



Walnut oral immunotherapy for desensitisation of walnut and additional tree nut allergies (Nut CRACKER): a single-centre, prospective cohort study

Arnon Elizur, Michael Y Appel, Liat Nachshon, Michael B Levy, Na'ama Epstein-Rigbi, Bo Pontoppidan, Jonas Lidholm, Michael R Goldberg

Summary

Background The safety and efficacy of oral immunotherapy for tree nut allergy has not been demonstrated to date, and its effectiveness is complicated by the high prevalence of co-allergies to several nuts. This study aimed to investigate the use of walnut oral immunotherapy in the desensitisation of walnut and additional tree nuts in patients who are co-allergic to several nuts.

Methods In a single-centre, prospective cohort study (the Nut Co-Reactivity ACquiring Knowledge for Elimination Recommendations study) at the Institute of Allergy, Immunology, and Paediatric Pulmonology at the Yitzhak Shamir Medical Centre, we recruited patients aged 4 years or older who were allergic to walnut, with or without co-allergy to pecan, hazelnut, and cashew. The diagnosis of each food allergy was based on a positive skin prick test or specific serum IgE (≥ 0.35 kU_A/L) to the corresponding nut together with a positive oral food challenge, unless an immediate (within 2 h of exposure) reaction in the past year had been documented. Patients with uncontrolled asthma or a medical contraindication to receive adrenaline were excluded. Patients were assigned to walnut oral immunotherapy or the control group (observation and strict dietary exclusion) on the basis of the order of presentation to the clinic. Oral immunotherapy began with a 4-day dose-escalation phase to establish the single highest tolerated dose, which was consumed daily at home for 24 days; subsequent monthly dose escalations were repeated until 4000 mg walnut protein was achieved. Patients who were desensitised to walnut continued to consume 1200 mg walnut protein daily for 6 months as maintenance. The primary outcome was walnut desensitisation (passing an oral food challenge with 4000 mg of walnut protein) at the end of the study, analysed by intention to treat. In patients who were co-allergic to pecan, hazelnut, and cashew, the proportion who achieved cross-desensitisation to these nuts in addition to walnut desensitisation was examined.

Findings 73 patients with a walnut allergy were enrolled between May 15, 2016, and Jan 14, 2018. 49 (89%) of 55 patients in the oral immunotherapy group were desensitised to walnut compared with none of 18 patients in the control group (odds ratio 9.2, 95% CI 4.3–19.5; $p < 0.0001$). Following walnut desensitisation, all patients who were co-allergic to pecan ($n=46$) were also desensitised to pecan. Additionally, 18 (60%) of 30 patients who were co-allergic to hazelnut or cashew, and 14 (93%) of 15 patients who were co-allergic to hazelnut alone, were either fully desensitised or responded to treatment. 47 (85%) of 55 patients had an adverse reaction (mostly grade 1 or 2) during up-dosing in the clinic; eight patients required intramuscular epinephrine in response to a dose at home. Of 45 patients who had follow-up data for the maintenance phase, all maintained walnut desensitisation and one patient required epinephrine during this period.

Interpretation Walnut oral immunotherapy can induce desensitisation to walnut as well as cross-desensitisation to pecan and hazelnut in patients who have tree nut co-allergies, with a reasonable safety profile. A low daily dose of the allergen maintains desensitisation.

Funding None.

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Introduction

Tree nut allergy is one of the most common food allergies. Its estimated global prevalence ranges from 0.05% to 4.9% and appears to be increasing.^{1–3} Unlike milk and egg allergy, as few as 9% of individuals with tree nut allergy experience a natural resolution.⁴ Tree nuts in general, and walnuts in particular, are a major cause of fatal and near-fatal reactions, as documented in US national self-reported registries.^{5,6} Walnut allergy is often associated with additional tree nut allergies, primarily pecan allergy, further increasing the risk of accidental exposures, dietary

limitations, and the emotional burden and anxiety in patients who are affected.^{7–9}

