

Egg and milk allergy in adults: comparison between fresh foods and commercial allergen extracts in skin prick test and histamine release from basophils

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Summary

The ability of skin prick test (SPT) and histamine release from basophils (HR) to diagnose clinical type I allergy to egg and milk was investigated as compared with double-blind, placebo-controlled food challenge (DBPCFC) in 17 adults suspected of type I egg and/or milk allergy. In both SPT and HR, commercial allergen extracts commonly used for SPT were compared with fresh, standardized foods. With commercial extracts the overall sensitivities of SPT and HR were 0.75 and 0.56 respectively, and none of the tests showed concordance with DBPCFC. With fresh, standardized foods the overall sensitivities of SPT and HR were 1.00 and 0.89 respectively, and both tests now showed a significant concordance with DBPCFC ($P < 0.05$). Specificity was only slightly improved in SPT, and unchanged in HR. Thus, the use of fresh, standardized foods significantly improved the outcome of both tests, as regards to sensitivity and concordance with DBPCFC. The diagnostic ability of SPT and HR appear to be strongly influenced by the allergen quality.

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Introduction

Previous studies have investigated the value of skin prick test (SPT), RAST and histamine release from basophils (HR) in the diagnosis of food allergy by comparing them with each other, with a convincing medical history, or with open challenges [1–4]. However, only 30–60% of the medical histories can be confirmed by double-blind, placebo-controlled food challenge (DBPCFC), which is now generally accepted for diagnosing food allergy in children and adults [5]. Large groups of children have recently been challenged in DBPCFC protocols [6–9], and there is a general agreement that DBPCFC cannot be substituted by either medical history, skin prick test (SPT), RAST or histamine release test (HR) alone or in combination. However, SPT is recommended for selecting children for DBPCFC [6,8]. SPT has long been claimed to be ineffective in the diagnosis of food allergy in adults, mainly because of a low SE [4,10]. Regarding the

diagnostic value of HR, conflicting results have been found in earlier studies [2,4,8,10,13,14]. However, only few studies have performed DBPCFC in adults, and results are conflicting, showing sensitivities of SPT between 0.30 and 1.00 [10–12], and sensitivities of HR between 0.15 and 1.00 [8,10].

These different sensitivities found in SPT and HR may be caused by many factors, such as patient selection and characterization, the quality of the different *in vitro/in vivo* tests, the quality of the allergen extracts, and the different types of food. The introduction of DBPCFC has permitted a standardized patient characterization, thus constituting a tool for evaluation of the diagnostic tests in food allergy. However, since the commercially available food allergen extracts are unstandardized and often poorly characterized, the influence of extract quality on the tests is still unknown.

The purpose of this study was therefore to investigate the ability of SPT and HR to identify clinically allergic individuals from among adults presenting a positive medical history, and to investigate the influence of extract quality on the test result. We selected a group of adults

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with strongly suspected type I allergic reaction to milk or egg, and performed titrated SPT and HR with commercial extracts, and fresh, standardized, liquid egg/milk. All patients were then challenged with the fresh unprocessed food by DBPCFC. Thus, by comparison with DBPCFC, we managed to test the outcome of SPT and HR performed with different allergen preparations in the same selected group of patients. Such studies are necessary for identification of optimal allergen preparations for SPT and HR with a view to predicting the outcome of DBPCFC.

Materials and methods

Patients

Among the patients attending the out-patient allergy clinic 17 adults representing 22 medical histories of immediate allergic reaction to egg or milk were selected by a medical history indicating type I food allergy: only patients reporting allergic symptoms/signs such as asthma, rhinitis, vomiting, diarrhoea, pruritus, urticaria and angioedema within 2 hr after intake of the suspected food item, on at least two separate occasions, were included in the study. The patients agreed to participate in this study before undergoing DBPCFC in another protocol. Blood samples for HR were collected and SPT was performed. Five adults with a clinical allergy to grass or birch pollen, defined as seasonal rhinitis or asthma accompanied by a positive SPT and RAST, and eight healthy volunteers, served as egg-and-milk-tolerant controls. The pollen allergic and normal controls were laboratory personnel. Moreover, patients only suspected of milk allergy served as egg-tolerant controls and vice versa. Prior to testing and challenging, all subjects discontinued medication: oral antihistamines for 5–7 days (astemizole 8 weeks), systemic steroid for 2 weeks and topical steroid on the SPT area for 72 hr. The number of patients/controls tested with each allergen is listed in Table 1.

