms were verified in the medical records and with the parents. All children were on a diet restricted in the suspected food, as was verified by a dietician. Children in whom there were indications by history that tolerance to the food may have occurred were excluded from the study. The children underwent DBPCFCs in our clinic between January 2003 and March 2007.

Sensitization to the allergenic food in question was determined by ImmunoCap RAST (Phadia AB, Uppsala, Sweden) and skin prick tests (SPTs) with commercially available extracts (ALK-Abelló, Hørsholm, Denmark) within 6 months prior to the DBPCFC. SPTs were expressed as Histamine Equivalent Prick (HEP).[17] This index

is computed by dividing the size of the wheal of the SPT of the food tested by the wheal of histamine. RAST-results of ≥ 0.35 kU/l and SPT-scores ≥ 0.3 were considered positive. Children showing either a positive SPT or specific IgE or both to the food tested were considered as sensitized to the food in question.
Previous determination of specific IgE in all children were investigated by searching their medical records and inquiring their general practitioners.
Symptoms during the most recent anaphylactic episode, as well as the time intervals between the most recent episode of anaphylaxis and the DBPCFCs were determined. This study was exempt from medical ethical approval, as DBPCFCs in children were performed as a routine diagnostic test.

Food challenges

Placebo and active challenges were administered in a random order, and were administered on separate days with at least two weeks interval in between. Randomisation was performed by the department of Dietetics of our center.
For the active challenge, the suspected allergenic food was disguised in a food matrix to which the patient was tolerant. Unequivocal tolerance to the food matrix was ascertained by dietary history by the dietician. Validation of adequate blinding of the test materials was achieved by sensory testing in a dedicated food laboratory.[18] The challenge doses were administered according to incremental scales for the allergenic food (active food) as described previously[19], preceded by a labial challenge in which the lip of the patient was rubbed with the test food. For safety reasons, the challenges started at very low doses, such as 0.05 ml of milk (1 drop), or 6 mg of peanut, which is a very small crumb. The subsequent doses were administered at time intervals of at least ½ hour. The challenge was discontinued when objective allergic symptoms occurred, when subjective allergic symptoms occurred twice on two successive administrations of the same dose of the challenge material.
Symptoms and signs during challenges were categorized as 1. Anaphylaxis (cardiovascular symptoms and signs), 2. Dermal symptoms, 3. Gastro-intestinal symptoms, 4. Local and upper airway symptoms, 5. Lower airway symptoms and 6. "Other" symptoms, such as pallor and cyanosis.
Symptoms were assessed until two hours after the last challenge dose, and the patient was discharged when symptoms had resolved. Immediate symptoms were defined as symptoms occurring during the challenge or within 2 hours after the last challenge dose. Late onset symptoms were defined as symptoms occurring between 2 and 48 hours after the last challenge dose. Two days after each challenge session late onset reactions were recorded by telephone questionnaire.
Forty-eight hours after the second challenge session, the code was broken and the outcome of the DBPCFC was assessed according to a protocol as described previously.[19] Food challenges with a clearly positive active session and a negative

placebo session were assessed as positive. Food challenges in which symptoms occurred during the placebo challenge, or during both active and placebo challenge sessions were assessed as negative. Patients with a positive DBPCFC were advised to continue the avoidance of the challenged food. Negative challenges were followed by an open food challenge or were advised to introduce the challenged food at home. Patients received written instructions explaining how to introduce the food at home, using incrementing amounts of allergenic food (or in equivalent amounts in their usual household forms, such as peanut butter or chocolate spread with hazelnut), and ranging from approximately the maximum challenge dose to normal daily food servings. Results of introduction at home were evaluated by telephone 1 month after the DBPCFC.

Statistics

Because of the small numbers median results of patient characteristics and test results are presented. Spearman's rho coefficient was used to calculate correlations between the cumulative eliciting doses (reactive dose in mg food) in DBPCFCs (not normally distributed) and specific IgE or SPT. Between children with positive and negative DBPCFCs, the following statistical tests were used to analyze differences: The Mann-Whitney test (two sided) for differences in age, specific IgE, SPTs, and time intervals between the anaphylactic reactions and the DBPCFCs, and the Chi-square test for the number of non-sensitized children and the presence of asthma.

RESULTS

Study population and sensitization

Twenty-one children were enrolled (13 males, 8 females, median age 6.1 years, range 0.8 – 14.4 years). At the time of challenge, 17 children had symptoms of atopic eczema (81%), 14 had asthma (67%) and 11 had rhinitis (52%). All but one (Table I, patient no 3) had at least one of these atopic symptoms. The remaining patient characteristics are presented in Table I.

Median specific IgE (n = 21) was 5.99 kU/l (range < 0.35 to > 100), median HEP (n = 19) was 0.9 (range 0.00 – 2.0). Data on previously determined specific IgE levels were incomplete.

History of anaphylaxis

Children were not always referred immediately to our clinic following their anaphylactic reactions and in some cases only after several years. The median time interval between the most recent anaphylactic reaction and the DBPCFC was 4.25 years, range 0.3 – 12.8 years.

Details of the most recent anaphylactic reaction to food are presented in Table I.

Table I. Characteristics of patients, anaphylactic reaction in dietary history, and results of DBPCFCs

Pat. no	Sex	Age (Yrs, Mo) at time of DBPCFC	Food	Symptoms during anaphylaxis (cat. 1to 6)*	SPT mm, (HEP)	Spec. IgE (KU/l)	Time interval (yrs)*	Results of DBPCFC	Valid symptoms during active challenge sessions (cat. 1 to 6)**	
									Imme-diate	Late onset
1	M	6,4	Cow's milk	1. Hy 2. U, gi, ad 3. N, vo 4. Oas	5 (0.9)	1.44	6.3	Positive Dose 3	2. U	3. Cr, d
2	M	6,0	Peanut	1. Co 2. U, gi, ad 3. N, vo 4. Oas, lo 5.Co, wh 6. A	7 (1.4)	78	3.5	Positive Dose 5	2. Gi,o 4. Rhi 6. A	-
3	F	3.2	Wheat	1. Dlc 3. cr, d	0 (0.0)	< 0.35	2.7	Positive Dose 6	-	3. Cr, d
4	M	6.1	Peanut	1. Dlc 2. U, o 3. Cr, n, vo, d 4. Oas, lo 5. Dys	8 (1.2)	> 100	3.1	Positive Dose 5	2. U 3. N, vo	-
5	F	13.6	Peanut	1. Syn 2. U, gi, o 3. Ab 4. Oas, lo, ts 5. Dys 6. Tach, a	4 (0.7)	0.41	12.1	Positive Mucosal	4. Ls	-
6	M	5.8	Hazel-nut	1. Dlc 2. R 3. N, vo 4. Oas, ls, ts 5. Dys, wh	5 (0.8)	23.5	2.7	Positive Dose 6	2. R 3. Cr	2. Ad 3. Cr 6. Dr
7	M	3.10	Hazel-nut	1. Syn 2. U, gi, o 3. N, vo 4. Oas, lo, ts stri	4 (0.9)	5.99	1.9	Positive Dose 3	4. Tt, ie	-
8	M	6.1	Egg	1.Co 2. O 6. Dr, leth	7 (1.3)	3.79	4.3	Negative	-	-

9	F	1.8	Cow's milk	1. Syn 2. U, gi, o 3. Cr, ab 4. Oas, lo, ts 6. Tach	-	8.78	1.1	Positive Dose 5	2. U, gi 3. Cr	-
10	M	0.8	Cow's milk	1. Hy 2. R 3. N, vo 5. Dys 6. Cy	8 (2)	> 100	0.3	Postive Dose 5	2. U 3. Vo	-
11	F	11.9	Egg	1. Dlc 2. U, o, ad 3. Cr, n, vo, d 4. Oas, ts	0 (0,0)	< 0.35	8.9	Positive Dose 6	2. U, gi, 4. Ie	6. Dr
12	M	5.8	Peanut	1. Syn 2. O 3. N, vo 4. Oas, lo	5 (0.9)	> 100	3.3	Positive Dose 4	2. O	3. D 4. Sne 5. Co
13	F	9.4	Cow's milk	1. Dlc 2. R, o 6. leth	- 	> 100	6.8	Positive Dose 4	2. R 3. N, vo, cr 4. Rhi	-
14	M	7.3	Cow's milk	1. Co 2. O 3. N, vo 3. Oas, lo 5. Dys 6. Pa	6 (1.3)	19.4	5.8	Positive Dose 5	3. Cr 4. Ls, st	
15	F	4.2	Cow's milk	1.Dlc 2.U, gi, 3.N,vo,cr,d	0 (0.0)	<0.35	1.0	Negative	-	-
16	M	4.9	Peanut	1. Syn 2 U, o	2 (0.6)	< 0.35	4.3	Negative	-	-
17	F	10.3	Peanut	1.Dlc 2. O 4..Oas 5..Dys	6 (1.3)	1.19	9.3	Postive Dose 1	4. Oas	-
18	M	3.2	Cow's milk	1. Syn, hy 3. N, vo, cr, d 4. Oas 6. Ta	2 (0.3)	< 0.35	2.6	Negative	-	-

Table I. continued

Pat. no	Sex	Age (Yrs, Mo) at time of DBPCFC	Food	Symptoms during anaphylaxis (cat. 1to 6)*	SPT mm (HEP)	Spec. IgE (KU/l)	Time interval (yrs)*	Results of DBPCFC	Valid symptoms during active challenge sessions (cat. 1 to 6)** Imme-diate	Late onset
19	F	13.2	Wheat	1. Dlc 2. R, u, gi, o, ad 3. N, vo, ab 4. Oas 6. Pa	4 (0.9)	6.85	10.3	Negative	-	-
20	M	12.2	Cow's Milk	1. Syn 2. R, u, gi, O, 3. N, vo, cr, d, 5. Dys 4. Oas, lo, ts 6. Pa	7 (1.2)	>100	11.8	Positive Mucosal	4 .Ls, tt	4. rhi
21	M	13.3	Cow's milk	1. As 2. R, u, gi, o 3. N, vo, cr, d 5. Dys 6 Pa	5 (0.8)	0.62	12.8	Negative	-	-

* time interval since most recent anaphylaxis to the food in question

**** Category 1: Cardiovascular symptoms:** As = anaphylactic shock (objectified hypotension), co = collapse, syn = syncope, hy = hypotonic reactions, dlc = decreased level of consciousness
Category 2: Dermal symptoms: r = rash/exanthema, u = urticaria, gi = generalized itch, o = (quincke's) oedema, ad = exacerbation of atopic dermatitis
Category 3: Gastro-intestinal symptoms: n = nausea, vo = vomiting, cr = cramp, d = diarrhoea
Category 4: Local and upper airway symptoms: oas = oral allergy symptoms, lo = laryngeal oedema, ls = lip swelling, ts = tongue swelling, rhi = rhinitis/ conjunctivitis, stri = stridor, tt = tight throat/itchy throat, ie = itchy ears, sne = sneezing, st = sore throat
Category 5: Lower airway symptoms: dys = dyspnoe, co = coughing, wh = wheezing
Category 6: Other symptoms: a =anxiety ta = tachycardia, dr = drowsiness, leth = lethargic, cy = cyanosis, pa = pallor

All children had reacted with cardiovascular symptoms and at least two or more concomitant allergic symptoms. All anaphylactic reactions occurred within 10 minutes following ingestion of the suspected food.

Results of DBPCFCs

The results of the DBPCFCs are shown in Table I. Twenty-one DBPCFCs were performed in 21 children with cow's milk (9 challenges), egg (2 challenges), peanut (6 challenges), hazelnut (2 challenges), and wheat (2 challenges).

