

LETTER TO THE EDITOR

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Sesame oleosins are minor allergens

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Abstract

Background: In daily practice, one-third of sesame allergic patients, confirmed by clinical history or food challenge, do not show any detectable specific IgE using current diagnostics. Currently used sesame extracts are water-based and therefore lacking hydrophobic proteins like oleosins. Oleosins, the stabilizer of lipid droplets in plants, are described as allergens in sesame, peanut and hazelnut. In this study, we examine the role of oleosins in sesame allergy and their potential cross-reactivity between sesame and (pea)nuts.

Methods: Specific IgE and IgG sensitisation to native and heterologously expressed sesame components and oleosins from other nuts, free of seed storage proteins, was assessed by line blot and sera from 17 sesame allergic patients without detectable specific IgE sensitisation to sesame, and compared to 18 sesame allergic and 13 tolerant patients with specific IgE sensitisation to sesame.

Results: Sesame allergic patients without sensitisation showed no specific IgE to the tested sesame oleosins or components. Low levels of specific IgE to sesame oleosins were detected in 17% of sesame allergic and 15% of tolerant patients with sIgE sensitisation. Oleosins were recognised by serum IgG from multiple patients confirming immune reactivity and excluding technical issues leading to lack of specific IgE-binding to oleosins.

Conclusion: Sesame oleosins are minor allergens and appear to have no additional value in diagnosing sesame allergy in adults based on sIgE and sIgG detection. There is a high need for additional diagnostic tools in those patients to minimize the number of required food challenges.

Keywords: Diagnostics, Food allergy, IgE hypersensitivity, Oleosins, Sesame

To the editor

Diagnosis of sesame allergy by measuring specific IgE (sIgE) is based on extracts or the major allergen: 2S albumin Ses i 1. However, this leads to false negative results in around 30% of sesame allergic patients, a high frequency compared to other food allergies [1]. To overcome this obstacle, we evaluated the role of oleosins in sesame allergy. Oleosins are oil-body stabilizing proteins and might be lacking in water-based extracts due to its hydrophobicity [2].

Adult sesame allergic and tolerant but sensitised patients who visited the outpatient clinic of the University Medical Centre Utrecht, The Netherlands, were retrospectively selected and allergy (n=35) or tolerance

(n=13) was confirmed by food challenge or an experienced physician diagnosis. Sesame allergic patients were subdivided into patients without and with detectable sIgE (ImmunoCAP sesame extract ≥ 0.35 kU/L; without n=17; with n=18). Patient characteristics are described in Table 1. Sera with sIgE against native and heterologously expressed oleosins from different nuts and seeds acted as positive controls. Ethical approval was acquired from the biobank committee of the University Medical Centre Utrecht, number 18-428.

Known sesame components and oleosins (from sesame, walnut, hazelnut, peanut and soy) with and without the hydrophobic domain based on the TMHMM model (prediction of transmembrane helices being not available for antibody binding) were heterologously expressed [3]. For comparisons to the native form, oil-body associated proteins (OAPs) were isolated from sesame, walnut and pecan using a modified previously described method

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Table 1 Patient demography of included sesame allergic and tolerant patients; allergy was defined by food challenge or an experienced physician

	Group 1a (n = 17) ^a	Group 1b (n = 18) ^a	Group 2 (n = 13) ^a	p values
Age (median)	53 (29–85)	35 (27–51)	32 (27–58)	0.001
Sex female	14 (82%)	10 (56%)	10 (77%)	0.1865
Food challenge	8 (47%)	1 (6%)	1 (8%)	0.0034
Total IgE (kU/l)	295 (39 to > 5000)	4470 (1010 to > 5000)	> 5000 (518 to > 5000)	< 0.0001
<i>Symptoms^b</i>				
Mild (Müller 0)	6 (35%)	6 (33%)	N.A.	0.9693
Moderate (Müller 1 + 2)	6 (35%)	6 (33%)	N.A.	
Severe (Müller 3 + 4)	5 (29%)	6 (33%)	N.A.	
<i>IgE measurements^c</i>				
Sesame extract (n; median, range)	17 (0, 0–0.32 kU/L)	18 (4.5, 0.5–75 kU/L)	13 (3.9, 0.4–48 kU/L)	0.5338
Ses i 1 ISAC (n; median, range)	6 (0 ISU)	10 (2.9, 0–22.5 ISU)	3 (0, 0–6 ISU)	0.4336

^a *G1a* sesame allergic patients without detectable sIgE sensitisation, *G1b* sesame allergic patients with sIgE sensitisation, *G2* sesame tolerant patients with sIgE sensitisation

^b Symptom distribution of each group is shown in Additional file 3: Figure S2

^c ImmunoCAP and ISAC data were compared between group 1b and 2 since group 1a was selected by lacking sIgE sensitisation; CAP > 0.35 kU/L was considered as positive

with an additional hydrophobic interaction chromatography (HIC) instead of a preparative gel electrophoresis to separate traces of seed storage proteins with similar molecular masses [4]. To examine the absence of seed storage proteins, enclosed proteins were identified by mass spectrometry and detected by western blot with anti-human IgE-alkaline phosphatase (AP). Sensitisation to these components was deeply investigated by measuring sIgE and sIgG levels using line blots (EUROLINE, EUROIMMUN, Luebeck, Germany) according to manufacturer's instructions. IgG subtypes were examined using an ELISA coated with different heterologously expressed oleosins (Ses i 4, Ses i Oleosin, Jug r Oleosin-1, Jug r Oleosin-2) and detected with anti-human IgG1-4 AP-conjugates. Detailed description of the methods is listed in Additional file 1.