Allergens

Three different allergen preparations were used: (i) glycerinated, commercial allergen extracts (Soluprick, ALK Laboratories, Hørsholm, Denmark): whole hen's egg 1:100 w/v and cow's milk 1:20 w/v, histamine dihydrochloride 10 mg/ml, diluent; (ii) fresh standardized foods: in order to obtain antigen mixtures representative of the different strains and feeding regimes, 40 000–50 000 eggs were collected from different parts of the country, broken and homogenized by slow stirring at 4°C for 3–5 hr. Likewise 40–70 tons of milk were collected from farms in different parts of the country. Samples from these

mixtures were stored at 4°C, and used within 36 hr for SPT and 48 hr for HR (from breaking the eggs and collecting the milk, respectively). The fresh, standardized egg was kindly supplied by DAN-EGG Products, Roskilde, Denmark. The fresh, standardized milk (unpasteurized and unhomogenized) was kindly supplied by MD Foods, Slagelse, Denmark. Commercially obtained fresh food: fresh hen's egg and fresh whole-fat, homogenized and pasteurized cow's milk bought locally.

SPT and HR were performed with commercial extracts and fresh, standardized foods. For SPT, commercial extracts were used both undiluted and diluted with diluent to 1/10, 1/100, and 1/1000 v/v, and fresh, standardized egg and milk were used both undiluted and diluted with 0.9% NaCl to 1/10, 1/100, and 1/1000 v/v. For HR, commercial extracts and fresh egg/milk were diluted in Pipes-AMC [60] to final concentrations of 1/10, 3/100, 1/100, 3/1000, 1/1000, and 1/10 000. Anti-IgE (Behringwerke, Germany) was used in final concentrations of 400, 40, and 4 U/ml. All dilutions were prepared immediately prior to use. For DBPCFC, fresh egg and whole-fat, homogenized and pasteurized milk bought locally were used.

Skin prick test

Each subject was tested with the commercial egg and milk extracts and the fresh standardized egg and milk in the dilutions described above, histamine dihydrochloride, saline, and commercial diluent. Single prick tests were performed on the volar surface of the forearm, using a DomeHollister-Stier prick test lancet with a 1 mm tip and shoulders. After 15 min (allergen) or 10 min (histamine), the inside of the weal was outlined with a felt-tipped pen and transferred to a recording sheet using transparent tape. The weal size was measured using the formula: $(D+d)/2$, where D is the maximum diameter and d its perpendicular diameter. A net weal diameter exceeding that of the negative control weal was regarded positive [15].

Histamine release from basophils

Histamine release test was performed as previously described [16]. Briefly, commercial allergen extracts and fresh egg and milk were used in the above mentioned dilutions. Samples of 25 µl of heparinized whole blood were incubated with 25 µl of allergen/anti-IgE for 1 hr at 37°C, the plates were rinsed, and protein residues were enzymatically removed [16]. Histamine bound to the microfibrils was measured spectrofluorometrically with a Gilson fluorometer. A histamine release of more than 15 ng/ml was considered positive. Patients with non-res-

ponding basophils, i.e. basophils releasing less than 15 ng/ml of histamine following anti-IgE challenge, were considered inconclusive.

Challenges

All 17 adults representing the 22 medical histories were tested by titrated, oral DBPCFC with fresh hen's egg/cow's milk bought locally. All challenges were performed in a double-blind, placebo-controlled fashion. Challenges comprised either tenfold increasing doses between 50 mg and 250 g of fresh, liquid allergen masked in placebo vehicle or pure placebo vehicle in identical volumes. All challenge doses were prepared and coded by the same dietitian. The order of active and placebo days was randomized by the dietitian, whereas the nursing staff serving the challenge doses and the physician evaluating the reactions did not know the constituents. The patients were challenged only when free of symptoms. All challenges were performed at the Food Allergy Unit with medication and resuscitation equipment readily available, and an intravenous line was established prior to all challenges. A positive reaction was defined as clearly objective allergic signs to an active challenge without symptoms to the placebo challenges. In case of moderate to severe allergic reaction, one active and one placebo challenge were considered sufficient. In case of milder reaction (e.g. only rhinoconjunctivitis or isolated decrease in FEV₁) or reaction to a placebo challenge, the patient was re-challenged, and if he correctly identified three active challenges among six randomized challenges, the reaction was scored as positive.