Fifteen DBPCFCs were positive (71%): 6 for cow's milk, 1 for egg, 5 for peanut, 2 for hazelnut, and 1 for wheat. Six DBPCFCs were negative (29%): 3 for cow's milk, 1 for egg, 1 for peanut, and 1 for wheat.

In positive DBPCFCs, a variety of symptoms occurred, such as dermal symptoms (9 cases), gastro-intestinal symptoms (9 cases), local symptoms (9 cases), and "other symptoms" (3 cases). No severe reactions occurred: we observed no immediate lower airway symptoms (except for coughing in patient no 12, Table I), no stridor, and no hypotension. No epinephrine was administered. An intramuscular antihistamine was administered in 1 patient, oral antihistamine in 4 patients.

In all positive challenges, symptoms occurred immediately (either alone or in combination with late onset symptoms), except in one patient (Table I, patient no. 3): this child reacted with late onset gastro-intestinal symptoms to wheat. In 13/15 positive challenges, children showed objective symptoms, whereas 2 children reported repeated subjective symptoms only, which resulted in termination of the challenges

In 4 children, placebo events occurred (patients' no.11, 14, 15 and 18). Patients no 11 and 14 reported vague feelings of a tight and sore throat during the placebo challenge. Because of clearly more convincing reactions during the active food challenge session, these challenges were assessed as positive. Patient no 15 reported late onset symptoms of cramp and diarrhoea following both the active food challenge session and the placebo challenge. This challenge was assessed as negative. Patient no 18 reported late onset cramps on the placebo day, but no symptoms on the active food challenge. This challenge was assessed as negative.

In figure 1, the cumulative eliciting doses (expressed in mg food) of the positive DBPCFCs are shown. The highest eliciting amounts were found for egg and wheat, whereas the lowest eliciting amounts were found for cow's milk and peanut.

Statistical analysis showed no correlation between the eliciting amount of food in positive food challenges and specific IgE or SPT. All children with a negative DBPCFC introduced the food successfully into their diets. The shortest time interval between anaphylaxis and negative DBPCFC was 1.0 years (patient no.15, Table I).

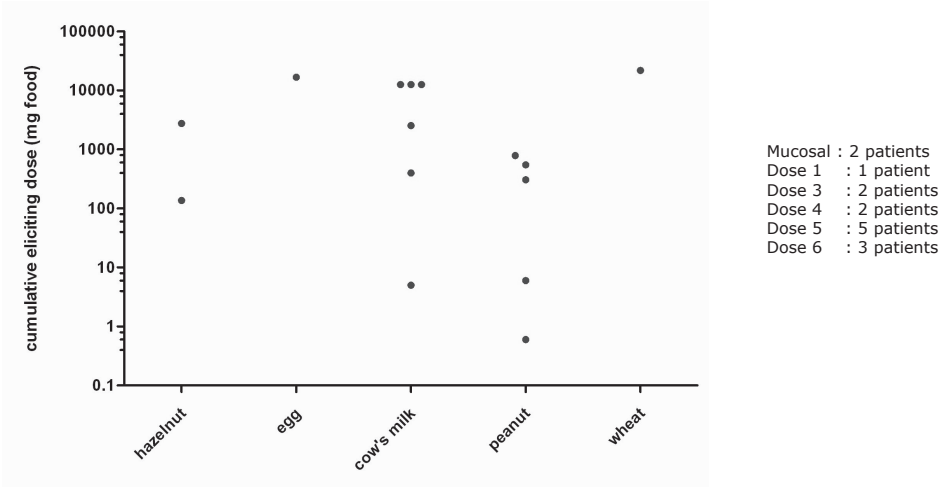

Figure 1. Cumulative eliciting dose in positive DBPCFCs in mg of food (n = 15)

Characteristics of negative versus positive DBPCFCs

We compared several characteristics of children with positive and negative DBPCFCs as predictors for the outcome of food challenge tests (Table II). Non-sensitized children were found in both groups (n.s.). In the negative group, 3 out of 6 patients were not sensitized, and in the positive food challenge group, 2 out of 15 patients were not. The latter children reacted to dose 6 (highest challenge

Table II. Characteristics of positive versus negative DBPCFCs.

	Positive DBPCFCs	Negative DBPCFCs
Sensitized (n)	13/15 (cow's milk, peanut, hazelnut)	3/6 (cow's milk, egg, wheat)
Non-sensitized (n)	2/15 (wheat, egg) (dose 6)	3/6 (cow's milk 2x, peanut)
Decreasing specific IgE	0/9	2/5
Increasing/unchanged specific IgE	9/0	3/5
Median specific IgE (kU/l	19.40* (<0.35 ->100)	0.46* (<0.35 – 6.85)
Median HEP	0.90 (0.0 – 2.0)	0.70 (0.0 – 1.3)
Median age (yrs)	6.1 (0.8 -13.6)	5.5 (3.2 -14.4)
Median time interval (yrs)	3.6 (0.3 – 12.1)	4.3 (1.0 – 12.8)
Asthma	11	3

* p = .029

dose). Also the other characteristics presented in Table II, such as size of SPT (HEP), age, time interval between anaphylaxis and DBPCFC, or the presence of asthma did not differ significantly between both groups. Only specific IgE to the food in question was significantly higher in the positive food challenge group (p =.029) (Table II).

DISCUSSION

To our knowledge, this is the first study using DBPCFCs in a consecutive series of children with a history of anaphylaxis to foods, and no indications in dietary history that tolerance to these foods might have supervened. Previous studies have reported the resolution of anaphylaxis to foods in individual patients.[13-16] In a study by Bock[15] serial food challenges were performed in a selected series of nine children who had experienced severe reactions to foods in their first year of life. Of these children, only three had experienced cardiovascular symptoms as part of their allergic reactions. One of these three children became tolerant to the food in question. The culprit foods in this study were cow's milk, egg and soy, and no children with peanut or nut allergy were reported, probably because of the young age of the subjects. Spergel et al.[20] found no development of tolerance to peanut by open food challenges in 5 children with a history of anaphylaxis, but in a subsequent report, one patient with a history of anaphylaxis was re-challenged and was found to be tolerant[16]. While these studies demonstrate that resolution of anaphylaxis to foods can occur, it is difficult to estimate how frequent this may be the case. Our results show that 29% (6 cases) of consecutively referred children did not react to the foods in question at the time of the challenges. Additionally, two (non-sensitized) patients reacted to the highest challenge dose (Table I, patients 3 and 11), and it is possible that they were in the process of outgrowing their food allergy.[22,23] Thus, in children with a history of anaphylaxis to food, re-evaluation of the clinical reactivity to food by challenge testing should be considered by the physician, even when there are no indications in dietary history that they have outgrown their anaphylaxis. Without this re-evaluation, patients may be unnecessarily diagnosed as being severely food allergic for prolonged periods of time. This may also have been the case in our study, as the duration between anaphylaxis and DBPCFC was up to 12.8 years.

An important limitation of this study is that we had very few children who had experienced their anaphylactic reaction to foods relatively shortly (i.e. weeks to months) before being tested. It therefore cannot be excluded that the frequency of negative test outcomes could be much lower in such cases. Further studies are needed to define minimum time intervals following anaphylaxis at which food challenges may be useful.

There are two possible explanations for the negative test results in 6 patients of the study population. Since the differential diagnosis of anaphylaxis is extensive, and initial food challenges were not performed to establish the diagnosis, it cannot be excluded that some of these children had another diagnosis initially. However, the same is true for other studies describing resolution of anaphylaxis to food in individual patients[13-16], as only one of these studies[15] utilized initial DBPCFCs. The other possibility is that previous anaphylactic reactions to foods have gone into spontaneous remission, as has been described for other systemic reactions to foods.[3-6] Several observations favour the latter explanation. First, in all children the time interval between consumption of the food and the onset of anaphylaxis was short: all children reacted within 10 minutes following ingestion of the causative food. Secondly, in all children the causative food or ingredient could be determined precisely by the parents and 3 out of 6 patients were still sensitized to the food in question (patients' no. 8,19,21, Table I). The other 3 children with a negative test result might have lost their sensitization to the food in question over time (patients' no 15, 16 and 18).

Thirdly, no recurrent episodes of anaphylaxis were reported by the children after the culprit food was eliminated from the diet. Thus, we think these children had true anaphylaxis to foods initially which resolved over time.

In the children with negative DBPCFCs, the dietary restrictions for the challenged foods could be terminated without recurrence of symptoms. Furthermore, these children were able to relinquish their epinephrine self-administration devices. This is important, because, while the availability of an epinephrine self-administration device during anaphylactic reactions is of unquestioned value, concerns have recently been raised about over-prescription of this medication.[24] These concerns seem warranted given the negative effects of carrying such a device on quality of life.[25]

We looked for predictors of the outcome of the DBPCFCs. In our analysis, only the levels of specific IgE were predictive. These were significantly higher in those with a positive than in those with a negative DBPCFC. This is in agreement with studies which have shown that the probability of clinical reactivity to food increases with increasing specific IgE levels.[21] As we found no negative challenge results in the 43% of children who had specific IgE values for cow's milk, egg, peanut beyond which 95% of the patients react[21], undertaking DBPCFCs may not be necessary in children with sensitisation levels beyond these decision points.

It has been reported that decrease in sensitization may predict development of tolerance of food allergy over time.[23] We carefully investigated previous determination of specific IgE in all children by searching their medical records and inquiring their general practitioners. We found that these data were incomplete. In 7/21 children, we did not find any data on previous specific IgE levels. In 14/21 children, previous data on specific IgE were available. In only 6 of these children,

specific IgE was determined at the time of anaphylaxis. The data on previous specific IgE in positive and negative challenges are presented in table II. No children (0/9) with a positive challenge test and only 2/5 children with a negative challenge test showed a decline in specific IgE to the food in question (Table II). However, in the other 3 children with a negative challenge, specific IgE was determined at the time the anaphylaxis in only 1 child, and it can not be excluded that in the other 2 children with a negative test, specific IgE may have been higher at the time of anaphylaxis.

DBPCFCs may be performed safely in children with a history of anaphylaxis. We observed no severe reactions, and no epinephrine was required in the treatment of reactions during challenge testing. Other studies also conclude that food challenges may be performed safely. [12,26] Elements of the challenge protocol which we feel contribute to the safety of the procedure are very low starting doses (in the 1.75 mg of protein range), a time interval between doses of at least ½ hour, and gradual increase of the amount of allergenic food (initially doubling, later 5-fold increases). Also, food challenge sessions were terminated when repeated subjective symptoms were reported by the patients, or when mild objective symptoms were observed, and the avoidance of higher doses in these situations may have prevented severe reactions from occurring. Although the numbers of our study population are small, to date, in more than 500 DBPCFCs performed in our center, no severe or life-threatening reactions have occurred.

In conclusion, in children with a history of anaphylaxis to foods and specific IgE levels below established decision points, resolution or non-recurrence of anaphylaxis is not uncommon, and re-evaluation of clinical reactivity to food by DBPCFC should be considered, even when there are no indications of tolerance to the food in question. Such challenge testing may not be necessary in children with sensitization above well established decision points. DBPCFCs can be performed safely in these children, allowing a substantial number of these children to return to a normal diet and to relinquish their epinephrine self administration devices. The value of challenge testing in children with recent anaphylactic reactions is presently unknown.