Oral immunotherapy has been shown to be effective in desensitising peanut, egg, and milk allergies, by raising the threshold of reaction to these allergenic foods.^{10–15} Clinical desensitisation is typically accompanied by a decrease in IgE and an increase in IgG4 to the allergenic food.^{10–15} The European Academy of Allergy and Clinical Immunology guidelines on allergen immunotherapy¹⁶ recommend oral immunotherapy for children with persistent milk, egg, or peanut allergy, but there are no

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Institute of Allergy, Immunology, and Paediatric Pulmonology, Yitzhak Shamir Medical Centre, Beer Yaakov, Israel (A Elizur MD, M Y Appel PhD, L Nachshon MD, M B Levy MD, N Epstein-Rigbi MD, M R Goldberg PhD); Department of Paediatrics, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel (A Elizur); and Thermo Fisher Scientific, Uppsala, Sweden (B Pontoppidan PhD, J Lidholm PhD)

Correspondence to:

Dr Arnon Elizur, Institute of Allergy, Immunology, and Paediatric Pulmonology, Yitzhak Shamir Medical Centre, Beer Yaakov 70300, Israel
elizura@gmail.com

Research in context

Evidence before this study

We searched PubMed with the search terms “tree nut desensitization” and “tree nut oral immunotherapy” for studies published on or before Nov 30, 2018, with no start date or language restrictions. A systematic review, published in April, 2018, identified a small study of eight patients, details of which were presented in an abstract only, suggesting that cross-desensitisation might occur following tree nut oral immunotherapy. In another study of patients treated with multiple food oral immunotherapy (including walnut), patients were desensitised to untreated nuts, but only in the context of receiving omalizumab. Walnut desensitisation without omalizumab was not achieved, and dissecting the effect of cross-desensitisation between two individual nuts in the setting of oral immunotherapy to multiple foods is not feasible.

Added value of this study

To our knowledge, this study is the first to show that walnut oral immunotherapy can induce desensitisation in patients who are allergic to walnut, as well as cross-desensitisation to additional tree nuts, primarily pecan and hazelnut, in patients who are co-allergic to these nuts. These clinical outcomes were accompanied by an immunological shift from allergy to a state of desensitisation, and this is the first study to show such an immunological shift in a food that is different from the one treated.

Implications for all the available evidence

These results will simplify the management of patients with multiple tree nut allergies and minimise the number of allergenic foods these patients are required to consume long term following oral immunotherapy. Understanding the molecular basis underlying this cross-desensitisation will require further studies.

sufficient data to recommend oral immunotherapy for other foods. Although peanut oral immunotherapy has not been shown to affect tree nut or sesame sensitisation,¹⁷ some data suggest that cross-desensitisation between tree nuts might occur.^{18–20} In a mouse model of food allergy, single tree nut immunotherapy was shown to decrease allergic responses in both cross-reactivity and multi-sensitisation mouse models.¹⁸ In a study of oral immunotherapy using multiple foods (including tree nuts) for co-allergic patients, desensitisation to walnut, as well as other untreated nuts, was successful, but only in conjunction with omalizumab; all the patients who were not treated with omalizumab did not show desensitisation to walnut, perhaps because of the higher number of adverse events.¹⁹ In another small study, desensitisation to walnut and another tree nut (pecan, cashew, hazelnut, or pistachio) was observed in seven of eight patients treated with walnut oral immunotherapy.²⁰ These data, together with our previous data⁷ showing a high prevalence of co-allergy to walnut in individuals also allergic to pecan (100%) and of co-allergy to walnut in those allergic to hazelnut (79%), suggest that walnut oral immunotherapy might be able to induce cross-desensitisation to other tree nuts. However, to date, no large controlled studies have investigated oral immunotherapy in patients who are allergic to walnut.²¹

We aimed to examine the safety and efficacy of walnut oral immunotherapy in desensitising walnut and co-existent pecan, hazelnut, and cashew allergies.