Calculations and statistics

SPT and HR were compared with DBPCFC, which was considered the golden standard of diagnosing clinical egg or milk allergy. Sensitivity of the diagnostic tests was calculated using the formula: DBPCFC-positive patients correctly identified by test/all DBPCFC-positive patients, and specificity by the formula: DBPCFC-negative patients correctly identified by test/all DBPCFC-negative patients. Thus, in this study, sensitivity and specificity denote the ability of the test to discriminate between DBPCFC-positive and DBPCFC-negative patients in a small and highly selected group of patients, and are not to be considered general statistic terms, as described by Nichaman, Sampson and Sheffer [17]. When evaluating the diagnostic tests, a high sensitivity was considered mandatory, since it was essential not to exclude any DBPCFC-positive patients in a group with a positive medical history. To permit comparison of different allergen preparations, the optimal allergen dilution

(OAD), i.e. the dilution giving maximum sensitivity without reducing specificity, was determined for each test and each allergen. Using the optimal dilution of each allergen preparation, two-by-two contingency tables were then established to compare the overall ability of tests with each allergen preparation to identify clinically allergic individuals. Concordance between test results and the results of DBPCFC was calculated using Fisher's exact test or Sign test with Yates correction for a small sample size. Calculations were made using the SPSS PC statistical software.

Ethics

All patients gave their informed consent to participate in this study. The study protocol was approved by the local ethical committee (journal no. V.100.1656/90).

Results

Challenges

Six of the 12 medical histories of immediate allergic reaction to egg and 3/10 to milk were confirmed by DBPCFC. During challenge these patients experienced a mean of 3.1 of the following symptoms: oral or pharyngeal itching and swelling, asthma, rhinoconjunctivitis, nausea, vomiting, abdominal pain, diarrhoea, universal pruritus, urticaria and angioedema. All reactions occurred within 40 min, and thresholds varied between 50 mg and 250 g of fresh food.

Titration of allergen preparations in SPT

The number of patients and controls tested is listed in Table 1. All subjects were scored as SPT-positive (weal diameter 3 mm larger than negative reference) or SPT-negative at each allergen dilution. The results of the SPT were compared to DBPCFC in the patients, giving the sensitivity and specificity at each allergen dilution. The OAD was then determined for each allergen preparation, as described in Calculations and Statistics.

Egg Sensitivity and specificity as computed in the six DBPCFC-positive patients and the six DBPCFC-negative patients at each dilution are shown in Fig. 1. The OAD of the commercial egg extract was the undiluted extract showing a sensitivity of 0.83 and a specificity of 0.67. The OAD of the fresh egg was determined 1/100 giving a sensitivity of 1.00 and a specificity of 0.67. No positive reactions to commercial egg extract or fresh egg were seen in the 21 egg-tolerant controls.

Milk Sensitivity and specificity as computed in the two DBPCFC-positive patients and the seven DBPCFC-

Table 1. Number of subjects tested with each allergen

	DBPCFC	HR	SPT
<i>Patients suspected of egg allergy</i>	12	12(9)	12
<i>Egg tolerant controls</i>			
Patients suspected of milk allergy	—	5	5
Pollen allergies	—	8(5)	8
Healthy volunteers	—	8(6)	8
<i>Patients suspected of milk allergy</i>	10	10(9)	9*
<i>Milk tolerant controls</i>			
Patients suspected of egg allergy	—	7(5)	7
Pollen allergies	—	8(5)	8
Healthy volunteers	—	8(6)	8

Numbers in parentheses indicate anti-IgE-responding subjects.
 *SPT was omitted in one patient due to extensive atopic dermatitis.

negative patients at each dilution are shown in Fig. 1. The OAD of the commercial milk extract was determined as 1/10 with a sensitivity of 0.50 and a specificity of 1.00. The OAD of fresh milk was 1/10 having a sensitivity of 1.00 and a specificity of 0.83. No positive reactions to commercial milk extract or fresh milk were seen in the 23 milk-tolerant controls.