Overall, the median age was 37 and allergic subjects suffered from typical symptoms being in line with an IgE-mediated hypersensitivity (characteristics are shown in Table 1). Gastro-intestinal symptoms were always accompanied by OAS or skin reactions (Additional file 2: Figure S1) and the allergic reactions took place within several minutes to half an hour after ingestion (data from 8/35), supporting the diagnosis 'food allergy'.

The applied sesame OAPs fraction (pool of HIC-fractions 4 and 5) was free of seed storage protein traces as confirmed by western blot analysis with sera positive for these proteins from the study cohort (Fig. 1, part a). Before performing a HIC, these sera reacted with proteins around 15 kDa, similar molecular masses as oleosins. After the additional purification step, only the positive control (PC) still recognised proteins at this

height. Additionally, no seed storage proteins but sesame oleosins were detected by mass spectrometry (Additional files 3 and 4: Figure S2 and Tables S1–S3).

Specific IgE-binding to sesame oleosins with levels above the detection limit (EAST-class 1–2) was detected in 17% of sensitised allergic and 15% of tolerant patients. However, none of the non-sensitised sesame allergic patients showed sIgE-binding neither to sesame oleosins nor to any other sesame components, except one serum showing low sIgE level to the 11S globulin Ses i 7 (G1a_6). Control sera, selected for sIgE binding to sesame (PC) or peanut oleosins (C1-3), showed sIgE binding to oleosins from different sources, confirming the binding capacity of heterologously expressed and native oleosins. Specific IgE-binding to oleosins in sensitised allergic patients was accompanied by recognising other sesame components, especially Ses i 1 and 2 whilst in tolerant patients was not (Fig. 1, part b and c). Patients showing IgE reactivity to heterologously expressed sesame oleosins were co-sensitised to heterologously expressed oleosins from walnut, hazelnut and peanut, indicating potential cross-reactivity (G1b_15, G1b_17). All oleosins expressed as full-length variant were recognised at least by one serum, except Cor a 12. In case of Cor a 13, the full-length and the hp-variant of Cor a 13 was recognised in parallel (G1b_17, PC).

Although oleosins were infrequently recognised by sIgE, they were bound by serum IgG from multiple patients across all groups confirming immune reactivity and excluding technical issues leading to lack of specific IgE-binding. Depletion of IgG by protein G columns in these sera did not result in sIgE binding (Additional file 5:

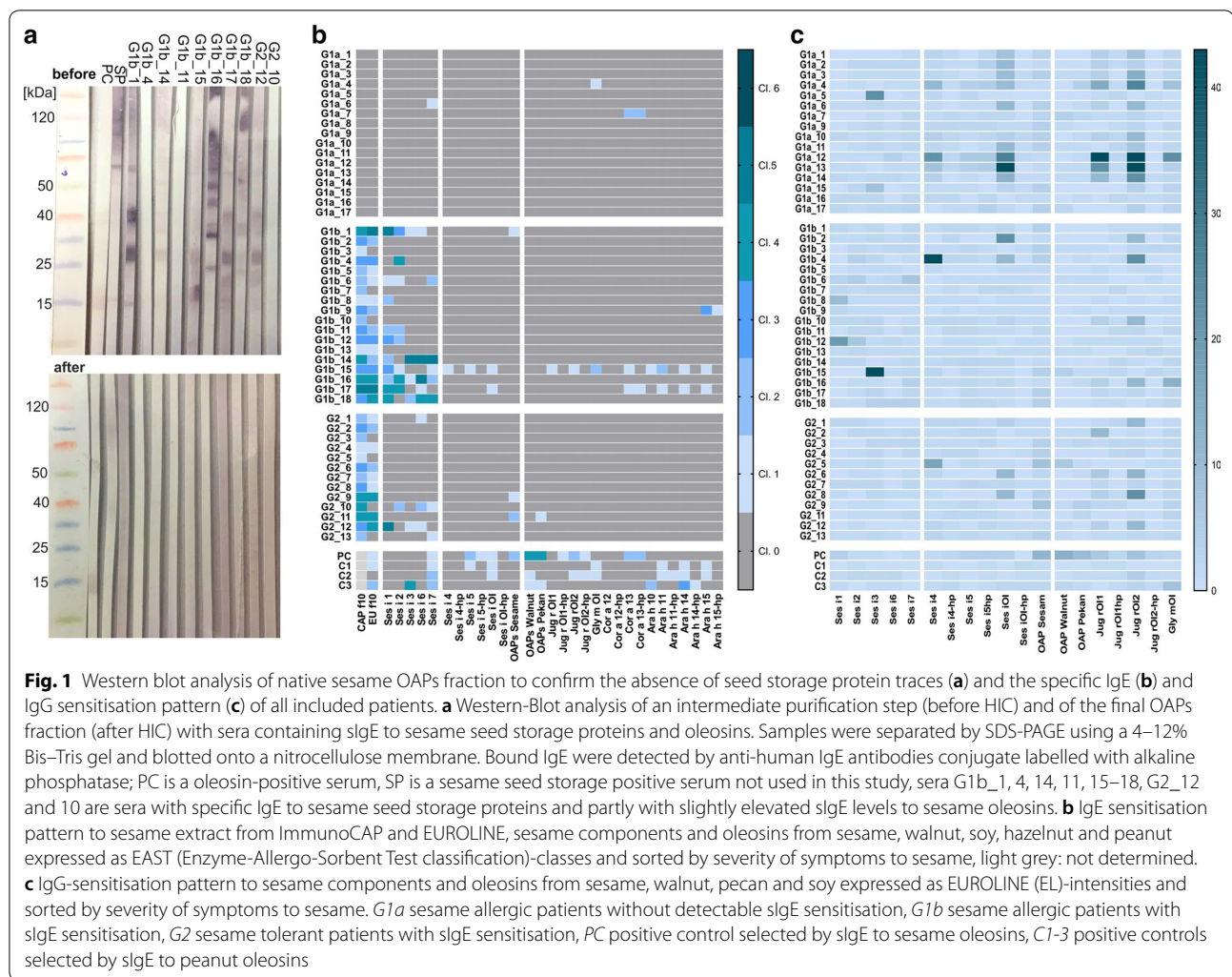


Figure S3), excluding competition between IgG and IgE. Specific IgG was mainly detected for Ses i 4, Ses i Ol, Jug r Ol-1 and Jug r Ol-2 and significantly increased in sesame allergic patients without detectable sIgE sensitisation compared with the other groups (Ses i 4: G1a vs. G1b, $p=0.0014$; Ses i Ol: G1a vs. G2, $p=0.03$; Jug r Ol-2: G1a vs. G1b, $p=0.0049$). The most prevalent subtype recognising oleosins was IgG1, while IgG2, IgG3 and IgG4 levels were dependent on the oleosin of interest (Additional file 6 and 7: Figures S4 and S5).

Up to 30% of sesame allergic patients, including those suffering from severe reactions, cannot be diagnosed by commercially available diagnostic tests [1, 5]. Launched aqueous sesame extracts do not contain hydrophobic proteins like oleosins. We demonstrated that oleosins were clearly recognized in 17% of sensitised sesame allergic patients, but did not have any additional diagnostic value compared to sesame extract and Ses i 1, especially in patients without detectable IgE sensitisation.

Contrary to our findings, 90% recognition in sesame allergic patients (31% without sIgE to sesame extract) was reported previously [6]. Moreover, OAPs reactivity was detected in 36% of hazelnut allergic patients and therefore considered relevant diagnostic markers, particularly in patients without detectable sIgE [7]. This discrepancy might be explained by the absence of seed storage proteins or fragments thereof [8] in our native preparation accomplished by HIC which was confirmed by western blot analysis and mass spectrometry.

Oleosins were recognised by sIgE levels slightly above the detection limit (EAST-class 1–2). This is in line with a hazelnut study across Europe showing a prevalence of 20% for the hazelnut oleosin Cor a 12, but low sIgE titre up to 1 kU/l ($\hat{=}$ EAST-class 2) [9]. In our test system, even lower sIgE titres might be explained by competition between different oleosins coated on the same line blot.

In conclusion, sesame oleosins are minor allergens and appear to have no additional value in diagnosing sesame

allergy in adults based on sIgE and sIgG detection. We propose a prospective study to evaluate the diagnostic value of direct basophil activation tests due to the high need for additional diagnostic tools in those patients.

Additional files

Additional file 1: Supplementary methods. Detailed description of the methods.

Additional file 2: Figure S1. Distinct symptom distribution between sesame allergic patients with and without detectable sIgE sensitisation. Sesame allergic patients without detectable sIgE sensitisation (G1a) showed more often skin related reactions (Mueller 1) compared to patients with sensitisation (G1b). The other way around, patients of G1b showed more often gastro-intestinal symptoms (Mueller 2) and cardiovascular reactions (Mueller 4).

Additional file 3: Figure S2. Gel images for mass spectrometry analyses of OAPs fractions. Gel images of elution fractions of OAPs from sesame, walnut and pecan nut after hydrophobic interaction chromatography used for mass spectrometry analyses.

Additional file 4: Table S1-S3. Enclosed proteins in native OAPs fractions. The native OAPs fractions were analysed by mass spectrometry. Table 1-3 show the identified proteins in those fractions.

Additional file 5: Figure S3. IgG depletion does not influence the recognition of sesame oleosins by sIgE. After IgG depletion, sera with high sIgG levels to oleosins (G1a_4, G1a_12, G1a_13 and G2_8) showed no increase in sIgE levels to sesame components or oleosins while sera with low sIgG levels but IgE sensitisation to sesame (G1b_1, G1b_15, G1b_17, G2_9 and G2_11) showed scarcely a decrease in sIgE levels, confirming no depletion of IgE during IgG depletion. One serum positive for sesame oleosins, G1b_15, displayed an increase in sIgE levels to oleosins although no sIgG to these components were detected.