Methods

Study design and participants

We did a single-centre, prospective cohort study (the Nut Co-Reactivity ACquiring Knowledge for Elimination Recommendations study) at the Institute of Allergy, Immunology, and Paediatric Pulmonology at the Yitzhak Shamir Medical Centre (Be'er Ya'akov, Israel;

formerly the Assaf Harofeh Medical Centre) and enrolled patients aged 4 years or older with IgE-mediated walnut allergy. The diagnoses of walnut allergy and co-allergies to pecan, hazelnut, and cashew were made at study entry, and were based on a positive skin prick test (SPT; appendix), with or without specific serum IgE (≥ 0.35 kU_A/L) to the corresponding nut, together with a positive oral food challenge, unless an immediate (within 2 h of exposure) recent (in the past year) reaction (cutaneous, gastrointestinal, respiratory, or systemic symptoms) had been documented. Open oral food challenges were done according to international guidelines (appendix).^{22,23} Patients with uncontrolled asthma or a medical contraindication to receive adrenaline were not eligible.

Treatment assignment was based solely on the order in which patients presented at our clinic, with the first patients entering the clinical walnut oral immunotherapy treatment programme and the remaining patients being managed by observation and strict dietary exclusion (control group). Patients were enrolled until the number calculated to provide the required power was exceeded. After the observation period, patients in the control group had a second oral food challenge, and were offered walnut oral immunotherapy.

The study was approved by the institutional review board (Helsinki number 235/14). Parental or patient (in individuals aged >18 years) consent was obtained from all participants.

Procedures

The treatment began with an initial dose-escalation phase of 4 days in an ambulatory care setting (appendix), as previously described.²⁴ During this phase, we established the single highest tolerated dose of each patient, defined as the maximal amount of walnut protein each patient could tolerate. This dose of walnut

See Online for appendix

protein was consumed once per day at home for 24 days. Subsequent monthly rounds of dose escalations were done in the clinic, followed by periods of 24 days of home treatment with fixed doses of walnut protein, until the goal of 4000 mg walnut protein was achieved (appendix). Patients or their caregivers were instructed to send a daily report of their home dose consumption, including any reactions and any treatments provided, and were encouraged to report any intercurrent illnesses or asthma exacerbations via a web-based reporting system or e-mail.²⁵ Adverse reactions during in-clinic treatment were categorised on the basis of the World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System.²⁶

Patients who were unable to consume 4000 mg of walnut protein at the end of the study were considered to be allergic to walnut. After reaching the desired desensitisation goal of 4000 mg walnut protein, participants with pecan, hazelnut, or cashew co-allergy had an oral food challenge with their respective nuts (2500 mg pecan protein, equivalent to 28 g pecan; 4000 mg hazelnut protein, equivalent to 26 g hazelnut; and 4000 mg cashew protein, equivalent to 22 g cashew). On the basis of our clinical experience with peanut oral immunotherapy, patients who were desensitised to walnut were instructed to consume 1200 mg walnut protein daily.²⁴ After 6 months, we did a supervised oral food challenge with 4000 mg of walnut protein.

SPT, basophil activation test (BAT),⁷ and serum IgE and IgG4 tests were done in both groups at study entry and upon completion (appendix). Measurements of specific IgE and IgG4 antibodies to walnut and its components (Jug r 1, Jug r 3, Jug r 4, and high-molecular-weight and low-molecular-weight vicilin fractions, with vicilin fractions comprising predominantly the naturally occurring processing products of Jug r 2 and Jug r 6) and to pecan, hazelnut, and cashew were done with ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden).

Outcomes

The primary outcome was walnut desensitisation—defined as the ability to consume 4000 mg walnut protein (ie, pass an oral food challenge), equivalent to 26 g walnut, without a reaction—at the end of the study. Three secondary outcomes were evaluated: the first was co-desensitisation to pecan (2500 mg), hazelnut (4000 mg), and cashew (4000 mg) in co-allergic patients following walnut desensitisation or responsiveness to the treatment programme (defined as a ≥ 10 times increase in reaction dose or to a dose of ≥ 1000 mg protein, but less than the maximal dose given for the tested nut).²⁷ The second was changes in immunological variables (SPT, BAT, and component-resolved diagnostics) from the start to the end of the study period, and the third was the ability of patients who were desensitised to walnut to maintain desensitisation after consuming a low walnut dose of 1200 mg protein

per day (equivalent to 8 g or about three halves of walnut) for 6 months.