Diagnostic ability of SPT

The overall ability of SPT to diagnose clinical egg and milk allergy within the study population (DBPCFC-positive/-negative patients) was determined for the following three allergen preparations:

- 1 Commercial, undiluted milk and egg extract (CE-SPT-routine).
- 2 Commercial extracts, OADs for egg and milk, respectively (CE-SPT-OAD).
- 3 Fresh foods, OADs for egg and milk, respectively (FF-SPT-OAD).

The correlation between SPT with these three preparations and DBPCFC is listed in Fig. 3 together with the overall sensitivities and specificities. CE-SPT-routine missed two DBPCFC-positive patients and showed no significant concordance with DBPCFC ($P > 0.05$). No improvement in sensitivity could be obtained by diluting the commercial extracts (CE-SPT-OAD), though specificity could be improved to 0.85, leading to a significant concordance with DBPCFC ($P < 0.05$). This improvement in specificity was due to exclusion of two false-positive patients by dilution of the commercial milk extract to OAD = 1/10 (Fig. 1). FF-SPT-OAD showed a significant concordance with DBPCFC ($P < 0.05$), and identified all DBPCFC-positive patients, i.e. showed a sensitivity of 1.00. The corresponding specificity was 0.77.

Titration of allergenic preparations in HR

The number of patients and controls assessable in HR is listed in Table 1. Anti-IgE non-responding subjects (3/17 patients, 5/16 controls). All subjects were scored as HR-positive (histamine release > 15 ng) or HR-negative at each allergen dilution. As for SPT, sensitivity and specificity as compared with DBPCFC was then computed in order to determine the OAD for each allergen preparation. Sensitivity and specificity at the different dilutions computed in the six DBPCFC-positive and three DBPCFC-negative patients assessable in HR, are shown in Fig. 2. Since some controls released histamine at the high allergen concentrations, the OAD was determined in a dilution range not inducing histamine release among the controls.

Egg No positive reaction to commercial egg extract was seen in the 16 egg-tolerant controls. The OAD of the commercial egg extract was the 1/10 dilution with a sensitivity of 0.50 and a specificity of 0.67. With fresh egg, 13/16 controls showed a positive reaction at the 1/10 dilution, decreasing to 3/16 at 1/100. No reactions were seen among the controls in the dilution range of 3/1000–1/10000, and the OAD of fresh egg was determined as 1/1000, with a sensitivity of 0.83 and a specificity of 0.67.

Milk Four of the 16 milk-tolerant controls showed a positive reaction at the 1/10 dilution of commercial milk extract, decreasing to 2/16 at the 1/100 dilution. No reactions were seen among the controls in the dilution range of 3/1000–1/10000, and the OAD of commercial milk extract was determined at 1/10000, with a sensitivity of 0.50 and a specificity of 1.00.

With fresh milk, positive reactions were seen in 9/16 milk tolerant controls at the 1/10 dilution. No reactions were seen among the controls in the dilution range 3/100–1/10000, and 1/100 was considered the OAD of fresh milk, giving a sensitivity of 1.00 and a specificity of 0.83.

Diagnostic ability of HR

The overall ability of HR to diagnose clinical egg and milk allergy in the study population was determined for the following two allergen preparations:

- (i) Commercial extracts, OADs for egg and milk, respectively (CE-HR-OAD).
- (ii) Fresh foods, OADs for egg and milk, respectively (FF-HR-OAD).

The correlation between these two preparations and DBPCFC is shown in Fig. 3 together with the overall sensitivities and specificities. CE-HR-OAD missed four DBPCFC-positive patients and showed no significant concordance with DBPCFC ($P > 0.05$).

