

Letter to the Editor

Identification of allergen-resolved threshold doses of carrot (*Daucus carota*) by means of oral challenge and ELISA

To the Editor:

An increasing number of publications focus on the determination of threshold doses for allergenic foods.^{1,2} Knowledge of the minimum dose that is able to elicit an allergic reaction is relevant for all food-allergic individuals and has implications for public health.²

So far these thresholds are in most cases calculated on the basis of the weight of ingested fresh material.^{1,3} However, the allergen content per gram of food may depend on the variability of protein biosynthesis and may vary over storage time because many foods (eg, carrots) are harvested once yearly and stored.

In an earlier double-blind, placebo-controlled food challenge in 20 carrot-allergic patients, an oral allergy syndrome occurred at a mean dose of 1.9 ± 1.0 g and systemic reactions at a mean dose of 1.7 ± 0.9 g of fresh carrot.³ Significantly lower threshold values have been found for other allergenic foods, prompting meticulous efforts to standardize the means of food delivery in food challenge protocols.^{1,2,4,5}

Scientifically, the most accurate approach for exposure assessment is quantification of the allergenic proteins present in the food used for challenge.¹ This study is a first approach to gain a deeper insight into the relationship between the content of allergens in fresh food and the clinical response at a certain threshold dose.

An open food challenge was carried out by a 2-step protocol until objective symptoms were detectable. The content of the major allergens Dau c 1 and Dau c 4 in the carrots used for the

challenge was subsequently quantified by allergen-specific ELISA assays.⁶

Eleven patients were selected by a positive case history of carrot allergy and the presence of IgE to carrot confirmed by skin prick testing or ImmunoCAP measurements (see this article's Online Repository at www.jacionline.org and see Table I). Approval by the local ethics committee (Faculty of Medicine, University of Erlangen-Nuremberg, No. 3494) for this study and written informed consent of all participants were obtained. The mean age of the patients was 43.5 ± 12.2 years (range, 20-58 years). Skin prick tests were performed as prick-to-prick tests with fresh carrot (variety *Rodelika*, Dottenfelder Hof, Bad Vilbel, Germany) as well as with commercial carrot (Bencard, Munich, Germany) and birch pollen (Allergopharma, Reinbek, Germany) extracts.

Oral challenges were done on 6 different days. Grated carrots (cv *Rodelika*) were freshly prepared in a food processor (BOSCH MUM 4405 ProfiMixx with continuous shredder MUZ 4DS2, Munich, Germany) 20 minutes before starting the oral provocation. Eleven increasing carrot doses (10, 25, 50 mg, 0.10, 0.25, 0.50, 1.0, 2.5, 5.0, 10.0, and 15.0 g) were administered in 15-minute intervals. The patients were required to retain the administered dose in their mouth for 1 minute and then swallow the sample. The symptoms after exposure under the tongue and 15 minutes after swallowing were documented. Depending on the reaction, the next dose was given or the challenge was stopped after observing objective allergic symptoms at 2 consecutive doses.

Already at the first dose (10 mg), 2 patients responded with pruritus of the oral cavity. This was the most frequently occurring first subjective symptom in our patient group (63.6%, 7 of 11). The first objective symptom was diagnosed at a discrete dose of

TABLE I. Clinical and serologic characterization of the carrot-allergic patients, and results of the open food challenge