REFERENCES

1. Sampson HA, Mendelson L, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. N Engl J Med 1992;327(6):380-4.
2. Bock SA, Muñoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. J Allergy Clin Immunol 2001;107:191-3.
3. Saarinen KM, Pelkonen AS, Makela MJ, Savilahti E. Clinical course and prognosis of cow's milk allergy are dependant on milk-specific IgE status. J Allergy Clin Immunol 2005;116:869-75.
4. Boyano-Martinez T, Garcia-Ara C, Diaz-Pena JM, Martin-Esteban M. Prediction of tolerance on the basis of quantification of egg white-specific IgE antibodies in children with egg allergy. J Allergy Clin Immunol 2002;110:304-9.
5. Skolnick HS, Conover-Walker MK, Koerner CB, Sampson HA, Burks AW, Wood RA. The natural history of peanut allergy. J Allergy Clin Immunol 2001;107;367-74.
6. Fleischer DM, Conover-Walker MK, Matsui EC, Wood RA. The natural history of tree nut allergy. J Allergy Clin Immunol 2005;116:1087-93.
7. Thong BY, Hourihane JO. Monitoring of IgE-mediated food allergy in childhood. Acta Pediatr 2004;93:759-64.
8. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J et al. Standardization of food challenges in patients with immediate reactions to foods – position paper from the European Academy of Allergology and Clinical Immunology. Allergy 2004;59:690-697.
9. Sampson HA. Update on food allergy. J Allergy Clin Immunol 2004;113:805-0.
10. Muraro A, Roberts G, Clark A, Eigenmann PA, Halken S, Lack G et al. EAACI task force on Anaphylaxis in children. The management of anaphylaxis in childhood: position paper of the European Academy of Allergology and Clinical Immunology. Allergy 2007;62:857-71.
11. Hourihane JO, Roberts SA, Warner JO. Resolution of peanut allergy: case-control study. BMJ 1998;316:1271-5.
12. Pucar F, Kagan R, Lim H, Clarke AE. Peanut challenge: a retrospective study of 140 patients. Clin Exp Allergy 2001;31:40-6.
13. Fleischer DM, Conover-Walker MK, Christie L, Burks AW, Rood RA. The natural progression of peanut allergy: Resolution and the possibility of recurrence. J Allergy Clin Immunol 2003;112:183-9.
14. Solensky R. Resolution of fish allergy: a case report. Ann Allergy Asthma Immunol 2003;91:411-2.
15. Bock SA. Natural history of severe reactions to foods in young children. J Pediatr 1985;107:676-80.
16. Spergel JM, Beasoleil JL, Pawlowski NA. Resolution of childhood peanut allergy. Ann Allergy Asthma Immunol 2000;85:473-6.
17. Malling HJ. Quantitative skin prck testing. Dose-response of histamine- and allergen-induced wheal reaction. Allergy 1987;42:196-204.
18. Vlieg-Boerstra BJ, Bijleveld CMA, van der Heide S, Beusekamp BJ, Wolt-Plompen SAA, Kukler J, Brinkman J, Duiverman EJ, Dubois AEJ. Development and validation of challenge materials for double-blind, placebo-controlled food challenges in children. J Allergy Clin Immunol 2004; 113:341-6.
19. Vlieg-Boerstra BJ, van der Heide S, Bijleveld CMA, Kukler J, Duiverman EJ, Dubois AEJ. Placebo reactions in double-blind, placebo-controlled food challenges in children. Allergy 2007;62:905-12.
20. Spergel JM, Fiedler JM. Natural history of peanut allergy. Curr Opin Peadiatr 2001;13:517-22.

21. Sampson HA. Utility of food-specific IgE concentrations in prediciting symptomatic food allergy. J Allergy Clin Immunol 2001;107:891-6.
22. Knight AK, Schreffler WG, Sampson HA, Sicherer SH, Noone S, Mofidi S, Nowak-Wegrzyn A. Skin prick test to egg white provides additional diagnostic utility to serum egg white-specific IgE antibody concentration in children. J Allergy Clin Immunol. 2006;117:842-7.
23. Shek LP, Soderstrom L, Ahlstedt S, Beyer K, Sampson HA. Determination of food specific IgE levels over time can predict the development of tolerance in cow's milk and hen's egg allergy. J Allergy Clin Immunol 2004;114:387-91.
24. Kemp AS. EpiPen epidemic: Suggestions for rational prescribing in childhood food allergy. J. Pediatr Child Health 2003;39:372-375.
25. Oude Elberink JN, de Monchy JG, van der Heide S, Guyatt GH, Dubois AE. Analysis of burden of treatment in patients receiving an EpiPen for yellow jacket anaphylaxis. J Allergy Clin Immunol 2006;118:699-704.
26. Perry TT, Matsui EC, Conover-Walker MK, Wood RA. Risk of oral food challenges. J Allergy Clin Immunol 2004;114:1164-8.

Chapter VIII General Discussion

The DBPCFC is characterized by several cardinal features, such as randomization, the use of adequately blinded challenge materials, and the administration of placebos. Other important variables and considerations are the assessment of symptoms, incremental scales used, adequate top dose and safety of the procedure. Improved, standardized and validated practical protocols for the performing of DBPCFCs with regard to these parameters are needed for the medical profession to date, as has been outlined in chapter I.

In part I of this discussion, we focus on standardization and validation of some of these parameters of the DBPCFC, as well as the clinical relevance of our findings regarding placebo events (chapters II-IV).

In part II of this discussion, we discuss the practical, clinical and diagnostic implications, as well as the safety of the challenge outcomes in patients having their first exposure to allergenic foods (Chapters V and VI), and in patients with a history of anaphylaxis to food respectively (Chapter VII). Finally, recommendations for future research are discussed.

Part I

Blinding

Chapter II describes the first sensory laboratory validated recipes for use in DBPCFC. These recipes are available for use in other centres. While it is essential to guarantee blinded conditions during DBPCFCs, efforts to validate blinding of recipes for DBPCFCs, other than by volunteer panels[1-5], are more recent[6]. The validation process in our study[6] consisted of sensory testing for difference with regard to adequate blinding of the active food of the developed recipes. This process consisted of a two-step procedure, in which the recipes were first tested by a volunteer hospital panel, and subsequently, if no statistical differences were detected between placebo and active test food samples, by a professional panel of food tasters (Figure 1).

The professional panel decoded 10 of the 27 recipes tested by detecting significant differences between the samples. By obtaining these results we have clearly demonstrated that the use of a professional panel is mandatory for optimal results in sensory testing for difference with regard to blinding of the active (allergenic) food. Utilizing volunteer panels in a non-controlled environment is an important first step in testing challenge materials, but is not sufficient to guarantee optimal blinding. Validating recipes in a non-professional environment is likely to overestimate the blinding capacity, and thus validity of recipes, as is outlined in Chapter III. In this chapter, we stressed the use of food laboratories and professional panellists in validating recipes for DBPCFC.

Recently, also Ronteltap[7] and Ballmer-Weber[8] reported on the use of sensory testing of recipes in professional food laboratories. This method of validation of recipes has now become the standard, and is being used in recipe development in

Figure 1. Panellists of the professional sensory panel in a sensory booth of the Food Laboratory of the University of Professional Education Groningen, doing the Triangle test

Europrevall, a European multi-centre research project of food allergy[9].

Top dose in DBPCFCs

The major challenge in developing and validating recipes for use in DBPCFCs is to disguise sufficiently great amounts of allergenic foods in an acceptable volume[2,6]. The final discrete dose, or the administered cumulative dose should be high enough to prevent false negative test results. In general, it is stated that a maximum dose of 8–10g of dried food (which is equivalent to 60–100g of wet food) should be taken as a single, maximum dose[10], or up to 15 – 20g of dried food as the cumulative dose. It is also stated that the top dose given should reflect a relevant amount of food[11], or should reflect the normal daily intake of the food[10, 12,13]. Although these statements seem reasonable, they are not based on evidence, but on the assumption and individual observations that individual patients may react to amounts of allergenic food up to normal food servings**.**

However, the total and maximum amounts of active allergenic food we administered in DBPCFCs were smaller than the amount of a single food serving, and were mainly determined and limited by the maximum amount we could disguise in an acceptable volume of the validated recipes. In view of the results of our study, large amounts of allergenic foods are very difficult to disguise in acceptable volumes of test foods[2,6,14], and the validity of high eliciting doses found in some studies may be questioned. For this reason Atkins et al.[14] started food challenges in adults in a double blind manner for the lower doses, ending with open food challenges for the higher doses. For reasons of safety, in children age six and older having a negative double-blind challenge with egg, peanut or nuts, currently, we also have the DBPCFC followed by an open challenge, and not by gradual introduction

at home as in younger children. It must be noted however, that, if reactions in these open food challenges occur, the validity of these observations remain to be assessed. Additional studies are needed to answer this question.

To date, there have been no studies specifically aimed at determining the top dose necessary to avoid false negative results of DBPCFC. Some studies describe the eliciting doses and the proportion of false negative results in detail[1,3,8,14,15,18]. In some of the previously mentioned studies, the (cumulative) eliciting dose is similar to or slightly higher than in our doses[11], while in other studies doses were much higher than those we administered[1,3,8,14,17,18]. However, these data are only reliable if absolute blinding has been guaranteed. Thus, it is possible that some of the responses to high doses may be biased by lack of blinding. Adequate blinding techniques could possibly result in lower final required test doses than currently proposed. To date, it is unclear what the highest top dose is which is sufficient to rule out false negative test results.

Finally, it has not been determined whether single or cumulative doses are of greater importance in the DBPCFC. In the latter approach (cumulative dose) all doses administered are summed and calculated as influencing each other, whereas the first approach treats individual doses as independent events[19]. The validity of one approach or the other from a biological point of view depends on factors such as the time spacing of doses, and the matrix in which the allergen is masked[19]. We suggest that both doses should be considered.

The diagnostic value of placebos

Our study on the diagnostic significance of placebo events shows that placebo events present with a variety of symptoms, which may be classified as subjective or objective, and immediate or late onset[20]. The total number of placebo events in sensitized children (12.9%) (Chapter IV), as well as the number of placebo events that reveals false positive test results (5.4%), are relatively low. These relatively low figures could possibly lead to the mistaken conclusion, that the administration of placebos and hence DBPCFCs are of marginal importance in sensitized children, and that the diagnosis might as well have been established by an open challenge. However, the active test food challenge session from which the potential false positive rate is calculated can be considered as an open food challenge, with the notable difference, however, that patients and observers are blinded for the challenge order. Thus, they do not know whether the active test food is being given. We argue that in our study true open food challenges would have rendered a significantly higher percentage of false positive results. This is based on the studies by several other authors[21-25]. Venter et al.[21] verified results of open food challenges in sensitized children by repeating positive open challenges by DBPCFCs. Remarkable differences were found in the proportions of test results. In children of 9 and 12 months of age, false positive rates of open food challenges

of 62% (5 out of 9 open challenges) can be calculated, and 50% (13 out of 25 open challenges) respectively. In six-year-old children, 3/6 (50%) children were overdiagnosed by open challenge as compared to DBPCFC[22]. In teenagers these proportions were 1/2 (50%) in 11-year-old children, and 4/7 (57%) in 15-year-old children[23]. It must be noted, that the results include both one day and one week challenges. In a recent paper by the same authors[24], it was shown that, with regard to the one day challenges in children with immediate symptoms, 8 out of 11 open challenges could be confirmed by one day DBPCFCs, which represents a false positive diagnosis of 3/11 = 27% by the open food challenge test. Also, Brouwer et al[25] found significantly high proportions of false positive diagnoses in children with atopic eczema, as is discussed in Chapter IV. Thus, from combining these data with the results of our study, the conclusion may be drawn that the most important contribution of the administration of placebos (in sensitized children) to the diagnostic accuracy of the DBPCFC is, that blinded administration of test food is made possible. Consequently, fewer events are observed during the active food challenge, because biased observations by patients and physicians are ruled out. It must be noted however, that these studies were population based[21-24] and conducted in a primary health care setting[25] respectively. In these populations, the proportions of self-perceived food allergy are higher than in tertiary care settings (7.2 – 14.2%)[21,23], which may increase the number of false positive observations in open challenges. As our study was conducted in a tertiary referral centre, the expected differences between open challenges and DBPCFCs are expected to be lower. However, our data show that the use of placebos significantly enhances the diagnostic reliability of DBPCFCs in a tertiary care referred population.