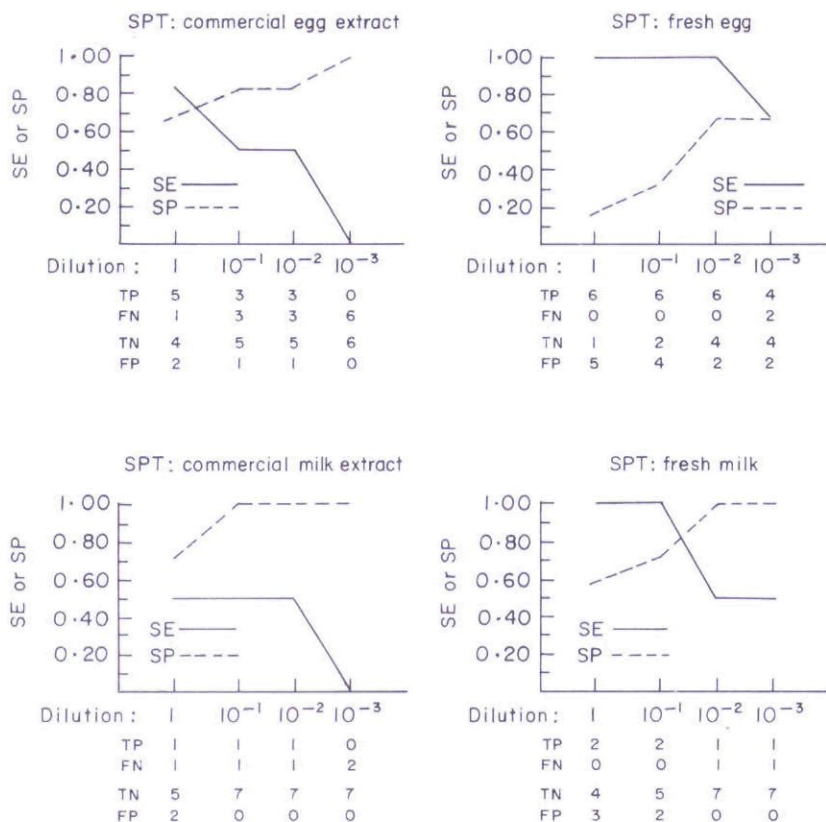


Fig. 1. SPT with egg and milk. Titration of allergen preparations. TP, true positive. FN, false negative. TN, true negative. FP, false positive.

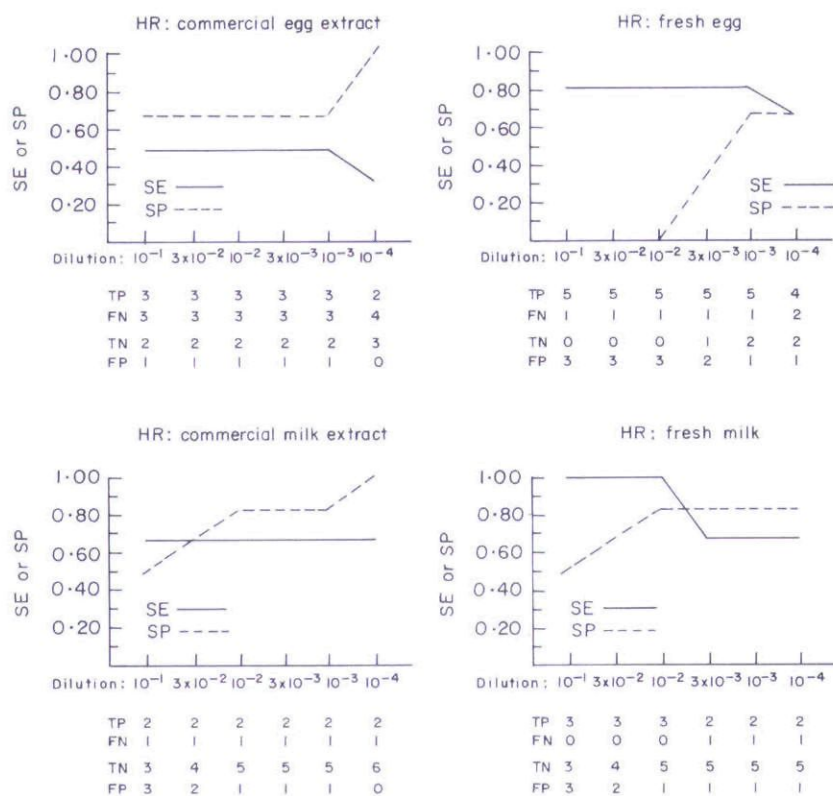


Fig. 2. HR with egg and milk. Titration of allergen preparations. TP, true positive. FN, false negative. TN, true negative. FP, false positive.