Additional file 6: Figure S4. Subtype analysis of sera with elevated sIgG levels for Ses i 4, Ses i Ol, Jug r Ol-1 and Jug r Ol-2. Detection of the IgG subtype bound to Ses i 4, Ses i Oleosin, Jug r Oleosin-1 and Jug r Oleosin-2 from serum with specific IgG to oleosins (EUROLINE-intensities > 8); Scatter-plot with the measured OD value ratios (sample/negative value [NV]) divided by subtype for each oleosin. Horizontal lines mark the mean value. Green: Group 1a - sesame allergic patients without detectable sIgE sensitisation; Red: Group 1b - sesame allergic patients with sIgE sensitisation; Black: Group 2 - sesame tolerant patients with sIgE sensitisation.

Additional file 7: Figure S5. Comparison of IgG subtype between allergic and tolerant patients. Detection of the IgG subtype bound to Ses i 4, Ses i Oleosin, Jug r Oleosin-1 and Jug r Oleosin-2 from serum with specific IgG to oleosins (EUROLINE-intensities > 8); the data were separated by allergic and tolerant patients, IgG subtype and protein (oleosin).

Abbreviations

EAST: Enzyme-Allergo-Sorbent Test; EL: EUROLINE; HIC: hydrophobic interaction chromatography; hp-variant: heterologous expressed oleosin without hydrophobic domain; NCBI: National Centre of Biotechnology Information; OAPs: oil-body associated proteins; sIgE: specific IgE; SPT: skin prick test; TMHMM: transmembrane prediction by hidden Markov model.

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Authors' contributions

AE, MR, WS, HO designed the experiments; AE, MB, TML, AK selected the retrospective patient cohort; AE, MR performed the experimental procedures; AE performed data collection and analyses and drafted the manuscript; MR, BB, MB, TML, WS, HO, AC contributed to data interpretation and critically revised the manuscript. EUROIMMUN was not involved in the overall design of the study. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its Additional information files.

Ethics approval and consent to participate

This study was carried out in accordance with the University Medical Centre Utrecht, Biobank Regulations, which are in compliance with the applicable national and international laws and regulations. These regulations permit the use of 'residual material from diagnostic testing' for research, unless the patient objects (Article 8, 'no objection' procedure). None of the included patients objected the use of their serum. The protocol was approved by the Biobank Research Ethics Committee of the University Medical Centre Utrecht under the protocol number 18-428.

Consent for publication

Not applicable.

Competing interests

B. Brix, M. Rossnagel and W. Suer are employees of EUROIMMUN. The research position of A. Ehlers is partially funded by EUROIMMUN. Other authors have no competing interests to declare.

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Methods

Patient selection

Sesame allergic and tolerant patients who visited the outpatient clinic of the University Medical Centre Utrecht, The Netherlands, between 2012 and 2017 were retrospectively selected. The selected patients were first divided into one of two groups regarding allergy (n=35) or tolerance (n=13) confirmed by food challenge or an experienced physician. Allergic patients were subdivided into patients without (n=17) and with (n=18) detectable sIgE sensitisation (ImmunoCAP sesame extract ≥ 0.35 kU/L). Tolerant patients without detectable sIgE sensitisation were excluded. Positive controls were sera with sIgE against native and heterologously expressed oleosins from different nuts and seeds. Ethical approval was acquired from the biobank committee of the University Medical Centre Utrecht under the number 18-428.

Purification of oil-body associated proteins

Oil-body associated proteins (OAPs) were isolated from sesame, walnut and pecan nut as described previously with the following modifications (1-3): Sesame seeds were ground 1:2 w/v in 50 mM NaH_2PO_4 buffer pH 7.4 containing 1 mM EDTA, 10 mM KCl, 2 mM DTT and 20% w/v sucrose (grinding buffer) using a blender. The ground sesame seeds were filtered over a gauze and layered 1:1 v/v with flotation buffer (50 mM NaH_2PO_4 , 1 mM EDTA, 10 mM KCl, 2 mM DTT, pH 7.4). The gradient was centrifuged for 30 min at 4 °C using a swing-bucket rotor at 16.000 x g. After centrifugation, a white fat pad was formed on top of the gradient. After resuspension of the fat pad in urea buffer (50 mM NaH_2PO_4 , 9 M urea, pH 7.4), the suspension was incubated for 15 min at room temperature, layered 1:1 v/v with 50 mM NaH_2PO_4 buffer pH 7.4 and centrifuged under the same conditions. This step was repeated twice, and the resulting fat pad was resuspended in grinding buffer containing 0,1 % Tween-20 and layered 1:1 v/v with 50 mM NaH_2PO_4 buffer pH 7.4. After centrifugation, the fat pad was resuspended in grinding buffer containing 2 M NaCl and layered with flotation buffer containing 2 M NaCl. After resuspension of the fat pad in grinding buffer, proteins were

precipitated with a fourfold volume of ice-cold acetone and washed twice. After air-drying, the pellet was solubilised in a detergent-containing buffer and dialyzed against a 50 mM TRIS buffer, pH 7.5. To separate traces of seed storage proteins, the protein solution was applied on a hydrophobic interaction chromatography column.