Assessment of DBPCFCs

Another important aspect of the DBPCFC is the assessment of symptoms, and the related challenge endpoints. To our knowledge, to date, no other protocols providing detailed criteria for the assessment of symptoms in DBPCFCs and the assessment of the final outcome of DBPCFCs have been published (Chapter IV).
With regard to assessment protocols, several features are important:
1. The diagnostic significance of placebo events, as discussed in the previous section;
2. History: DBPCFCs are assessed as positive when allergic symptoms occur following the active food challenge, or when symptoms from dietary history are reproduced, even if these symptoms are characterized as non-allergic symptoms (Figure 1, Chapter IV). This may implicate that subjective symptoms, as well as symptoms which are unlikely to cause food allergy, such as headache or an overall feeling of distress, if reproduced by DBPCFC, may be assessed as positive. However, in the individual patient, for scientific purposes, these latter observations can only be validated by repeated DBPCFCs according to the so-called N=1 trial.

This design is discussed later in this chapter.
Safety may also be achieved by termination of the challenge when subjective symptoms occur (see below), and the use of prolonged time intervals between subsequent doses, if necessary in the individual patient;
3. Criteria to terminate a challenge (challenge endpoints): it is a matter of debate whether the challenge should be terminated in case of (repeated) subjective or objective symptoms, or in case of mild or more pronounced symptoms. Niggemann et al[26], for example, argue that objective symptoms should be induced to terminate a challenge. Considering both subjective and objective symptoms as valid will lead to an earlier termination of the test, which is likely to avoid more severe reactions. As in our clinic children with severe anaphylaxis in history are not excluded from challenge tests, we have decided to adhere to a challenge scheme giving optimal safety during DBPCFCs. This may be different for other centres, where severe reactors are excluded from food challenge testing. In our protocol, the challenge is terminated in case of objective symptoms, ongoing subjective symptoms for more than 30 minutes, or repeated transient subjective symptoms. In the latter situation, the same challenge dose is repeated. Also mild symptoms may be assessed as positive, since the purpose of the DBPCFC is not to reproduce severity of symptoms during DBPCFCs, but to demonstrate a causal relationship between the food and symptoms.
The question can be raised if, for scientific purposes, results of DBPCFCs using different challenge endpoints may be compared[19], because earlier termination of a challenge session might result in a higher proportion of false positive test results.
4. The clinical relevance of late onset symptoms: Late onset symptoms may be particularly relevant in children with atopic dermatitis. In a position paper on eczematous reactions to food, late onset symptoms have recently been considered as valid[27]. A rate of 25% of late onset symptoms following food challenges in children with atopic dermatitis has been reported[28, 29]. However, in these protocols, active and placebo challenges were interspersed, making assessment of placebo "reactions" difficult. In our study on placebo reactions (Chapter IV) it is shown that including late phase symptoms in the assessment of DBPCFCs enhances the proportion of placebo events significantly, as approximately as many late onset placebo events as immediate placebo events were observed. Significantly, we observed similar numbers of late onset symptoms following active food challenges and following placebo challenges (unpublished data). In our opinion, further studies are mandatory to elucidate the clinical relevance of these late onset symptoms.

The DBPCFC: the best available test

To date, the DBPCFC is the best available test in diagnosing food allergy. During recent international conferences on allergy, the question has been raised if other

diagnostic tools can replace the DBPCFC. Much work has been done on the diagnostic value of skin prick tests (SPTs) and specific IgE during the last 10 -15 years. Current knowledge of the predictive value of specific IgE and SPTs has shown that increasingly higher values of food-specific IgE and increasing size of skin prick tests are associated with an increasing probability of clinical reactivity to food[30]. Decision points with regard to immediate reactions to food have been established for cow's milk, egg, and peanut above which 95% of patients were found to have positive challenge tests[30,31]. In these patients many physicians consider the performance of DBPCFCs not necessary. However, many patients show sensitization levels below these decision points and thus need to be challenged, and different predictive values are being generated from emerging studies, which might represent nuances of diet, age, disease and challenge protocols[29,32,33]. Recent data have shown that levels of specific IgE clearly increase with age[34]. Furthermore, these decision points are determined on the basis of immediate reactions to foods, and have not been determined for late onset reactions to foods[27]. Finally, it may be debated if a 5% chance of diagnostic error is acceptable, particularly if the diagnosis results in long term dietary avoidance for an individual patient. Thus, to date, the DBPCFC remains the best available test we have in diagnosing food allergy.

Validation of the outcome of the DBPCFC

The DBPCFC is considered the gold standard for the diagnosis of food allergy. However, this test is not perfect. As discussed before, false negative outcomes may occur, and as assessment protocols have not been validated yet, we might be unaware of false positive outcomes. The question is how to validate the outcome of a DBPCFC?

Reliable biomarkers could theoretically validate the outcome of DBPCFCs, but so far, no biomarkers have been identified, that distinguish between responders and non-responders[35-37]. Recently, Clark et al[38] showed that facial thermography as measured during a challenge might provide a sensitive method to determine the outcome of food challenge tests. A significant early rise in nasal temperature correlated with a positive challenge outcome. Such novel methods may aid interpretation of challenge outcomes in future, but need to be validated first.

Validation of a positive outcome of a DBPCFC in an individual patient, and thus the causative effect of the food, can be validated by repeated challenges[13,38]. In a so-called "N of 1" trial (single patient randomized trial)[39-41], 3 placebo and 3 active food challenges are administered in a double-blind fashion and in a random order. In an N of 1 trial, in which 3 active and 3 placebo challenges are administered, a total of 6!/3!3! = 20 different sequences can be made. Using this number of challenges, the chance that the patient guesses the right sequence of all possible sequences is 0.05. Additionally, in this procedure, the chance that

appropriate responses will occur to all six challenges by chance (in the worst case scenario, where the chance of reacting on any given test day is 50%), may be calculated as 0.5 * 0.5 * 0.5 * 0.5 * 0.5 * 0.5 = 0.015, which is less than 0.05. In this model, appropriate responses are defined as symptoms on active challenge, and no symptoms on placebo challenge. Thus, positive test results in the individual patient are validated if a patient reacts to all 3 active challenge sessions, and not to one or more placebo challenges. This calculation may be used when there are no baseline symptoms.

Alternatively, if baseline symptoms are unavoidable or cannot be reduced to zero, allergic reactions to the active challenges may be validated if statistically significant differences can be calculated between total mean or median symptoms scores during active challenges and placebo challenges. A stable baseline situation is of great importance to avoid placebo events in either situation, as this could make the test false negative.

However, in daily clinical practice, single patient randomized trials for every individual patient are not practical, too labour intensive and very time consuming, and may yield an unacceptable burden for the patient. Therefore, DBPCFCs are usually performed in patients once. Thus, when performing DBPCFCs only once, one should be aware that some positive results might be false positive. In practice, follow-up challenges are performed to verify the persistence or resolution of food allergy. Possible false positive challenge results may be refuted in these follow-up challenges. Also, equivocally positive DBPCFCs, in which the results remain questionable (usually mild) after the highest challenge dose may be repeated. Unequivocal *negative* DBPCFCs can be validated by a negative open challenge or a successful introduction of the challenged food into the diet of the patient. In the latter case patients should be monitored carefully for actual introduction of the food in normal servings into their diet. Equivocal negative DBPCFCs, in which late onset symptoms occur and are related to the introduction of the challenged food into the diet of the child, can either be validated by a subsequent period of elimination and renewed introduction, by repeated challenges, or by a blinded prolonged DBPCFC. In our experience, a subsequent period of elimination and a renewed introduction does usually not result in recurrence of symptoms (unpublished data).

Part II

Practical implications of DBPCFCs in children having their first exposure to the challenged food

Our finding in Chapter V in children having their first exposure to an allergenic food by DBPCFC, that a significant proportion (51%) of children reacts with allergic symptoms, is not new. The underlying reason for conducting this study was the need for practical guidelines in children at risk for food allergy regarding

the introduction of allergenic foods at home as a first exposure. In every day allergy practice, as well as in the literature, allergic reactions to first exposure are common[43-47], but very unwanted. Therefore, guidelines with improved safety for home introduction of allergenic food are needed. Even if these reactions are not severe, and present as mild or moderate symptoms such as oedema, rash, diarrhoea, and/or vomiting, they are often experienced as very frightening by the parents of these infants. As a result, parents of children at risk for atopic disease are reluctant to introduce these foods into the diet of their children.

As it is generally assumed that the lower the dose, the less severe the symptoms[19], the reason for the occurrence of relatively severe reactions at first exposure may be that the first dose administered by the parents at home is much too high in sensitive children. Our study showed that, without detailed instructions, parents would administer median doses which are approximately at least 8 times higher than the first doses of the incremental scales used in DBPCFCs, and for peanut even 25 times higher. The potential hazard that such quantities of allergenic foods may pose is further suggested by our finding, that even very small amounts of foods in their usual household form, such as 1 sip of milk, 1 bite of a sponge finger or muffin, 1 small cube of bread with peanut butter, contain considerable amounts of allergenic foods, comparable to doses 4 or higher of our incremental scales. Thus, detailed instructions on low-dose administration of allergenic foods are needed for introduction at home. We succeeded in designing such instructions because, when using the written instructions of the introduction schedule and the reference photograph (Appendix 1 and Figure 1, Chapter V), it seemed feasible by the parents to administer the median required amounts of food for all doses.

As discussed in Chapter V, the introduction schedules we devised can be utilized by physicians and dieticians in children at risk for food allergy, but who do not, according to the physician's assessment, warrant first exposure under medical supervision. We assume that when using these guidelines, safety is improved, as the incrementing amounts are based on the doses steps we administered in DBPCFCs in which severe reactions were absent. Future studies regarding the usage of these introduction schedules could validate the safety of these guidelines.

It is a matter of debate in which children these guidelines are to be used. As stated in Chapter V, there is no consensus about which children who should introduce allergenic foods at home, and which children who should be challenged under medical supervision because of a significant risk for (severe) allergic reactions. As discussed in Chapter V, based on the literature, it could be argued that children with two or more of the following risk factors should not introduce allergenic foods at home as a first exposure: coexistent asthma or other significant comorbidity, adolescence or young adult age, introduction of peanuts or tree nuts, and distance to emergency medical care.

Thus, these guidelines for first exposure to allergenic foods could be utilised for

those children not having two or more of these risk factors, but who are at risk for food allergy generally, and wish to introduce allergenic foods at home as safely as possible. Whether all atopic children should use these guidelines requires further study, and this would have major implications for health care delivery in the area of food allergy.