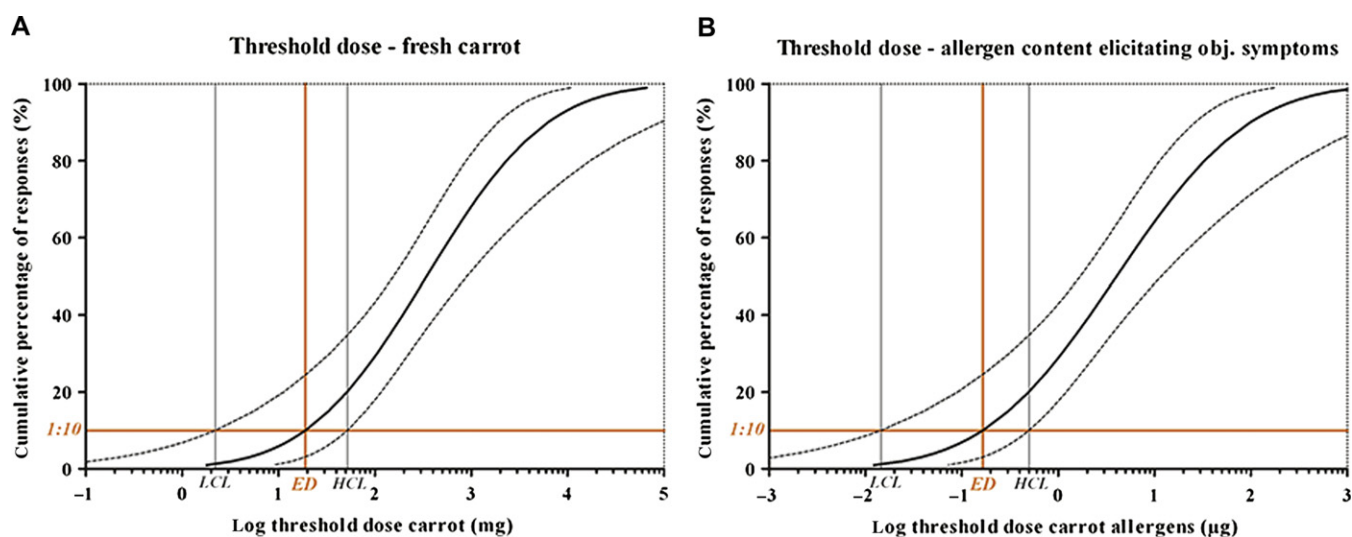
Patient No.	Age (y)	Case history Carrot	ImmunoCAP (kU _A /L) (CAP class)				Experimental ImmunoCAP (kU _A /L) (CAP class)			Oral food challenge (cumulative doses)			
			Carrot	rBet v 1	rBet v 2	Bromelain	rDau c 1.0104	rDau c 1.0201	rDau c 4	First subjective symptom	At carrot dose (mg)	First objective symptom	At carrot dose (mg)
1	20	OAS	0.07 (0)	43.10 (4)	7.46 (3)	0.28 (0)	5.25 (3)	4.32 (3)	2.11 (2)	Pruritus (oral)	85	Hoarseness	185
2	58	OAS	2.82 (2)	35.90 (4)	0.03 (0)	0.88 (2)	3.70 (3)	4.32 (3)	0.09 (0)	No*	–	Tearing	1935
3	55	OAS	5.20 (3)	55.80 (5)	2.70 (2)	0.18 (0)	10.50 (3)	4.90 (3)	1.48 (2)	Pruritus (oral)	35	Redness of conjunctiva	185
4	34	OAS	2.20 (2)	9.28 (3)	1.30 (2)	0.02 (0)	2.53 (2)	1.55 (2)	0.88 (2)	No*	–	Swelling (oral cavity)	35
5	45	OAS	2.51 (2)	11.90 (3)	2.90 (2)	0.21 (0)	1.95 (2)	0.77 (2)	5.31 (3)	Pruritus (oral)	10	Swelling (throat)	85
6	44	OAS	0.62 (1)	5.35 (3)	0.00 (0)	0.08 (0)	0.64 (1)	0.91 (2)	0.05 (0)	Pruritus (oral, ears)	35	Redness of conjunctiva, swelling (lips)	435
7	52	OAS, dyspnea	0.44 (1)	3.16 (2)	0.26 (0)	0.03 (0)	0.59 (1)	0.45 (1)	0.22 (0)	Pruritus (oral)	10	Tearing	185
8	33	OAS	1.05 (2)	10.50 (3)	0.00 (0)	0.07 (0)	0.69 (1)	1.06 (2)	0.04 (0)	No*	–	Cough	935
9	32	OAS, diarrhea	0.42 (1)	29.40 (4)	0.00 (0)	0.17 (0)	1.29 (2)	2.28 (2)	0.03 (0)	No*	–	Swelling (lips)	1935
10	49	OAS	24.00 (4)	>100 (6)	0.13 (0)	2.90 (2)	28.40 (4)	16.60 (3)	0.54 (1)	Pruritus (throat)	35	Hoarseness	85
11	56	OAS	8.16 (3)	0.06 (0)	0.14 (0)	0.36 (1)	4.86 (3)	14.40 (3)	0.18 (0)	Pruritus (throat)	85	Redness of conjunctiva, swelling (ears)	185