Changes in quality of life from start to end of treatment were compared between patients who were desensitised for all tested allergenic tree nuts (walnut, pecan, hazelnut, and cashew) and those who remained allergic to any of these nuts. Parents of children aged 4–12 years in the oral immunotherapy group completed the Food Allergy Quality of Life Questionnaire (FAQLQ)–Parental Form at the start of the study and after reaching desensitisation, as previously described.²⁸ Parents of children aged 13–17 years completed the FAQLQ–Parental Form Teenager and those aged 18 years or older self-completed the FAQLQ–Adult Form, again at the start of the study and after reaching desensitisation.

Statistical analysis

Assuming a successful desensitisation in 60% of people in the oral immunotherapy group and 10% in the control group, we calculated that 50 participants (40 receiving oral immunotherapy and ten being allocated to the observational control group) would provide 90% power with a two-sided α of 5%. All clinical outcomes were assessed by intention-to-treat analysis. Differences between the oral immunotherapy group and the control group were analysed with a Fisher's exact test for categorical variables and Mann-Whitney tests for continuous variables. A Wilcoxon signed-rank test was used to examine changes in SPT, BAT, and serum levels of antibodies in patients treated with oral immunotherapy and those in the control group between the beginning and the end of the study, and changes in FAQLQ responses in patients treated with oral immunotherapy between the beginning and the end of the study. Patients with missing answers in at least 80% of the questions in the FAQLQ were excluded in the quality-of-life analysis.

All analyses were two-tailed and a p value of less than 0.05 was considered significant. Statistical analyses were done using SPSS 20.

Role of the funding source

There was no funding source for this study. The corresponding author had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

Results

73 patients aged 4–20 years with a walnut allergy were enrolled between May 15, 2016, and Jan 14, 2018 (figure 1). The first 55 patients entered a clinical walnut oral immunotherapy treatment programme (oral immunotherapy group), and the remaining 18 patients on the waiting list were managed by observation and strict dietary exclusion (observational control group). Baseline demographics, asthma