The guidelines for first exposure of major allergenic foods may become even more important because currently, our concept about the prevention of food allergy is changing[48]. Delay in the introduction of highly allergenic foods was generally regarded to be an effective preventive measure with respect to the development of atopic disease[49]. However, these recommendations on the prevention of food allergy with respect to the delayed introduction of major allergenic foods were merely based on only two, non-randomized, prospective studies[50,51]. Additionally, a delayed introduction of allergenic foods might even increase the prevalence of atopic disease[52]. Thus, there are little epidemiological data to support this belief[53]. As a result, depending on future study results, advice on timing of introduction of allergenic foods in young infants may change in the near future, promoting timely introduction of these common allergenic foods instead of delayed introduction. Studies show that it is an illusion to expect that a total avoidance of an allergenic food is feasible. Unintentional exposure and sensitisation may occur in utero[54], through breast-feeding[55], or by environment[56]. These mechanisms are supported by the observations that many children are sensitized to foods which they have never consumed before in their lives. Thus, primary prevention avoidance strategies result in low-level exposure rather than no exposure at all, because obviously, sensitization can not be prevented. In fact, low-dose, intermittent exposure may be the trigger for developing IgE-mediated food allergies[57], and it is possible that the current practice of allergen avoidance may have contributed to the increased prevalence of food allergy[57].

Unintentional exposure may also occur through the diet, despite efforts to totally avoid an allergenic food. In our study on avoidance of allergic foods in children adhering to a food allergen avoidance diet for allergy prevention (Chapter VI), we found that it was difficult to totally avoid allergenic foods. In only one third of the children, unintentional ingestion was thought to be unlikely following a thorough dietary history. Despite the attempt to adhere to dietary measures in all other children of the study population, unintentional ingestion could not be ruled out or was revealed by questioning. Additionally, cross-contamination with the allergenic food cannot be ruled out, even in the one third of children without unintentional ingestion. Thus, in all of these children, a low-level exposure and no-total avoidance by diet was very likely the result of the avoidance diet.

Practical implications of DBPCFCs in children with a history of anaphylaxis to food

In chapter VII, we have clearly shown that assessment of clinical reactivity to food by challenge testing in children with a history of anaphylaxis is not unnecessary or unsafe. In our study, a substantial proportion (29%) of children had a negative test result. To the great relief of the children and their parents, the diagnosis of anaphylaxis to food was removed from these children. These children would probably not have been aware of this, if they had not been challenged. Not unimportantly, these children could relinquish their Epinephrine self administration devices given for the food in question. As the impact of having a self administration device is enormous for the patient and his/her environment[58], the prescription of this device should be based on stringent diagnostic criteria, which, in the case of food allergy, should be obtained by double-blind challenge testing.

This figure is probably higher than many health care professionals had expected. In general, it is assumed that resolution of anaphylaxis is rare, but only few data exist on the natural history of anaphylaxis to food, as is discussed in chapter VII. This is probably due to the fear for severe reactions during DBPCFCs in children with anaphylaxis to food, and due to the statement, that food challenges are contraindicated in patients with a history of anaphylaxis[60], unless the patient is believed to have outgrown the food allergy[60-62]. Therefore, DBPCFCs in children with a history of anaphylaxis are often not performed. In our study, no children had histories suggesting resolution, but nevertheless in a significant proportion of children the anaphylaxis had resolved.

There is no consensus on the long-term management with regard to the diagnostic work-up of anaphylaxis to foods to ascertain for resolution, except for the statement that children, who are believed to have outgrown their food allergy, may be challenged[60-62]. This might be the case in, for example recent unintentional ingestion without subsequent reactions, as well as in cases of reduction or disappearance of sensitization. However, our data show, that re-evaluation of the initial diagnosis of anaphylaxis is worthwhile, even in the absence of such suspicion. These children should be referred to centres where DBPCFCs can be performed safely. Based on the results of our study it is not justified to formulate recommendations about regular time intervals following anaphylaxis on which DBPCFCs should be performed, as in our study, these prospective challenges were not performed at fixed time intervals following the last reaction. A different study design is warranted to answer this question.

We want to stress the fact that these children should only be challenged in centres experienced in performing high-risk food challenge tests. Aside from the elements of the challenge protocol which we feel contribute to the safety of the procedure, as described in Chapter VII, experienced staff is mandatory in making crucial clinical decisions while observing the patient. This is specifically important with respect

to decisions to terminate or continue the challenge, delaying the administration of the subsequent dose for safety reasons, and the administration of the required medical care. Of course, all necessary medical treatment should be available.
Apart from the necessity of re-evaluation of anaphylaxis to food, it is important that all patients who have experienced anaphylaxis to food should be referred to a specialist physician knowledgeable about anaphylaxis. Patients should be referred to have the causative food identified, for education regarding avoidance strategies to avoid future anaphylactic reactions, as well as for the management of anaphylactic reactions[61,63]. Additionally, dietary advice by a dietician knowledgeable about anaphylaxis to food, and information from consumers associations such as the Food Allergy and Anaphylaxis Network in the USA (www.foodallergy.org), the Anaphylaxis Network in the Netherlands (www.anafylaxis.net), or the Anaphylaxis Campaign in the UK (www. anaphylaxis.org.uk) is essential.

Is there a sufficient basis for the use of the DBPCFC?
The Health Council of the Netherlands [64] stated that the DBPCFC is the diagnostic procedure of choice for diagnosing food allergy, and that this test should become available for diagnosing food allergy in primary care. Currently, many paediatricians and allergists are undertaking initiatives to initiate DBPCFCs, supported by workshops and educational sessions on DBPCFCs provided by paediatric departments of the UMCG and UMCU. Obviously, performing DBPCFCs seems feasible for many centres as soon as they are convinced about the necessity of this procedure. Similar initiatives in other countries could enhance the initiation of DBPCFCs.
However, not all physicians are convinced of the added diagnostic value of the DBPCFC as compared to open food challenge tests. Especially in case of a convincing history with immediate, objective reactions to food and sensitization to the food in question, many health care providers state that double-blind challenges are not necessary in these patients, and prefer open challenges in these cases[59]. Others might even consider any oral food challenge unnecessary in these cases. However, there are no data to support these assertions. On the contrary, as discussed in Chapter IV and earlier in this chapter, studies have shown that open food challenge render many false positive results, even in case of immediate reactions in open food challenges. False positive open food challenges may be explained by a number of factors, the most important of which is bias due to lack of blinding, as is discussed in Chapter IV.

Recommendations for future research with respect to the performance and validation of DBPCFCs
In order to make the DBPCFC feasible for daily clinical practice, more specific practical standardized protocols are required. These protocols should include a

larger variety of validated challenge materials (recipes) for a broad range of foods, ready-to-use conversion of recipes to incremental scales to be administered, as well as broadly accepted guidelines for the assessment of symptoms, termination of challenges, and medical safety measures.

For scientific purposes, adequate top doses could be determined by comparing results of open food challenges to DBPCFCs using different top doses, while using validated recipes to guarantee optimal blinding of such doses. Also, matrix effects on the clinical effect of the putative top dose should be studied. The clinical relevance of immediate *vs.* late onset symptoms, as well as subjective *vs.* objective symptoms should be validated by repeated challenges. The availability of biomarkers for the confirmation of allergic responses to challenge tests would be of great help in the avoidance of false positive test results.

Indications for DBPCFCs could be studied in several subgroups of patients, such as in children younger than 3 years old, in non-sensitized children, and in children with immediate, objective symptoms in dietary history. This could be done by comparing results of open food challenges to those of DBPCFCs, by studying the clinical relevance of placebo events in DBPCFCs, and by examining the clinical relevance of the dietary history.

Future studies regarding the use of introduction schedules at first exposure could validate the safety of these guidelines, and in which children there schedules are to be utilized.

Finally, studies on the natural history of anaphylaxis to food prospectively utilizing DBPCFCs at different time points after such reactions are needed.

REFERENCES

1. Norgaard A, Bindslev-Jensen C. Egg and milk allergy in adults. Allergy 1992;47:503-9.
2. Carter C. Double-blind food challenges in children: a dietitian's perspective. Current Medical Literature (Allergy). The Royal Society of Medicine 1995;3:95-99.
3. Wensing M, Penninks AH, Hefle SL, Koppelman SJ, Bruijnzeel-Koomen CAFM, Knulst AC. The distribution of individual threshold doses eliciting allergic reactions in a population with peanut allergy. J Allergy Clin Immunol 2002;110:915-20.
4. Huijbers GB, Colen AAM, Niestijl Jansen JJ, Kardinaal AFM, Vlieg-Boerstra BJ, Martens BPM. Masking foods for food challenge: practical aspects of masking foods for a double-blind placebo-controlled food challenge. J Am Diet Assoc 1994;94:645-9.
5. Van Odijk J, Ahlstedt S, Bengtsson U, Borres MP, Hulthén L. Double-blind placebo-controlled challenges for peanut allergy. The efficiency of blinding procedures and the allergenic activity of peanut availability in the recipes. Allergy 2005:60;602-5.
6. Vlieg-Boerstra BJ, Bijleveld CMA, van der Heide S, Beusekamp BJ, Wolt-Plompen SAA, Kukler J, Brinkman J, Duiverman EJ, Dubois AEJ. Development and validation of challenge materials for double-blind, placebo-controlled food challenges in children. J Allergy Clin Immunol 2004; 113:341-6.
7. Ronteltap A, van Schaik J, Wensing M, Rynja FJ, Knulst AC, de Vries JH. Sensory testing of recipes masking peanut or hazelnut for double-blind placebo-controlled food challenges. Allergy 2004;59:457-60.
8. Ballmer-Weber BK, Holzhauser Th, Scibilia J, Mittag D, Zisa G, Ortolani C et al. Clinical Characteristics of soybean allergy in Europe: A double-blind, placebo-controlled food challenge study. J Allergy Clin Immunol 2007;119:1489-96.
9. Mills EN, Mackie AR, Burney P, Beyer K, Frewer L, Madsen C et al. The prevalence, cost and basis of food allergy across Europe. Allergy 2007;62:717-722.
10. Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: A manual. J. Allergy Clin.Immunol 1988;82:986-997.
11. Sicherer SH, Morrow EH, Sampson HA. Dose-response in double-blind placebo-controlled oral food challenges in children with atopic dermatitis. J Allergy Clin Immunol 2000;105:582-6.
12. Niggemann B, Wahn U, Sampson HA. Proposals for standardization of oral food challenge tests in infants and children. Pediatr Allergy Immunol 1994;5:11-3.
13. Bindslev-Jensen C. Standardisation of double-blind, placebo-controlled food challenges. Allergy 2001:56; suppl. 67:75-7.
14. Atkins FM, Steinberg SS, and Metcalfe DD. Evaluation of immediate adverse reactions to foods in adult patients. II. A detailed analysis of reaction patterns during oral food challenge. J Allergy Clin Immunol 1985;75:356-63.
15. Bock SA, Atkins FM. Patterns of food hypersensitivity during sixteen years of double-blind, placebo-controlled food challenges. J Pediatr 1990;117:561-7.
16. Caffarelli C, Petroccione T. False-negative food challenges in children with suspected food allergy. Lancet 2001;358:1871-2.
17. Wensing M, Penninks AH, Hefle SL, Akkerdaas JH, Ree R van, Koppelman SJ, Bruijnzeel-Koomen CAFM, Knulst AC. The range of minimum provoking doses in hazelnut-allergic patients as determined by double-blind, placebo-controlled food challenges. Clin Exp

Allergy 2002;32:1757-62.