Heterologous expression and purification of recombinant allergens

Sesame components (Ses i 1, 2, 3, 6 and 7) and oleosins (sesame, walnut, hazelnut and peanut) were expressed as fusion proteins with N-terminal His(6x) in *E. coli* as previously described (4, 5). The hp-variants of oleosins were expressed without the hydrophobic part determined by using the TMHMM model (6). All heterologous expressed proteins were purified by immobilised metal ion chromatography under denaturing conditions. Full-length oleosins were dialyzed against citrate buffer pH 5.5 for applying on the line blot.

Mass spectrometry

Enclosed proteins of the OAPs fractions from sesame, walnut and pecan nut were identified by mass spectrometry. Reduced (0.02 M DTT) and alkylated (0.06 M iodoacetamide) proteins were separated in a 4-12% polyacrylamide gel stained with colloidal Coomassie. After excising and achromatising, these proteins were digested in-gel by trypsin for 3 hours at 37 °C. The resulting peptides were subsequently extracted and spotted with α -cyano-4-hydroxycinnamic acid onto a MTP Anchor Chip 384 TF target (Bruker, Billerica, Massachusetts, US). Spotted peptides were measured by matrix-assisted laser desorption/ionisation-time of flight/TOF mass spectrometry (MALDI-TOF/TOF) using an Autoflex III smartbeam TOF/TOF200 System (Bruker, Billerica, Massachusetts, US) combined with the flexControl 3.4 software. MS spectra for peptide mass fingerprinting (PMF) were acquired in a positive ion reflector mode with 6000 shots ranging from 600 to 4.000 Da. For spectra calibration, external commercially available Peptide Calibration Standard II was processed with flexAnalysis 3.4 and the resulting peak lists were examined with BioTools 3.2. MS spectra for protein identification were analysed by submitting them to the MASCOT search engine MASCOT Server 2.3 (Matrix Science, London, U.K.) searching

against the NCBI database (2016/12/19) including 33.467 proteins from *Sesamum indicum*, 56.036 proteins from *Juglans regia* and 278 proteins from *Carya illinoensis*. Search parameters were set as follows: mass tolerance of 80 ppm, acceptance of one missed trypsin cleavage site, carbamidomethylation of cysteine residues as fixed modification, and oxidation of methionine residues as variable modification. For evaluation of protein hits, significance was defined as $p < 0.05$. PMF hits were confirmed by selecting two to five peptides of each identified protein with the WARP feedback mechanism of BioTools for MS/MS measurements. Recording of parent and fragment masses were performed with 400 and 1,000 shots, respectively. Resulted spectra were processed and analysed as described above with a fragment mass tolerance of 0.7 Da. These measurements were performed twice with different preparations in duplicates.

Line Blot

Sensitisation was defined by measuring sIgE and sIgG levels using a line blot (EUROLINE, EUROIMMUN, Luebeck, Germany) according manufacturer's instructions. For IgE detection, line blots were incubated overnight with 1 ml diluted serum (1:11 in universal buffer), some of them IgG-depleted (Protein G columns, GE Healthcare, Buckinghamshire, Great Britain), at room temperature on a rocket shaker. For detection of sIgG levels, 0.51 ml diluted serum (1:51 in universal buffer) was incubated for one hour at room temperature. Bound serum antibodies were detected with anti-human IgE or IgG alkaline phosphatase-coupled conjugate and visualised by adding nitro-blue tetrazolium/5-bromo-4-chloro-3'-indolyphosphate substrate. The binding of antibodies was evaluated using the EUROLINEScan software. For IgE measurement, band intensities were reported as intensity levels or classes, leaned on the Enzyme-Allergo-Sorbent Test (EAST) classification (19). EUROLINE intensities (EL-intensities) of 3 or greater were considered as positive, corresponding to classes from 1 to 6. For IgG measurement, band intensities smaller than 8 were considered as negative. These measurements were performed once.

Total IgE levels

Total IgE levels were determined using the ELISA technique (EUROIMMUN, Lübeck, Germany). In brief, 100 µl of diluted serum (1:10 in universal buffer) were applied per well and incubated for 30 min at room temperature. Bound IgE was detected by applying anti-IgE horse radish peroxidase-coupled conjugate and visualised by adding tetramethylbenzidine. Optical intensities read at 450 nm were evaluated with a calibration curve and converted into IU/ml. These measurements were performed once in duplicates.

IgG subtype determination

Microtiter plates were coated with heterologously expressed full-length oleosins (9 µg/mL in PBS, pH 7.5) over night at 4 °C. After blocking, 100 µl of diluted serum (1:25 in blocking buffer) was applied in duplicates and allowed to react for 1 hour at room temperature on an orbital shaker. Bound antibodies were detected using anti-human IgG1-4 AP-conjugates and stained with p-Nitrophenyl Phosphate Disodium salt (ThermoFisher Scientific, Waltham, MA, USA). The optical density (OD) was read at 405 nm using an automated spectrophotometer (iMark, Bio-Rad, Hercules, CA, USA). OD values of each IgG subtype measured for sera previously considered as positive (line blot) were divided by the OD value of the negative control. These measurements were performed twice in duplicates.