CE-SPT routine		
DBPCFC		
	POS	NEG
SPT POS	6	4
SPT NEG	2	9
SE : 0.75 SP : 0.69 $P > 0.05$		

CE-SPT OAD		
DBPCFC		
	POS	NEG
SPT POS	6	2
SPT NEG	2	11
SE : 0.75 SP : 0.85 $P < 0.05$		

FF-SPT OAD		
DBPCFC		
	POS	NEG
SPT POS	8	3
SPT NEG	0	10
SE : 1.00 SP : 0.77 $P < 0.05$		

CE-HR OAD		
DBPCFC		
	POS	NEG
HR POS	5	2
HR NEG	4	7
SE : 0.56 SP : 0.78 $P > 0.05$		

FF-HR OAD		
DBPCFC		
	POS	NEG
HR POS	8	2
HR NEG	1	7
SE : 0.89 SP : 0.78 $P < 0.05$		

Fig. 3. Overall diagnostic abilities of SPT and HR with different allergen preparations. CE-SPT, SPT with commercial egg and milk extracts. Routine, use of undiluted commercial extract as performed in routine SPT. FF-SPT, SPT with fresh, standardized egg and milk. CE-HR, HR with commercial egg and milk extracts. FF-HR, HR with fresh, standardized egg and milk. OAD, optimal allergen dilution, see text 'Calculations and Statistics'. SE, sensitivity. SP, specificity.

The sensitivity of FF-HR-OAD was 0.89, representing a diagnostic gain of three patients (two DBPCFC-positive to egg, and one to milk) when using fresh standardized food instead of commercial extracts. The corresponding specificity of FF-HR-OAD was 0.78, i.e. similar to that of CE-HR-OAD. FF-HR-OAD showed a significant concordance with DBPCFC ($P < 0.05$).

Discussion

Diagnostic value of SPT

Several recent studies have evaluated the use of SPT and RAST in large groups of children challenges in DBPCFC protocols [6–9]. These studies generally agree that DBPCFC cannot be substituted by neither medical history, SPT, RAST or HR alone nor in combination. In selecting patients for DBPCFC, Bock in 1976 found that SPT was a valuable tool for identification of children with immediate hypersensitivity to food, showing a sensitivity of 1.00 in 76 children [8]. In 1982, Sampson recommended SPT as a major diagnostic test in children with atopic dermatitis, though sensitivities varied between 0.73 and 1.00 and never exceeded 0.80 for milk and egg [6]. Both studies used commercial extracts for SPT and freeze-dried food products from a different commercial source for DBPCFC. However, Sampson found that the sensitivity of the SPT varied within the same group of

DBPCFC-positive children tested with different food allergens from the same supplier [6]. This variation probably reflects the limited knowledge of preservation of the allergenic properties of the different foods during extraction, and this lack of proper allergens limits the potential value of any test.

SPT has long been claimed to be ineffective in the diagnosis of food allergy in adults, mainly because of a low sensitivity [4,10]. However, studies comparing SPT to DBPCFC in adults are few. Bernstein found a sensitivity of only 0.30 in adults with a positive DBPCFC using commercial extracts in SPT and freeze-dried food products from a different commercial source in DBPCFC [10]. Atkins found a sensitivity of 0.90 in 10 DBPCFC-positive adults challenged with fresh foods and skin prick tested with commercial extracts [12]. Daul found a sensitivity of 1.00 using the same self-prepared shrimp extract for both testing and DBPCFC [11]. These results suggest that a good correlation between SPT and DBPCFC can be seen in adults, when using fresh foods in the food challenge or an identical extract of good quality in both SPT and DBPCFC.