18. Flinterman AE, Pasmans SG, Hoekstra MO, Meijer Y, van Hoffen E, Knol EF et al. Determination of no-observed-adverse-effect levels and eliciting doses in a representative group of peanut-sensitized children. J Allergy Clin Immunol 2006;117:448-54.
19. Crevel RW, Briggs D, Hefle SL, Knulst AC, Taylor SL. Hazard characterisation in food allergen risk assessment: The application of statistical approach and the use of clinical data. Food and Chemical Toxicology 2007;45:691-701.
20. Vlieg-Boerstra BJ, van der Heide S, Bijleveld CMA, Kukler J, Duiverman EJ, Dubois AEJ. Placebo reactions in double-blind, placebo-controlled food challenges in children. Allergy 2007;62:905-12.
21. Venter C, Pereira B, Grundy J, Clayton CB, Roberts G, Higgins B et al. Incidence of parentally reported and clinically diagnosed food hypersensitivity in the first year of life. J Allergy Clin Immunol 2006;117:1118-24.
22. Venter C, Pereira B, Grundy J, Clayton CB, Arshad SH, Dean T. Prevalence of sensitization reported and objectively assessed food hypersensitivity amongst six-year-old children: a population-based study. Pediatr Allergy Immunol 2006;17:356-63.
23. Pereira B, Venter C, Grundy J, Clayton CB, Arshad SH, Dean T. Prevalence of sensitization to food allergens, reported adverse reaction to foods, food avoidance, and food hypersensitivity among teenagers. J Allergy Clin Immunol 2005;11:884-92.
24. Venter C, Pereira B, Voigt K, Grundy J, Bernie CB, Gant C et al. Comparison of open and double blind placebo controlled food challenges in diagnosis of food hypersensitivity amongst children. J Hum Nutr & Diet. In press
25. Brouwer M.L., Wolt-Plompen SAA, Dubois AEJ, Heide S van der, Jansen DF, Hoijer MA et al. No effects of probiotics on atopic dermatitis in infancy: a randomized placebo-controlled trial. Clin Exp Allergy 2006;36:899-906.
26. Niggeman B, Beyer K. Pitfalls in double-blind, placebo-controlled oral food challenges. Allergy 2007;62:729-32.
27. Werfel T, Ballmer-Weber B, Eigenmann PA, Niggemann B, Rancé F, Turjanmaa K et al. Eczematous reactions to food in atopic eczema: position paper of the EAACI and GA^{2-}len. Allergy 2007;62:723-28.
28. Niggemann B, Sielaff B, Beyer K,Binder C, Wahn U. Outcome of double-blind, placebo-controlled food challenge tests in 107 children with atopic dermatitis. Clin Exp Allergy 1999;29:91-6.
29. Celik-Bilgili S, Mehl A, Verstege A, Staden U, Nocom M, Beyer K. et al. The predictive value of specific immunoglobulin E levels in serum for the outcome of oral challenges. Clin Exp Allergy 2005;35:268-73.
30. Sampson HA, Ho DG. Relationship between food-specific IgE-concentrations and the risk of positive food challenges in children and adolescents. Allergy Clin Immunol 1997;100:444-51.
31. Garcia-Ara C, Boyano-Martinez T, Diaz-Pena JM, Martin-Munoz F, Reche-Frutos M, Martin-Esteban M. Specific IgE levels in the diagnosis of immediate hypersensitivity to cow's milk protein in the infant. J Allergy Clin Immunol 2001;107:185-90.
32. Osterballe M, Bindslev-Jensen C. Threshold levels in food challenge and specific IgE in patients with egg allergy: is there a relationship? J Allergy Clin Immunol 2003;112:196-201.
33. Sicherer SH, Sampson HA. Food allergy. J Allergy Clin Immunol 2006;117:S470-75.
34. Komata T, Söderström L, Borres MP, Tachimoto H, Ebisawa M. The predictive relation-

ship of food-specific serum IgE concentrations to challenge outcomes for egg and milk varies by patient age. J Allergy Clin Immunol 2007;119:1272-4.

35. Odijk J, Peterson CG, Ahlstedt S, Bengtsson U, Borres MP, Hulthén L et al. Measurements of eosinophil activation before and after food challenges in adults with food hypersensitivity. Int Arch Allergy Immunol 2006;140:334-41.
36. Vila L, Sanz ML, Sanchez-Lopez G, Garcia-Avilez C, Dieguez I. Variations of serum eosinophil cationic protein and tryptase, measured in serum and saliva, during the course of immediate allergic reactions to foods. Allergy 2001; 56: 568-72.
37. Simons FE, Frew AJ, Ansotegui IJ, Bochner BS, Golde DB, Finkelman FD et al. Risk assessment in anaphylaxis: Current and future approaches. J Allergy Clin Immunol 2007;120:S2-24.
38. Clark AT, Mangat JS, Tay SS, King Y, Monk CJ, White PA et al. Facial thermography is a sensitive and specific method for assessing food challenge outcome. Allergy 2007;62:744-9.
39. Metcalfe DD, Sampson HA. Workshop on experimental methodology for clinical studies of adverse reactions to foods and food additives. J Allergy Clin Immunol 1990;86:421-42.
40. Guyatt G, Sackett D, Taylor DW, Chong J, Roberts R, Pugsley S. Determining optimal therapy – randomized trials in individual patients. N Engl J Med 1986;314:889-92.
41. McLeod RS. Taylor DW, Cohen Z, Cullen JB. Single-patient randomised clinical trials. Use in determining optimum treatment for patient with inflammation of Kock continent ileostomy reservoir. Lancet. 1986;1 (8483):726-8.
42. Single-patient trials. Lancet1986;1 (8492):1254-5.
43. Sicherer SH, Burks AW, Sampson HA. Clinical features of acute allergic reactions to peanut and tree nuts in children. Pediatr 1998;102:e6.
44. Monti G., Muratore MC, Peltran A, Bonfante G, Silvestro R, Oggero R, Mussa GC. High incidence of adverse reactions to egg challenge on first known exposure in young atopic dermatitis children: predictive value of skin prick test and radioallergosorbent test to egg proteins. Clin Exp Allergy 2002;32:1515-19.
45. Kagan R, Hayami D., Joseph L, St. Pierre Y, Clarke AE. The predictive value of a positive prick skin test to peanut in atopic, peanut-naïve children. Ann Allergy Asthma Immunol. 2003;90,640-5.
46. 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; 95:1215-20.
47. Lack G, Fox D, Northstone K, Golding J. Factors associated with the development of peanut allergy in childhood. N Engl J Med 2003;348(11):977-85.
48. Brand PLP, Vlieg-Boerstra BJ, Dubois AEJ. Dietary prevention of allergic disease in children: Are current recommendations really base don good evidence? Pediatr Allergy Immunol 2007;18:475-79.
49. American Academy of Pediatrics, Committee on Nutrition. Hypoallergenic infant formulas. Pediatrics 2000;106:346-9.
50. Kajosaari M. Atopy prophylaxis in high-risk infants: prospective 5-year follow-up study of children with six months exclusive breast feeding and solid food elimination. Adv Exp Med Biol 1991;310:453-8.
51. Fergusson DM, Horwood. LJ. Early solid food diet and eczema in childhood: a 10-year longitudinal study. Pediatr Allergy Immunol 1994; (Suppl 1)5:44-7.

52. Zutavern A., von Mutius E., Harris J, Mills P, Moffatt S, White C, Cullinan P. The introduction of solids in relation to asthma and eczema. Arch Dis Child 2004**;**89,303-8.
53. Lack G. The concept of oral tolerance induction to foods. Nestle Nutr Workshops Ser Pediatr Program 2007: 63-8;discussion 68-72.
54. Jones CA, Kilburn SA, Warner JA, Warner JO. Intrauterine environment and fetal allergic sensitization. Clin Exp Allergy 1998;28:655-9.
55. Fukushima Y, Kawata Y, Onda T, Kitagawa M (1997): Consumption of cow milk and egg by lactating women and the presence of β-lactoglobulin in breast milk. Am J Clin Nutr 1997;65:30-5.
56. Perry TT, Conover-Walker MK, Pomes A, Chapman MD, Wood RA. Distribution of peanut allergen in the environment. J Allergy Clin Immunol 2004;113:973-6.
57. Liem JJ, Kozyrskyj AL, Huq SI, Becker AB. The risk of developing food allergy in premature or low-birthweight children. J Allergy Clin Immunol 2007;119:1203-9.
58. Oude Elberink JN, de Monchy JG, van der Heide S, Guyatt GH, Dubois AE. Analysis of burden of treatment in patients receiving an EpiPen for yellow jacket anaphylaxis. J Allergy Clin Immunol 2006;118:699-704.
59. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J et al. Standardization of food challenges in patients with immediate reactions to foods – position paper from the European Academy of Allergology and Clinical Immunology. Allergy 2004;59:690-697.
60. Sicherer SH. Food allergy: When and how to perform oral food challenges. Pediatr Allergy Immunol 1999;10(4):226-34.
61. Muraro A, Roberts G, Clark A, Eigenmann PA, Halken S, Lack G et al. EAACI task force on Anaphylaxis in children. The management of anaphylaxis in childhood: position paper of the European academy of allergology and clinical immunology.Allergy 2007;62:857-71.
62. Sampson HA. Update on food allergy. J Allergy Clin Immunol 2004;113:805-19.
63. Simons FE. Anaphylaxis, killer allergy: long-term management in the community. J Allergy Clin Immunol 2006;117:367-77.
64. Gezondheidsraad. Voedselallergie (Health Council of the Netherlands. Food Allergy). 2007.

Chapter IV Summary & Samenvatting

SUMMARY

In a double-blind, placebo-controlled food challenge (DBPCFC), the patient is challenged with sequentially increasing amounts of the active suspected allergenic food (or "verum") and with a placebo food. The active and placebo challenges are conducted in random order and preferably on separate days. Both the patient and the physician are blinded for the sequence of the challenges, until the code is broken at the end of the test. In this test, the patient serves as his/her own control.

The DBPCFC is currently the only objective test to ascertain the presence of food allergy. However, this test is not perfect. Despite this, during the past several decades, the DBPCFC has been regarded as the gold standard for diagnosing food allergy. Several attempts have been made to standardize and validate the test procedure for clinical and scientific purposes. To date, no (universal) protocol for the performance of the DBPCFC has been established. In daily practice, the DBPCFC is conducted in only a limited number of centres. In the Netherlands, there is an increasing interest in performing DBPCFCs. Many centres are currently attending workshops and educational sessions on DBPCFCs, predominantly provided by the UMCG. Specific ready-to-use standardized protocols, including a broad range of validated challenge materials (recipes), incremental scales, as well as guidelines for the assessment of symptoms and termination of challenges are much needed, which may help physicians and dieticians in initiating and performing food challenge tests.

This thesis has been written in the framework of the Food Challenge Unit (FCU) of the University Medical Centre Groningen (UMCG). The aims of this thesis were first, to standardize the procedure of the DBPCFC in children for the FCU of the UMCG and to validate several parameters of the challenge procedure. Secondly, to examine the outcome of approximately 500 DBPCFCs performed from 2002 until 2007 and to formulate practical guidelines and recommendations for the management of food allergy in children.

In chapter II, the development and validation or challenge materials (recipes) for use in DBPCFCs are described. For every recipe, a placebo recipe and an active test food recipe were developed. Recipes with cow's milk, soymilk, egg, peanut, hazelnut, and wheat were first tested by volunteers from the hospital staff using sensory tests for difference, and subsequently by a professional panel of food tasters in a food laboratory designed for sensory testing. Twenty-seven recipes were developed and tested as valid by the volunteer panel, whereas only 17 of these recipes could be validated by the professional panel. These latter recipes are currently used in DBPCFCs in the UMCG, as well as in some other centres in

the Netherlands.