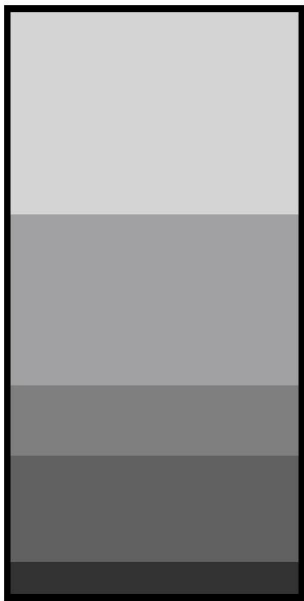
Data analysis

The baseline data were statistically analysed using one-way ANOVA or Mann-Whitney-U-test for continuous data and Fisher's exact test for categorical data. For comparison of sIgG levels between the patient groups, Kruskal–Wallis and Dunn's post-hoc tests were used. Statistical evaluation was performed with GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA) and SPSS Statistics 21 (IBM Corporation, Armonk, NY, USA). P values ≤ 0.05 were considered as statistically significant.

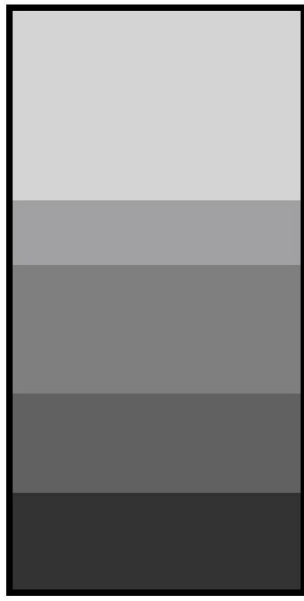
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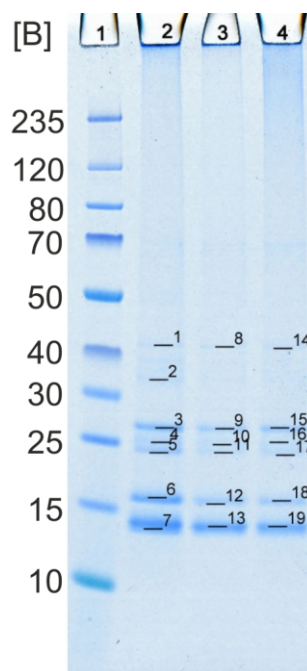
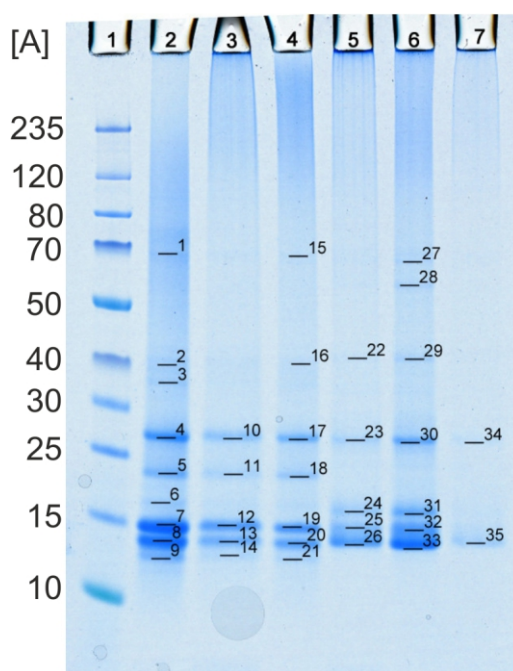
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Group 1a



Group 1b





- [A]
 1 - Marker
 2 - OAPs sesame HIC-fraction 3
 3 - OAPs sesame HIC-fraction 4
 4 - OAPs sesame HIC-fraction 5
 5 - OAPs walnut HIC-fraction 3
 6 - OAPs walnut HIC-fraction 4
 7 - OAPs walnut HIC-fraction 5
- [B]
 1 - Marker
 2 - OAPs pecan nut HIC-fraction 3
 3 - OAPs pecan nut HIC-fraction 4
 4 - OAPs pecan nut HIC-fraction 5

Figure S2: Enclosed proteins of the native OAPs fractions from sesame, walnut and pecan nut were identified by mass spectrometry. The proteins were separated by gel electrophoresis using 4-12% bis-tris gels and the bands were excised and in-gel digested with trypsin.

[A] Elution fractions of hydrophobic interaction chromatography (HIC) from sesame (line 2-4) and walnut (line 5-7) OAPs

[B] Elution fractions of hydrophobic interaction chromatography (HIC) from pecan nut OAPs (line 2-4)

Table S1: The native sesame OAPs fractions was analysed by mass spectrometry using peptide mass fingerprint. The table shows the identified proteins in this fraction.