OAS, Oral allergy syndrome; r, recombinant.

*Objective symptom occurred without preceding subjective symptoms.

TABLE II. Results of ELISA measurements adapted to carrot threshold doses for objective symptoms

Patient no.	Oral food challenge	At discrete dose		At cumulative dose	
	First objective symptoms	Carrot (mg)	Sum of single allergens (μ g)	Carrot (mg)	Sum of single allergens (μ g)
1	Hoarseness	100	0.57	185	1.06
2	Tearing	1000	17.81	1935	34.46
3	Redness of conjunctiva	100	0.93	185	1.73
4	Swelling (oral cavity)	25	0.39	35	0.55
5	Swelling (throat)	50	0.78	85	1.32
6	Redness of conjunctiva, swelling (lips)	250	3.39	435	5.89
7	Tearing	100	1.36	185	2.51
8	Cough	500	6.77	935	12.67
9	Swelling (lips)	1000	7.60	1935	14.71
10	Hoarseness	50	0.42	85	0.71
11	Redness of conjunctiva, swelling (ears)	100	0.30	185	0.56

**FIG 1.** Cumulative distribution (%) of objective threshold doses ($n = 11$) for carrot (**A**) and objective responses against the cumulative dose of relevant carrot allergens (**B**), including the 95% CI (dashed lines). Estimated threshold doses (EDs) at patient proportions of 1:10 (ED_{10}) are depicted in orange. LCL/HCL, Lower and higher confidence limits.

25 mg (ie, cumulative dose of 35 mg) of carrot. Seven patients (63.6%) first reacted with objective symptoms at a cumulative dose below 190 mg. Objective symptoms observed most frequently were edema in the oral cavity or of the ears (45.5%, 5 of 11), followed by symptoms of the eye (redness of conjunctiva and tearing; see Table I).

After ELISA⁶ measurement (see additional information and Table E1 in this article's Online Repository at www.jacionline.org), the allergen content was calculated for each sample used for challenge. The correlation between the amount of carrot (mg) and the content of the relevant allergens at the individual threshold doses is shown in Table II. Taking into account the sensitization profile of individual patients in terms of IgE-reactivity to Dau c 1.01, Dau c 1.02, and Dau c 4, threshold doses for objective symptoms corresponded to a cumulative amount of 0.55 to 34.46 μ g of carrot allergens (see Table II).

Using a statistical model proposed by Bindslev-Jensen et al,⁷ all data underwent a probit analysis using SAS/STAT software (SAS System for Windows, version 9.2) to estimate threshold levels of carrots and the corresponding content of the main allergens in relation to the sensitization profile of the patients.

Fig 1 displays cumulative distribution functions generated by plotting the proportion of objective responses against log dose of carrot (see Fig 1, A) or against the sum of relevant carrot allergens (see Fig 1, B). Because of the small number of patients, the 95% CIs of both curves cover a rather broad range.