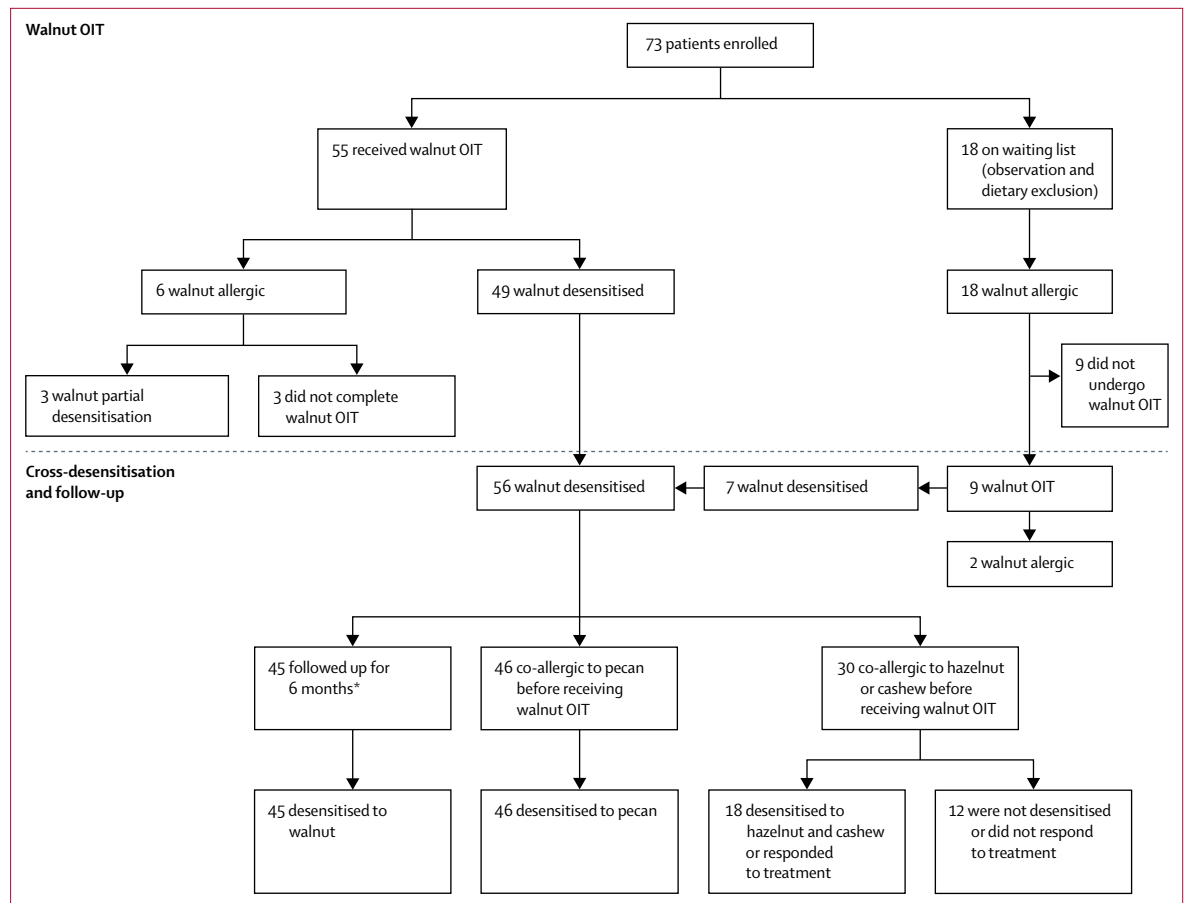


Figure 1: Study enrolment and clinical outcomes

Nine patients who completed their observation period subsequently received oral immunotherapy. Desensitisation was defined as ability to pass an oral food challenge with 4000 mg walnut protein, 2500 mg pecan protein, 4000 mg hazelnut protein, or 4000 mg cashew protein. Partial desensitisation was defined as a 10 times increase in the patient's single highest tolerated dose or a tolerated dose of at least 1000 mg but less than 4000 mg walnut protein. Treatment responsiveness was defined as at least a 10 times increase in reaction dose or a tolerated dose of at least 1000 mg but less than 4000 mg of hazelnut or cashew protein. OIT=oral immunotherapy. *During follow-up, patients consumed 1200 mg walnut protein.

comorbidity, and severity of reaction before oral immunotherapy were similar between the two groups (table 1). Baseline immunological parameters (SPT wheal size, IgE levels to walnut and its components, and BAT reactivity) were also similar between the two groups (table 1).

The results of the intention-to-treat analysis revealed that 49 (89%) of the 55 patients in the oral immunotherapy group were desensitised to 4000 mg of walnut protein during the study, compared with none of 18 patients in the control group (odds ratio [OR] 9.2, 95% CI 4.3–19.5; $p<0.0001$). Median duration of treatment in the oral immunotherapy group was 5.9 months (IQR 3.8–8.9) and median observation period in the control group was 7.1 months (3.8–9.2; $p=0.51$). In the immunotherapy group, of the six patients who did not achieve desensitisation to 4000 mg of walnut protein, three patients were partially desensitised to walnut (≥ 10 times increase in their single highest tolerated dose or desensitisation to a dose of ≥ 1000 mg but <4000 mg

walnut protein) and three patients did not complete treatment (two because of food aversion and one because of anaphylactic reactions; figure 1). The outcomes of oral immunotherapy according to the range of walnut doses that induced a reaction before oral immunotherapy are shown in table 2. Of the patients with a single highest tolerated dose of more than 10 mg walnut protein at the beginning of treatment, 42 (98%) of 43 patients were desensitised to walnut, compared with seven (58%) of 12 patients with a single highest tolerated dose of up to 10 mg walnut ($p=0.001$; appendix).