Band	Protein (Accession number)	Cut-Off	Protein Score	Sequence coverage [%]	Molecular Mass [kDa]
Sesame OAPs: HIC-Elution-Fraction 3					
1	uncharacterized protein LOC105177100 (NP_001292915.1)	49	45	10	17,4
	15 kDa oleosin (AAD42942.1)	49	23	6	15,2
	PREDICTED: oleosin 1-like (XP_011076526.1)	49	23	4	24,0
2	11-beta-hydroxysteroid dehydrogenase-like 5 (NP_001291330.1)	79/50	169/55	54/4	41,3
3	11-beta-hydroxysteroid dehydrogenase 1B-like (NP_001291322.1)	79/50	125/31	50/2	39,7
	11-beta-hydroxysteroid dehydrogenase-like 5 (NP_001291330.1)	79/50	87/20	41/4	41,3
4	peroxygenase (NP_001291323.1)	79/50	76/115	40/23	27,8
5	PREDICTED: legumin B-like (XP_011083024.1)	79/50	93/59	35/8	52,1
	Peroxygenase (NP_001291323.1)	79/50	80/81	32/10	27,8
	11S globulin precursor isoform 4 (ABB60055.1)	79/50	80/59	32/8	53,0
6	PREDICTED: legumin B-like (XP_011083024.1)	79	72	33	52,1
	11S globulin precursor isoform 4 (ABB60055.1)	79	60	28	53,0
	15 kDa oleosin (AAD42942.1)	50	22	6	15,2
	PREDICTED: oleosin 1-like (XP_011076526.1)	50	22	4	24,0
7	15 kDa oleosin (AAD42942.1)	50	61	6	15,2
	PREDICTED: oleosin 1-like (XP_011076526.1)	50	61	4	24,0
8	uncharacterized protein LOC105177100 (NP_001292915.1)	51	43	10	17,4
9	uncharacterized protein LOC105177100 (NP_001292915.1)	49	40	10	17,4
	15 kDa oleosin (AAD42942.1)	49	35	6	15,2
	PREDICTED: oleosin 1-like (XP_011076526.1)	49	35	4	24,0
	PREDICTED: oleosin 16.4 kDa (XP_011097414.1)	49	20	10	17,4

Sesame OAPs: HIC-Elution-Fraction 4

10	Peroxygenase (NP_001291323.1)	79/49	108/27	41/8	27,8
11	Peroxygenase (NP_001291323.1)	51	81	10	27,8
12	uncharacterized protein LOC105177100 (NP_001292915.1)	79	57	39	17,4
	15 kDa oleosin (AAD42942.1)	50	47	6	15,2
	PREDICTED: oleosin 1-like (XP_011076526.1)	50	47	4	24,0
	uncharacterized protein LOC105177100 (NP_001292915.1)	51	24	10	17,4
13	PREDICTED: oleosin 1-like (XP_011081346.1)	51	22	7	14,8
	15 kDa oleosin (AAD42942.1)	51	21	6	15,2
	PREDICTED: oleosin 1-like (XP_011076526.1)	51	21	4	24,0
	PREDICTED: DDB1- and CUL4-associated factor 8 (XP_011089371.1)	79	58	22	54,1
14	uncharacterized protein LOC105177100 (NP_001292915.1)	49	15	10	17,4

Sesame OAPs: HIC-Elution-Fraction 5

15	-	-	-	-	-
16	11-beta-hydroxysteroid dehydrogenase-like 5 (NP_001291330.1)	79/50	190/70	53/7	41,3
17	Peroxygenase (NP_001291323.1)	79/50	119/73	53/13	27,8
18	Peroxygenase (NP_001291323.1)	79/50	80/63	27/10	27,8
19	uncharacterized protein LOC105177100 (NP_001292915.1)	50	67	10	17,4
	15 kDa oleosin (AAD42942.1)	50	52	6	15,2
	PREDICTED: oleosin 1-like (XP_011076526.1)	50	52	4	24,0
	PREDICTED: oleosin 1-like (XP_011081346.1)	51	46	7	14,8
20	uncharacterized protein LOC105177100 (NP_001292915.1)	51	43	10	17,4
21	PREDICTED: DDB1- and CUL4-associated factor 8 (XP_011089371.1)	79	91	36	54,1
	uncharacterized protein LOC105177100 (NP_001292915.1)	49	17	10	17,4

Cut-off, protein-score and sequence coverage are highlighted in green if the results were obtained from MS/MS experiments

Table S2: The native walnut OAPs fractions was analysed by mass spectrometry using peptide mass fingerprint. The table shows the identified proteins in this fraction.

Band	Protein (Accession number)	Cut- Off	Protein Score	Sequence coverage [%]	Molecular Mass [kDa]
Walnut OAPs: HIC-Elution-Fraction 3					
22	-	-	-	-	-
23	-	-	-	-	-
24	-	-	-	-	-
25	-	-	-	-	-
26	Oleosin (AET74077.1)	79	40	38	14,7
Walnut OAPs: HIC-Elution-Fraction 4					
27	-	-	-	-	-
28	-	-	-	-	-
29	-	-	-	-	-
30	-	-	-	-	-
31	-	-	-	-	-
32	-	-	-	-	-
33	Oleosin (AET74077.1)	79	48	38	14,7
Walnut OAPs: HIC-Elution-Fraction 5					
34	-	-	-	-	-
35	Oleosin (AET74077.1)	79	53	35	14,7

Cut-off, protein-score and sequence coverage are highlighted in green if the results were obtained from MS/MS experiments

Table S3: The native pecan nut OAPs fractions was analysed by mass spectrometry using peptide mass fingerprint. The table shows the identified proteins in this fraction.