In chapter III, we commented on the method of sensory testing of recipes for DBPCFCs by other authors, using a non-professional panel of food tasters, and stressed the importance of professional taste panels for reliably validated recipes.
In Chapter IV, we examined the occurrence and features of placebo events in DBPCFCs in children sensitized to the challenged food, and assessed their diagnostic significance in the DBPCFC. For optimal consistency of assessment of challenge results, we devised a standardized algorithm to assess immediate and late onset events following each challenge session. The outcome of the DBPCFC was assessed according to a standardized protocol. In 12.9% of all challenges, placebo events occurred, while 5.4% of the positive active challenges were revealed to be false positive by administration of a placebo challenge. Based on our results and on studies comparing results of open food challenges to DBPCFCs, we concluded that the diagnostic significance of the administration of placebo challenges is not only to identify false positive test results, but more importantly to allow for blinding of the active food challenge. Consequently, fewer events occur during the active food challenges, due to unbiased observations.

In young infants at risk for food allergy, it has been proposed to introduce allergenic foods, such as egg and peanut, gradually into their diets, but no practical dietary advice has been devised. However, severe reactions at first exposure are not uncommon, probably because the doses administered at home are likely to be relatively high for sensitive children. Therefore, in Chapter V**,** we devised introduction schedules for major allergenic foods for use at home, to be administered in children who are, according to the physician's assessment, eligible to introduce these foods at home. The amounts of foods to be administered were derived from the incremental scales of DBPCFCs as performed in children never exposed to these foods. Detailed written instructions and a reference photograph of the required incrementing amounts of allergenic foods were developed for use at home. Using these introduction schedules, parents portioned initial doses significantly lower than without these introduction schedules. We concluded that the use of these ready-to-use introduction schedules may improve the safety of introduction at home at first exposure, and may be utilized by physicians and dieticians for this purpose.

It is known that complete dietary avoidance is hardly feasible in food allergic patients. In chapter VI, we studied the rate of complete avoidance (before the new European labelling rules of November 2005) of allergic foods in children adhering to a food allergen avoidance diet from birth for allergy prevention. Utilizing food

frequency questionnaires for common allergenic foods, we found that only one third of these children succeeded in avoiding unintentional ingestion of the allergenic foods. All of these children underwent DBPCFCs, 39% were positive. Tolerance of small amounts of allergenic foods did not preclude positive challenge reactions. Dietary assessment does not seem a useful tool in predicting the outcome of DBPCFC in children adhering to an elimination diet.

It is generally assumed, that resolution of anaphylaxis to food is rare, and that challenges should only be performed if the patient is to be believed to have outgrown the food allergy. The purpose of the study in chapter VII was to determine whether the frequency of negative challenge tests in children with anaphylaxis to food is frequent enough to warrant challenge testing, and to document the safety of this procedure. Children with a clear-cut history of anaphylaxis to foods with no indications for resolution of anaphylaxis underwent DBPCFCs. Of the 21 challenges performed, 6 DBPCFCs were negative (29%): 3 for cow's milk, 1 for egg, 1 for peanut, and 1 for wheat. No severe reactions occurred, and no adrenaline was administered. To our knowledge, this is the first study in an otherwise unselected population of children with a history of anaphylaxis to foods, in whom resolution of anaphylaxis to food is assessed by DBPCFCs. We concluded that resolution of anaphylaxis in children may occur, and that assessment of clinical reactivity to food by DBPCFC should be considered in such children, also when there are no indications that they have outgrown their anaphylaxis. DBPCFCs can be performed safely in these children, providing that a very careful protocol is used, and if conducted in centres experienced in performing high-risk food challenges.

In chapter VIII, the main results of this thesis are discussed, and recommendations for future research are made.

SAMENVATTING

In een dubbelblinde placebogecontroleerde voedselprovocatie test (DBPCFC) krijgt een kind oplopende doses van een testvoeding toegediend met daarin het te testen verdachte allergene voedingsmiddel (verum), of zonder het verum (placebovoeding). De volgorde van de verum- en placeboprovocaties worden at random bepaald en bij voorkeur op twee verschillende dagen uitgevoerd. Zowel de patiënt als de behandelaar zijn geblindeerd voor de volgorde van de testvoedingen (zij zijn niet op de hoogte van de volgorde van de testvoedingen), totdat aan het einde van de test de code wordt verbroken. Bij deze test fungeert de patiënt als zijn eigen controle.
Tot op de dag van vandaag is de DBPCFC de enige objectieve test om de diagnose voedselallergie te kunnen stellen. Hoewel deze test niet volmaakt is, geldt de DBPCFC sinds enkele decennia als de goud standaard (beste test) voor de diagnostiek van voedselallergie. Er zijn verschillende pogingen ondernomen om deze test te standaardiseren en te valideren om klinische en wetenschappelijke redenen, maar tot op heden bestaat er geen universeel protocol voor het uitvoeren van "de dubbelblinde". In de praktijk wordt de DBPCFC nog slechts in een beperkt aantal centra uitgevoerd. In Nederland is er in toenemende mate belangstelling voor de DBPCFC. Veel centra volgen momenteel workshops en voorlichtingsbijeenkomsten over de DBPCFC, die voor een belangrijk deel worden verzorgd door het UMCG. Voor gebruik op grotere schaal zijn echter specifieke, direct bruikbare, en gestandaardiseerde protocollen nodig (en inmiddels beschikbaar), met daarin opgenomen gevalideerde provocatiematerialen (receptuur), doseerschema's en criteria ter beoordeling van symptomen of beëindiging van een provocatie. Deze protocollen kunnen artsen en diëtisten op weg helpen en ondersteunen bij het (gaan) uitvoeren van voedselprovocatie tests.

Dit proefschrift is geschreven in het kader van de Voedsel Provocatie Unit (VPU) van het Universitair Medisch Centrum Groningen (UMCG). Het doel van dit proefschrift was ten eerste om de procedure van de DBPCFC op onderdelen te standaardiseren en te valideren voor kinderen, die in de VPU van het UMCG worden getest op voedselallergie. Het tweede doel was om op basis van uitkomsten van ongeveer 500 DBPCFCs, die van 2002 tot 2007 zijn uitgevoerd, aanbevelingen te doen voor de diagnostiek en behandeling van voedselallergie bij kinderen.

In hoofdstuk II wordt de ontwikkeling en validatie van provocatie materiaal (recepten) voor DBPCFCs beschreven. Hierbij is onderzocht of het te testen allergene voedingsmiddel (verum) onherkenbaar kon worden verwerkt in een testvoeding. Van elk recept zijn een placebo en een verum variant ontwikkeld. Recepten voor provocaties met melk, sojamelk, ei, pinda, hazelnoot en tarwe

werden eerst getest door middel van sensorische verschiltesten door vrijwilligers uit het ziekenhuis, vervolgens door professionele panelleden van een smaakcentrum voor sensorisch onderzoek.
Zevenentwintig recepten werden ontwikkeld en valide bevonden door het vrijwilligers panel. Dat wil zeggen dat het panel de testvoedingen met het verum niet kon onderscheiden van de placebo voedingen. Slechts 17 van deze recepten konden worden gevalideerd door het panel van het smaakcentrum. Deze laatst genoemde recepten worden thans gebruikt bij het uitvoeren van DBPCFCs in het UMCG, en in verschillende andere centra in Nederland.

Hoofdstuk III bestaat uit een door ons ingezonden brief aan een tijdschrift, als reactie op een artikel van andere auteurs, waarin receptuur voor DBPCFCs door een vrijwilligers panel is gevalideerd. Wij hebben benadrukt, dat recepten uitsluitend betrouwbaar kunnen worden gevalideerd met gebruik van een professioneel proefpanel in een professioneel smaakcentrum.

In hoofdstuk IV wordt beschreven hoe vaak wij tijdens DBPCFCs bij kinderen, die voor het geteste voedingsmiddel gesensibiliseerd waren (aantoonbare IgE-antistoffen hadden in het bloed of een reactie hadden in de huidtest), placeboreacties hebben waargenomen. Ook wordt de aard van de placeboreacties beschreven. Verder wordt de betekenis van deze placebo "reacties" voor de uiteindelijke uitslag van de DBPCFCs beschreven. De waargenomen symptomen tijdens provocaties werden zo uniform en onbevooroordeeld mogelijk beoordeeld aan de hand van een nieuw ontwikkeld en gestandaardiseerd stroomdiagram. Ook de eindbeoordeling van de DBPCFCs vond plaats volgens een in dit hoofdstuk weergegeven protocol. In 12.9% van alle DBPCFCs traden placeboreacties op. Hierdoor kon 5.4% van alle positieve verumprovocaties als fout-positief worden ontmaskerd. Op basis van deze resultaten en op basis van studies waarin resultaten van open provocatie onderzoek werden vergeleken met die van DBPCFCs, concludeerden wij dat de diagnostische waarde van het gebruik van placebo's niet alleen bestaat uit het ontmaskeren van fout-positieve reacties op verumprovocaties, maar vooral ook om blindering van de verumprovocatie mogelijk te maken. Hierdoor worden minder (fout-positieve) reacties waargenomen bij de verumprocaties, doordat bevooroordeling (bias) tijdens de observaties is uitgesloten.

Voor jonge kinderen met een verhoogd risico op voedselallergie geldt het algemene advies om allergene voedingsmiddelen, zoals ei en pinda, voorzichtig in opklimmende hoeveelheden te introduceren in het dieet. Specifieke richtlijnen bestaan hiervoor niet Het is echter bekend dat een eerste inname van een allergeen voedingsmiddel kan leiden tot ernstige allergische reacties, waarschijnlijk omdat de eerste porties voor gevoelige kinderen relatief hoog zijn.

Om die reden hebben wij in hoofdstuk V introductieschema's voor deze allergene voedingsmiddelen voor thuis gebruik ontwikkeld. Deze schema's kunnen worden gebruikt door kinderen met een verhoogd risico op voedselallergie, maar die, ter beoordeling van de behandelend arts, geen provocatie behoeven. Dit kunnen bijvoorbeeld kinderen zijn met eczeem of met koemelkallergie. De hoeveelheden van de introductieschema's zijn gebaseerd op de doseerschema's van DBPCFCs, uitgevoerd bij kinderen die nog niet eerder het desbetreffende voedingsmiddel hadden gegeten/gedronken. De schema's bestaan uit schriftelijke richtlijnen met daarbij een begeleidende foto, waarop de te verstrekken voedingsmiddelen in opklimmende doses staan afgebeeld. Met behulp van deze schema's bleken de ouders de mediane hoeveelheden van de bedoelde porties nauwkeurig te kunnen portioneren, en de laagste doses significant lager te portioneren dan zonder deze schema's. Wij concludeerden dat het gebruik van deze schema's de veiligheid van thuisintroductie van allergene voedingsmiddelen kan verhogen. Artsen en diëtisten kunnen deze schema's voor dit doel gebruiken.