At the individual basis, the summarized carrot allergen threshold of our patient group started at 0.55 μ g (see Table II, patients 4 and 11). By probit analysis, this was down-calculated to an estimated threshold dose of 0.165 μ g (lower confidence limit, 0.015 μ g; higher confidence limit, 0.500 μ g) for 10% of the patients (ED_{10}) on population level (see Fig 1, B). Also, 10% of the carrot-allergic patients are predicted to react with objective symptoms at a cumulative dose of 18.98 mg of carrot (lower confidence limit, 2.22 mg; higher confidence limit, 51.79 mg; see Fig 1, A). This value is in a range comparable to the results of other recent studies, revealing an ED_{10} for whole hen's egg (43 mg),⁸ whole hazelnut (143 mg),⁸ and whole peanut (106 and 12 mg),^{8,9} respectively. In these foods, the main allergens are known to represent a substantial proportion of the total protein composition.

To our knowledge, this is the first threshold dose study including a quantification of the individual allergens in the food used for challenges. This seems to be relevant because allergen contents contained in fresh foods may vary considerably, for example, because of storage time and conditions (see Table E1).

In an upcoming study, it is planned to repeat this approach by using double-blind, placebo-controlled food challenge and to determine the “true lowest observed adverse effect level” and the “no observed adverse effect level” thresholds for carrot-allergic patients.

Kay Foetisch, PhD^{a,*}

Stephan Scheurer, PhD^a

Stefan Vieths, PhD^a

Kay-Martin Hanschmann, MSc^b

Jonas Lidholm, PhD^c

Vera Mahler, MD^{d,*}

From ^athe Division of Allergology, Paul-Ehrlich Institut, Langen, Germany; ^bDivision of Microbiology, Biostatistics Section, Paul-Ehrlich Institut, Langen, Germany; ^cThermo Fisher Scientific, Uppsala, Sweden; and ^dthe Department of Dermatology, University Hospital, Erlangen, Germany. E-mail: foeka@pei.de and vera.mahler@uk-erlangen.de.

*These authors contributed equally to this work.

This study was supported by the German Research foundation (DFG VI 165/6-1 and DFG MA1997/3-1).

Disclosure of potential conflict of interest: K. Foetisch has been supported by one or more grants from the German Research Foundation (DFG VI 165/6-1). S. Scheurer has been supported by one or more grants from the German Research Foundation (DFG VI 165/6-1). J. Lidholm is employed by Thermo Fisher Scientific. V. Mahler has been supported by one or more grants from the German Research Foundation (DFG MA1997/3-1). S. Vieths has consultant arrangements with the Food Allergy Resource and Research Program, the Institute for Product Quality, and Fresenius Academy; is employed by Johann Wolfgang Goethe-Universität; has provided expert testimony for the Medical University of Vienna; has received grants from the Monsanto Company and Pioneer Hi-Bred International; has received payment for lectures from Deutsche Dermatologische Gesellschaft, the Spanish Society of Allergy and Clinical Immunology, Westdeutsche Arbeitsgemeinschaft für Pädiatrische Pneumologie und Allergologie e.V., Gesellschaft für Pädiatrische Allergologie und Umweltmedizin, and the American Academy of Asthma, Allergy & Immunology; has received royalties from Schattauer Allergologie Handbuch and Elsevier Nahrungsmittelallergien und Intoleranzen; and has received travel expenses from the German Research

Foundation, the Federal Institute for Risk Assessment, the Austrian Society for Allergology and Immunology, the French Society of Allergology, the European Directorate for the Quality of Medicines and Health Care, the European Academy of Allergy and Clinical Immunology, the World Allergy Organization, Technical University of Munich, Deutscher Allergie und Asthmapund, Association Monégasque pour le Perfectionnement des Connaissances des Médecins, the Federal Office of Consumer Protection and Food Safety, the German Chemical Society, the Austrian Society for Dermatology and Venerology, and AKM Allergiekongress. K.-M. Hanschmann declares that she has no relevant conflicts of interest.