Band	Protein (Accession number)	Cut- Off	Protein Score	Sequence coverage [%]	Molecular Mass [kDa]
Pecan nut OAPs: HIC-Elution-Fraction 3					
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	-	-
Pecan nut OAPs: HIC-Elution-Fraction 4					
8	-	-	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-
11	-	-	-	-	-
12	-	-	-	-	-
13	-	-	-	-	-
Pecan nut OAPs: HIC-Elution-Fraction 5					
14	-	-	-	-	-
15	-	-	-	-	-
16	-	-	-	-	-
17	-	-	-	-	-
18	-	-	-	-	-
19	-	-	-	-	-

Cut-off, protein-score and sequence coverage are highlighted in green if the results were obtained from MS/MS experiments

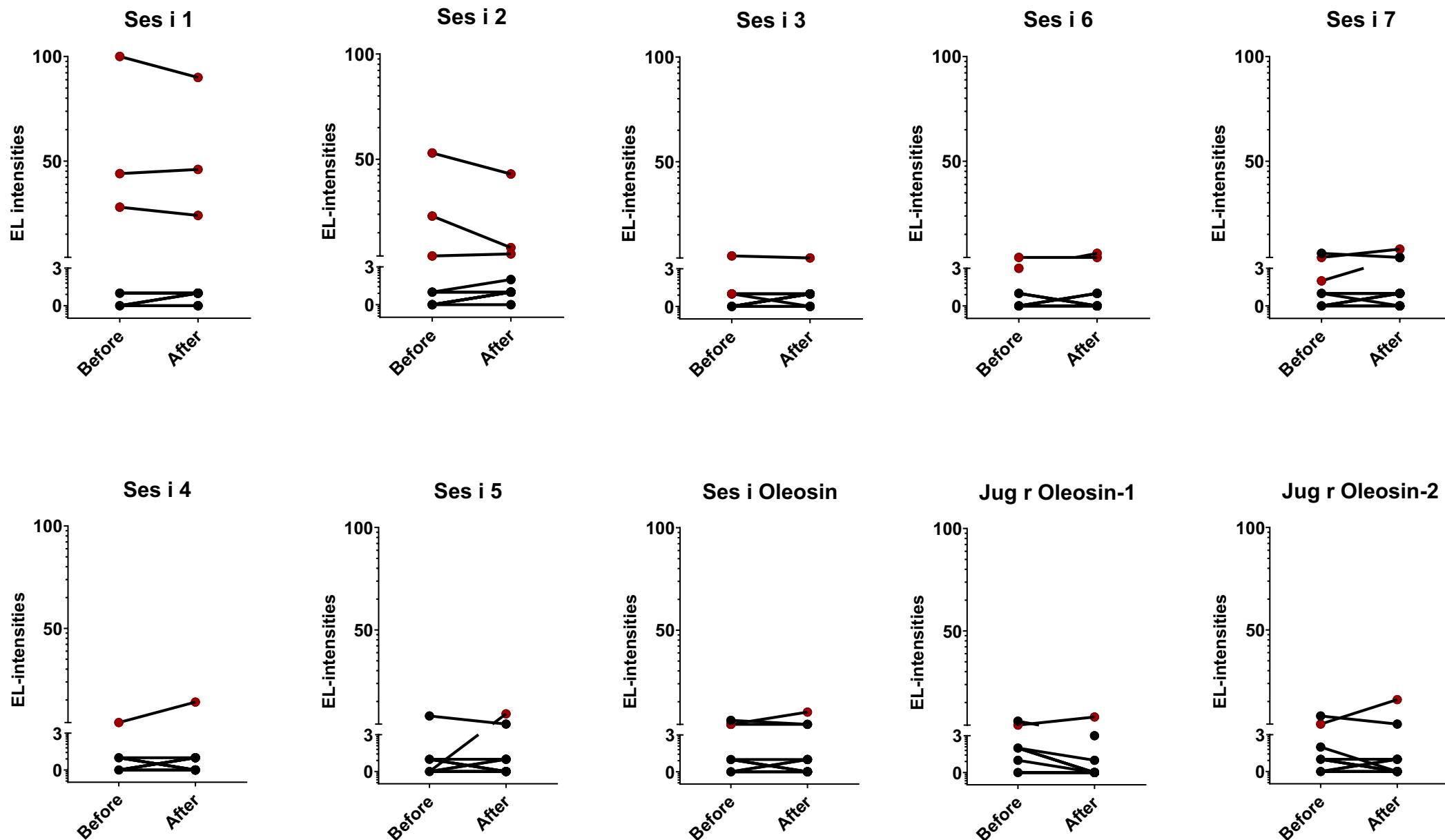


Figure S3: Sera with sIgE levels greater than an EUROLINE (EL-) intensity of 3 were marked accordingly to their study group (green: group 1a – sesame allergic without sensitisation, red: group 1b – sesame allergic with sensitisation, black: group 2 – sesame tolerant with sensitisation).