Changes in immunological variables were compared between the time of study entry and completion point in patients who were desensitised to walnut after oral immunotherapy and in patients in the observational control group. In the oral immunotherapy group, walnut SPT wheal size, allergen-induced CD63 expression in BAT, walnut serum IgE levels, and IgE:IgG4 ratio significantly decreased, whereas walnut IgG4 levels increased (figure 2). Similarly, IgE to most walnut

components and IgE:IgG4 ratio to all walnut components decreased significantly, whereas IgG4 to all walnut components increased during oral immunotherapy (appendix). By contrast, in the control group, no significant changes were noted for SPT wheal size, allergen-induced CD63 expression in BAT, serum levels of IgE and IgG4, or IgE:IgG4 ratio to walnut (figure 2) or walnut components (appendix) from study start to end.

2432 doses were given to patients in the clinic and 10605 doses were self-administered during the home treatment phase. Although 47 (85%) of 55 patients in the oral immunotherapy group had some adverse reactions during the in-hospital up-dosing phase, and 40 (73%) during the home-dosing phase, the reactions were mostly mild (grade 1 or 2) and occurred in response to only 109 (4%) of the doses in clinic and 244 (2%) of home doses (table 3). Upper respiratory and gastrointestinal manifestations were the most common. No severe reactions (defined as at least grade 3 in severity) were recorded during the study. Most reactions required treatment with antihistamines only, but 11 (20%) patients required intramuscular epinephrine during the in-hospital study phase (a single patient had a reaction that required two epinephrine injections) and eight (15%) patients administered intramuscular epinephrine during the home treatment phase. Three (5%) of the 55 patients treated with oral immunotherapy had gastrointestinal symptoms that were not temporally related to administered doses and were associated with increased eosinophil counts, compatible with oral immunotherapy-induced gastrointestinal and eosinophilic responses, as previously described.²⁹ All three patients were managed with temporary dose reduction, as previously described,²⁹ with resolution of symptoms and eosinophilia, and were subsequently desensitised to walnut without additional symptoms.

Patients in the control group had a second walnut oral food challenge after the observation period, and all 18 patients remained allergic to walnut. Nine (50%) began walnut oral immunotherapy, and seven (78%) were desensitised to 4000 mg walnut protein. Thus, 64 patients (55 in the initial oral immunotherapy group and nine in the control group who crossed over after the observation period) received walnut oral immunotherapy; of those 64 patients, 56 (88%) were successfully desensitised to walnut, and were included in the follow-up and cross-desensitisation tests (figure 1).

Of the 56 patients who achieved walnut desensitisation, 46 (82%) were co-allergic to pecan (as established in 41 patients on the basis of a positive oral food challenge and in five patients on the basis of a recent reaction to pecan). The median pecan reaction dose before walnut oral immunotherapy was 540 mg (IQR 125–1250 mg). Following walnut oral immunotherapy, all 46 patients, regardless of reaction dose before oral immunotherapy, tolerated 2500 mg of pecan protein on oral food challenge (figure 1; table 4). On comparison between the start of