Het is bekend dat het voor patiënten met voedselallergie bijna niet haalbaar is om allergene ingrediënten volledig te vermijden. In hoofdstuk VI hebben wij onderzocht of ouders van kinderen, die om preventieve redenen allergene voedingsmiddelen vanaf de geboorte uit het dieet van hun kind weglieten, hier in slaagden. Verder hebben wij onderzocht of de mate van eliminatie voorspellend was voor de uitslag van de DBPCFC. Dit onderzoek vond plaats toen de Europese wetgeving voor etikettering van vóór november 2005 nog van kracht was. Met behulp van voedsel frequentie vragenlijsten voor deze allergene voedingsmiddelen stelden wij vast dat maar 1/3 van deze kinderen er in slaagde om deze voedingsmiddelen volledig te vermijden. Alle kinderen uit deze onderzoeksgroep ondergingen DBPCFCs, en 39% reageerde positief. Ook een deel van de kinderen die, zonder dat zij dat merkten, geringe hoeveelheden allergeen voedingsmiddel verdroegen, hadden positieve provocaties. De mate van eliminatie hield geen verband met de uitslag van de provocatie.
Over het algemeen wordt aangenomen, dat anafylaxie voor voeding (een ernstige, in principe levensbedreigende reactie) zelden verdwijnt, en dat voedselprovocaties bij kinderen met anafylaxie alleen dan uitgevoerd moeten worden, als er aanwijzingen zijn dat de voedselallergie is verdwenen. Het doel van het onderzoek (hoofdstuk VII) was om met behulp van DBPCFCs vast te stellen, hoe vaak dubbelblinde voedselprovoaties negatief zijn bij kinderen met anafalaxie voor voeding, of het doen van voedselprovocatie onderzoek dus nodig is, en om te onderzoeken of voedselprovocaties bij deze kinderen veilig kunnen worden uitgevoerd. Kinderen met een duidelijke voorgeschiedenis van anafylaxie voor voeding ondergingen een DBPCFC. Bij geen van de kinderen waren er aanwijzingen dat de anafylaxie was verdwenen, omdat het voedingsmiddel strikt werd gemeden. Van de 21 uitgevoerde

provocaties, waren er 6 negatief (29%): 3 melk provocaties, 1 ei provocatie, 1 pinda provocatie, en 1 tarwe provocatie. Deze kinderen waren hun anafylaxie en hun voedselallergie voor het geteste voedingsmiddel kwijtgeraakt. Er traden geen ernstige reacties op, noch was toediening van adrenaline noodzakelijk.

Voor zover wij kunnen vaststellen is dit de eerste opéénvolgende reeks van kinderen met een voorgeschiedenis van anafylaxie voor voeding, bij wie met behulp van DBPCFCs is vastgesteld hoe vaak de anafylaxie is verdwenen. Wij concludeerden dat anafylaxie voor voeding bij een aanzienlijk deel van de kinderen kan verdwijnen, en dat om die reden bij deze kinderen een voedselprovocatie overwogen moeten worden, ook al zijn er geen indicaties in de dieetvoorgeschiedenis dat de anafylaxie is verdwenen. Dit geldt niet voor kinderen bij wie grote hoeveelheden IgE tegen het voedingsmiddel worden gevonden. Verder concludeerden wij dat DBPCFCs veilig kunnen worden uitgevoerd bij deze kinderen, mits deze volgens een zorgvuldig protocol worden uitgevoerd en plaatsvinden in een centrum met ervaring in het doen van hoogrisico provocaties.

In hoofdstuk VIII worden de belangrijkste resultaten van dit proefschrift bediscussieerd, en zijn aanbevelingen voor toekomstig onderzoek geformuleerd.

DANKWOORD (ACKNOWLEDGEMENTS)

Ik kan me die email nog goed herinneren: Of ik soms geïnteresseerd was in het doen van "wat" onderzoek voor de duur van een jaar? "Dat doet ze nooit", zouden sommigen hebben gezegd, maar dat deed ze wel. Eén van de uitspraken van mijn man Henk is, dat je op de trein moet springen als die langs komt, omdat die anders zonder jou voorbij rijdt. Ik ben er op gesprongen.

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ABOUT THE AUTHOR

Berber Vlieg-Boerstra grew up in The Hague and Leidschendam, The Netherlands. After High School she studied Nutrition and Dietetics (BSc) at the Hogeschool van Arnhem en Nijmegen. She has a long standing interest in food allergy. Since 1987, she has been working as a dietician in private practice in the field of food allergy. Nationally, from the very beginning that food allergy was put on the agenda in the 1980's and 1990's, she has been involved in the field of food allergy. She participated in numerous publications, workshops and seminars for patients, dieticians, physicians, and other health care professionals in the Netherlands.
She has been a member of the Scientific Advisory Board of the Dutch National Foundation for Food Allergy (Stichting Voedselallergie), as well as a member of the Advisory Board of the Dutch Patient Organisation for Food Allergy (LIVO) for many years.
Because of her increasing interest in research, and challenged by many unanswered questions in the field of food allergy, in 2002, she completed a course in methodology and statistics at the Dutch Institute for Allied Health. Her scientific career started in 2001 at the Beatrix Children's Hospital of the University Medical Center Groningen, where she has been working part time on this doctorate thesis on double-blind, placebo-controlled food challenges, as well as on (hypo)allergenic apple cultivars and quality of life in food allergy.

LIST OF PUBLICATIONS

Publications in peer reviewed journals

1. Jansen JJ, Kardinaal AF, Huijbers G, **Vlieg-Boerstra BJ**, Martens BP, Ockhuizen T. Prevalence of food allergy and intolerance in the adult Dutch population. J Allergy Clin Immunol 1994;93:446-56
2. Huijbers GB, Cohen AA, Janssen JJ, Kardinaal AF, **Vlieg-Boerstra BJ**, Martens BP. Masking foods for food challenge: practical aspects of masking foods for a double-blind, placebo-controlled food challenge. J Am Diet Assoc 1994;94:645-9.
3. **Vlieg-Boerstra BJ**, Bijleveld CM, van der Heide S, Beusekamp BJ, Wolt-Plompen SA, Kukler J, Brinkman J, Duiverman EJ, Dubois AEJ. Development and validation of challenge materials for double-blind, placebo-controlled food challenges in children. J Allergy Clin Immunol 2004;113:341-6.
4. **Vlieg-Boerstra BJ**, Dubois AEJ. Published recipes for double-blind, placebo-controlled food challenges. Allergy 2005;60:1212.
5. **Vlieg-Boerstra BJ**, van der Heide S, Oude Elberink JN, Kluin-Nelemans JC, Dubois AEJ. Mastocytosis and adverse reactions to biogenic amines and histamine-releasing foods: what is the evidence? Neth J Med 2005 ;63:244-9.
6. **Vlieg-Boerstra BJ**, van der Heide S, Bijleveld CMA, Kukler J, Duiverman EJ , Wolt-Plompen SAA, and Dubois AEJ. Dietary assessment in children adhering to a food allergen avoidance diet for allergy prevention. Eur J Clin Nutr 2006;60:1384-1390.
7. Asero R, Ballmer-Weber BK, Beyer K, Conti A, Dubakiene R, Fernandez-Rivas M **et al.** IgE-mediated food allergy diagnosis: Current status and new perspectives. Mol. Nutr. Food Res. 2007;51:135-47.
8. de Blok BM, **Vlieg-Boerstra BJ**, Oude Elberink JN, Duiverman EJ, DunnGalvin A, Hourihane JO, et al. A framework for measuring the social impact of food allergy across Europe: a EuroPrevall state of the art paper. Allergy 2007;62:733-7.
9. Brand PLP **Vlieg-Boerstra BJ**, Dubois AEJ. Dietary prevention of allergic disease in children: are current recommendations really based on good evidence?. Pediatr Allergy Immunol 2007;18:475-9.
10. **Vlieg-Boerstra BJ**, van der Heide S, Bijleveld CMA , Kukler J, Duiverman EJ, Dubois AEJ. Placebo reactions in double-blind, placebo-controlled food challenges in children. Allergy 2007;62:905-12
11. Kootstra HS, **Vlieg-Boerstra BJ**, Dubois AEJ. Assessment of the reduced allergenic properties of the Santana apple. Ann Allergy Clin Immunol. Accepted.
12. **Vlieg-Boerstra BJ**, Dubois AEJ, van der Heide S, Bijleveld SMA, Wolt-Plompen SAA, Oude Elberink JNG, et al. Ready-to-use introduction schedules for first exposure to allergenic foods in children at home. Accepted.

13. **Vlieg-Boerstra BJ** , Duiverman EJ , van der Heide S, Bijleveld CMA , Kukler J, Dubois AEJ. Should children with a history of anaphylaxis to foods undergo challenge testing? Submitted.
14. Flokstra-de Blok BMJ, DunnGalvin A, **Vlieg-Boerstra BJ**, Oude Elberink JNG, Duiverman EJ, Hourihane JO et al. Development and validation of a self-administered health-related quality of life questionnaire for food allergic children. Submitted.

Publications in Dutch peer reviewed journals

1. Kneepkens CMF, Kapitein B, Gevers S, Meijer Y, **Vlieg B**, Neijens HJ, Hoekstra MO. Zuigelingenvoeding en allergie. Preventie en behandeling van koemelkallergie bij jonge kinderen. Tijdschr Kindergeneeskd 2004;72:219-225.
2. **Vlieg-Boerstra BJ**, Bijleveld CMA, van der Heide S, Duiverman EJ, Rings EHHM, Beusekamp BJ, Kukler K, Dubois AEJ. De dubbelblinde placebogecontroleerde voedselprovocatietest bij kinderen. Ned Tijdschr Allergie 2005;5:129-135.
3. Kootstra HS, Dubois AEJ, **Vlieg-Boerstra BJ**. De (hypo)allergene eigenschappen van de Santana appel. Ned. Tijdschr Allergie 2007;7:192-8.
4. **Vlieg-Boerstra BJ**, Y. Meijer. Dubbelblinde voedselprovocaties. Wie kunnen en gaan ze doen? Tijdschr Kindergeneeskd. 2008. Invited submission.
5. **Vlieg-Boerstra BJ**., van der Heide S. Dubbelblinde voedselprovocaties bij kinderen. Tijdschr Klinisch Chemici. 2008. Invited submission.

Publications in Text-books

1. Martens BPM., **Vlieg-Boerstra BJ**. Dieetbegeleiding van voedselallergie en –intolerantie bij volwassenen. Niet experimenteren maar weloverwogen elimineren en introduceren. In JGR de Monchy e.a. (red). Voedselallergie en –intolerantie. Wat doen we ermee? Bohn Stafleu van Loghum, 1989.
2. **Vlieg-Boerstra BJ**, Martens BPM. Voedselallergie en –intolerantie. Wat doen we ermee? In JGR de Monchy e.a. (red). Bohn Stafleu van Loghum, 1989.
3. **Vlieg-Boerstra BJ**. Voeding en Constitutioneel eczeem. In: Oranje AP en de Waard-van der Spek FB. Voeding en eczeem. Handboek Kinderdermatologie. De Tijdstroom. Utrecht. Tweede druk. 2005.
4. **Vlieg-Boerstra BJ**. Herziening: Voedselovergevoeligheid bij oudere kinderen en volwassenen. In: Informatorium voor Voeding en Diëtetiek. Bohn Stafleu van Loghum 2005;Dieetleer VII.
5. **Vlieg-Boerstra BJ**, Y. Meijer. Voedselprovocaties. Werkboek kinderallergologie. VU University Press. 2006.
6. **Vlieg-Boerstra BJ**, Carbasius Weber EC. Diëtistische begeleiding van kinderen met voedselallergie. Werkboek kinderallergologie. VU University Press. 2006.
7. **Vlieg-Boerstra BJ**. Food challenges. In Skypala I, Venter C (editors): Food hypersensitivity – diagnosis, management and treatment of food allergy and intolerance. Blackwell Publishing Limited. Invited submission 2007.

Other publications

1. Martens BPM, Vlieg-Boerstra BJ. Klinische diagnostiek. In: Consensusbijeenkomst voedselovergevoeligheid. Utrecht:CBO,1990.
2. Vlieg-Boerstra BJ. Dieetadviezen. In: Consensusbijeenkomst voedselovergevoeligheid. Utrecht:CBO,1990.
3. Vlieg-Boerstra BJ. Hypoallergene zuigelingenvoedingen. Modern Medicine, 2007.