REFERENCES

1. Buchanan R, Dennis S, Gendel S, Acheson D, Assimon SA, Beru N, et al. Threshold Working Group. Approaches to establish thresholds for major food allergens and for gluten in food. *J Food Protection* 2008;71:1043-88.
2. Crevel RWR, Ballmer-Weber BK, Holzhauser T, Hourihane JO'B, Timmermans F, Taylor SL. Thresholds for food allergens and their value to different stakeholders. *Allergy* 2008;63:597-609.
3. Ballmer-Weber BK, Wüthrich B, Wangorsch A, Fötisch K, Altmann F, Vieths S. Carrot allergy: double-blinded, placebo-controlled food challenge and identification of allergens. *J Allergy Clin Immunol* 2001;108:301-7.
4. 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;34:689-95.
5. Cochrane SA, Salt LJ, Wantling E, Rogers A, Coutts J, Ballmer-Weber BK, et al. Development of a standardized low-dose double-blind placebo-controlled challenge vehicle for the EuroPrevall project. *Allergy* 2012;67:107-13.
6. Foetisch K, Dahl L, Jansen B, Becker WM, Lidholm J, van Ree R, et al. Development and in-house validation of allergen-specific ELISA tests for the quantification of Dau c 1.01, Dau c 1.02 and Dau c 4 in carrot extracts (*Daucus carota*). *Anal Bioanal Chem* 2011;399:935-43.
7. Bindslev-Jensen C, Briggs D, Osterballe M. Can we determine a threshold level for allergenic foods by statistical analysis of published data in the literature? *Allergy* 2002;57:741-6.
8. Eller E, Hansen TK, Bindslev-Jensen C. Clinical thresholds to egg, hazelnut, milk and peanut: results from a single-center study using standardized challenges. *Ann Allergy Asthma Immunol* 2012;108:332-6.
9. Taylor SL, Moneret-Vautrin DA, Crevel RW, Sheffield D, Morisset M, Dumont P, et al. Threshold dose for peanut: risk characterization based upon diagnostic oral challenge of a series of 286 peanut-allergic individuals. *Food Chem Toxicol* 2010;48:814-9.

<http://dx.doi.org/10.1016/j.jaci.2012.12.1577>

APPENDIX

All patients had a positive skin prick test result to carrot and birch pollen. Determination of specific IgE to carrot (f31), recombinant (r)Bet v 1, rBet v 2, and bromelain was done by using ImmunoCAP analysis (Thermo Fischer, Uppsala, Sweden), and experimental ImmunoCAP tests were used for rDau c 1.0104, rDau c 1.0201, and rDau c 4. Specific IgE level of 0.35 KU_A/L or more was considered positive. All patients showed specific IgE to rDau c 1.0104 and rDau c 1.0201, 91% to rBet v 1 (10 of 11), 36.4% to rBet v 2

(4 of 11), 27.3% to bromelain (3 of 11), and 45.5% to rDau c 4 (5 of 11).

Carrots were stored at 4°C prior to oral provocation. To evaluate the influence of carrot storage at 4°C on the allergen content, aliquots of all carrot samples used for oral provocation (no. 1-6) were stored at −20°C and analyzed by ELISA assays for the main carrot allergens (Table E1). The results revealed a slow but steady decrease in the content of Dau c 1.0104 over time of carrot storage (11 months in total) whereas the other 2 allergens, Dau c 1.0201 and Dau c 4, remained rather stable.

TABLE E1. Content of the main carrot allergens ($\mu\text{g/g}$ of carrot) in the oral provocation samples used over a time range of 11 months (derived from the same carrot harvest stored at 4°C)

Carrot sample no.	Allergen content ($\mu\text{g/g}$ of carrot)		
	Dau c 1.0104	Dau c 1.0201	Dau c 4
1	17.73	0.08	2.23
2	13.50	0.05	2.02
3	7.56	0.04	1.89
4	6.19	0.02	3.12
5	6.03	0.04	2.29
6	3.00	0.04	2.69