	Oral immunotherapy (n=55)	Control (n=18)	p value
Demographics			
Age (years)	7.9 (6.0–9.7)	6.8 (5.7–11.4)	0.88
Sex			0.24
Male	36 (65%)	15 (83%)	..
Female	19 (35%)	3 (17%)	..
Other food allergies (not tree nuts)	15 (27%)	7 (39%)	0.39
Pecan allergy	45 (82%)	16 (89%)	0.72
Cashew or hazelnut allergy	29 (53%)	12 (67%)	0.41
Atopic dermatitis	29 (53%)	10 (56%)	1.0
House dust mite sensitivity	37 (67%)	14 (77%)	0.71
Asthma	28 (51%)	13 (72%)	0.17
Previous reaction severity			
Reaction to walnut	38 (69%)	10 (56%)	0.39
Anaphylaxis	26 (47%)	7 (39%)	0.59
Epinephrine use	20 (36%)	3 (17%)	0.15
Emergency room visit for allergy reaction	24 (44%)	4 (22%)	0.16
Previous hospital admission for allergy reaction	2 (4%)	0	1.0
Walnut reaction dose (mg)	210 (50–465)	100 (35–420)	0.71
Immunological variables			
Walnut skin prick test (mm)	9 (7–11)	10 (6–14)	0.31
Walnut-induced CD63 (%)	32.2% (8.6–61.8)	54.3% (16.6–72.3)	0.21
Walnut IgE (kU _A /L)*	6.4 (2.5–19.8)	6.3 (1.9–58.9)	0.52
rJug r 1 IgE (kU _A /L)*	3.6 (0.5–9.4)	2.0 (0.4–25.2)	0.75
rJug r 3 IgE (kU _A /L)*†	<0.10 (<0.10–0.14)	0.10 (<0.10–0.19)	0.15
nJug r 4 IgE (kU _A /L)*	0.5 (0.1–3.7)	0.9 (0.5–6.6)	0.21
Low-molecular weight vicilin IgE (kU _A /L)*†	1.6 (<0.10–4.0)	1.5 (0.2–22.0)	0.39
High-molecular-weight vicilin IgE (kU _A /L)*†	0.7 (<0.10–4.7)	1.3 (0.4–9.0)	0.34

Continuous variables are presented with medians (IQR), discrete variables as n (%). *n=42 for the OIT group and n=18 for the control group. †0.10 kU_A/L is the detection ability threshold, therefore the exact value is not reported if <0.10 kU_A/L.

Table 1: Baseline demographic and immunological characteristics

	Patients (n=55)	Desensitisation* (n=49)
Recent anaphylaxis†	1 (2%)	1 (100%)
<20 mg	5 (9%)	4 (80%)
20–99 mg	17 (31%)	13 (76%)
100–249 mg	14 (25%)	13 (93%)
250–499 mg	5 (9%)	5 (100%)
500–1249 mg	6 (11%)	6 (100%)
≥1250 mg	7 (13%)	7 (100%)

*Able to consume 4000 mg walnut protein after oral immunotherapy.
†Immediate (within 2 h of exposure) reaction (cutaneous, gastrointestinal, respiratory, or systemic symptoms) to walnut in the past year.

Table 2: Proportion of patients who achieved desensitisation to walnut, by reaction doses before oral immunotherapy

walnut oral immunotherapy to the end, significant reductions were noted in pecan SPT, pecan IgE, and pecan IgE:IgG4 ratio and induced CD63 expression on