In our study the ability of SPT to diagnose clinical type I food allergy was investigated in a small group of patients, presenting medical histories suggesting an immediate allergic reaction to egg or milk. The patients were tested with titrated commercial extracts and titrated fresh foods, and underwent DBPCFC with fresh foods.

SPT performed like in routine, i.e. using undiluted, commercial extracts showed no concordance with DBPCFC and missed 2/9 DBPCFC-positive patients. This low sensitivity could not be improved by dilution, though some improvement in specificity was obtained. SPT with fresh, standardized foods identified all DBPCFC-positive patients, and showed significant concordance with DBPCFC. Our results suggest that the value of SPT, even in a well-characterized study population, varies with the allergen concentration and the nature of the allergen preparation. SPT with fresh egg and milk correlated better with DBPCFC than SPT with commercial extracts, which suggests that the fresh food extracts are of a better quality than the commercial food allergen extracts currently available. This may be due to either a higher allergen concentration or to the presence of more allergens or less degraded native allergens in fresh food.

Diagnostic value of HR

Bernstein found no correlation between HR and DBPCFC in adults [10], using glycerinated food allergen extracts prepared for SPT, and HR has therefore been claimed useless in the diagnosis of food allergy in adults. However, in his study of 76 children Bock found that when using self-prepared allergen extracts for HR, all DBPCFC-positive children also had a positive HR [8]. These results suggest that the value of HR depends on the allergen extract used. In a study not using DBPCFC in the diagnosis of food allergy, good agreement between HR and suspected food allergy have been established by Clinton [13], who found that 83% of the patients with a positive history and positive SPT to egg also reacted positively in HR. Recently, Pastorello also found that 66% of apple-intolerant patients reacted positively in HR [14].

In this study we used an automated, glassfibre-based, whole-blood method developed by Skov [16]. With this method, good correlation between HR and bronchial challenges in children with asthma has been shown [16,18]. In our study the value of HR was investigated using commercial extracts and fresh foods. Histamine release induced by commercial extracts showed no significant concordance with DBPCFC, even though the correlation was better than that found by Bernstein, Day and Welsh [10], and comparable with the results obtained by Pastorello [14]. When fresh, standardized egg/milk was used for histamine release, the overall SE was improved, and a significant concordance with DBPCFC was obtained, which suggests that HR strongly depends on the quality of the allergen. False positive reactions were seen among atopic and healthy controls at high allergen concentrations, and this may be an important source of

error, which can be eliminated by proper dilution of the allergens. Properly diluted fresh foods improved the HR test significantly, rendering it comparable with FF-SPT.

Allergen preparations

As mentioned in the introduction, the different sensitivities and specificities found in different studies may be caused by several factors, such as patient characterization, test quality, allergen quality and potency, and the single food allergens. In our study both patient characterization and calculation of sensitivity are similar to those of previous DBPCFC studies. However, in the previous studies allergen preparations used for SPT and HR have been different from the ones used in challenges, except in the study by Daul [11], where the same self-prepared allergen preparation was used in both the tests and the challenges, giving a sensitivity of 1.00. Using fresh foods in both SPT and DBPCFC also produced a sensitivity of 1.00 in our study, and this result suggests that it may be important to use identical allergens when comparing SPT with DBPCFC. Regarding HR, Bock found a high sensitivity using a self-prepared extract [8], and in our study sensitivity significantly improved when commercial extracts were substituted by fresh foods. These results demonstrate the importance of allergen extracts and indicate that the commercial egg and milk allergen extracts somehow decrease the sensitivity of the HR test.

In conclusion, comparison of commercial extracts and fresh egg and milk showed that the diagnostic ability of both SPT and HR varied with the concentration of allergen and could be improved by using fresh foods. Considering the small number of patients, this study produces no conclusive evidence of the predictive diagnostic value of these tests in a wide, unselected population. However, the patients in this study are well-characterized and have been challenged by DBPCFC, and the results suggest that the allergen quality is an important factor influencing the diagnostic value of both SPT and HR, in particular the sensitivity of the tests. Improvement of the quality of allergen preparations may reveal SPT and HR to be valuable methods in the diagnosis of type I food allergy and more exact predictors of clinical allergy than hitherto thought.

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