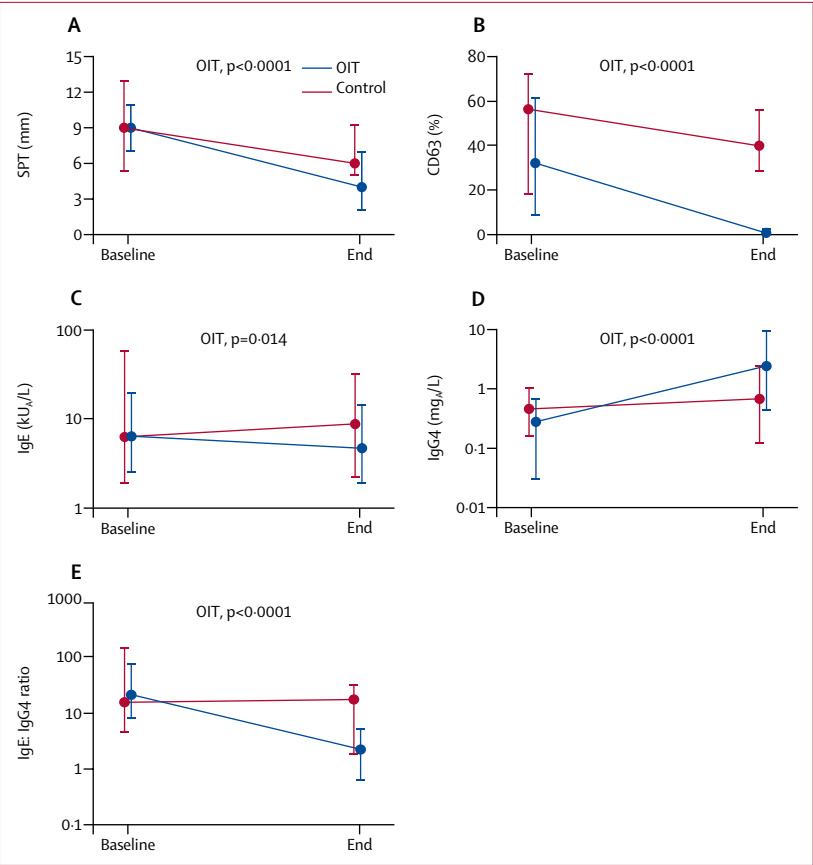


Figure 2: Changes in immune variables during oral immunotherapy versus control observation
Medians (IQR) of walnut SPT (A), basophil activation (CD63 expression; B), IgE (C), IgG4 (D), and IgE:IgG4 ratio (E) in patients desensitised to walnut after oral immunotherapy versus controls from study start to end date. Data are available for SPT in 45 patients, BAT in 44 patients, and IgE, IgG4, and IgE:IgG4 ratio in 42 patients in the oral immunotherapy group, and for SPT in 12 patients, BAT in 16 patients, IgE in 18 patients, and IgG4 and IgE:IgG4 ratio in 17 patients in the control group. p values for the difference between baseline and study end are shown for the OIT group only; none of the differences was significant in the control group. BAT=basophil activation test. OIT=oral immunotherapy. SPT=skin prick test.

BAT, whereas pecan IgG4 concentrations significantly increased (figure 3).
Of the 56 patients who desensitised to walnut, 30 (54%) patients had a co-allergy to either hazelnut ($n=15$) or cashew ($n=19$; four patients were allergic to both). Before walnut oral immunotherapy, their median reaction doses were 120 mg (IQR 20–540) for hazelnut and 100 mg (20–388) for cashew. After successful completion of walnut oral immunotherapy, 18 (60%) of 30 patients were either desensitised ($n=12$; able to consume a dose of 4000 mg of the corresponding hazelnut or cashew nut protein) or responded to treatment ($n=6$; figure 1, table 4; appendix). Cross-desensitisation was observed primarily for hazelnut, with eight (53%) of 15 patients being desensitised and 14 (93%) of 15 patients being either desensitised or responding to treatment. Pre-oral immunotherapy hazelnut reaction dose was similar between patients who were desensitised to hazelnut following walnut oral immunotherapy (median 150 mg,

	Patients (n=55)	Doses
Hospital (2432 doses)		
Total reactions	47 (85%)	109 (4%)
Perioral	5 (9%)	5 (<1%)
Skin	21 (38%)	33 (1%)
Upper respiratory	29 (53%)	43 (2%)
Lower respiratory	8 (15%)	11 (1%)
Eyes	15 (27%)	20 (1%)
Gastrointestinal	26 (47%)	42 (2%)
Cardiovascular system	0	0
Upper airway block	0	0
Non-responding asthma	0	0
Grade 1	35 (64%)	63 (3%)
Grade 2	27 (49%)	46 (2%)
Antihistamine use	39 (71%)	76 (3%)
Bronchodilator use	10 (18%)	14 (1%)
Epinephrine use	11 (20%)	12 (<1%)
Home (10 605 doses)		
Total reactions	40 (73%)	244 (2%)
Single reaction	10 (18%)	NA
Two to four reactions	18 (33%)	NA
A least five reactions	12 (22%)	NA
Epinephrine use	8 (15%)	9 (<1%)
6 month follow-up*		
Total reactions	11 (24%)	23 (<1%)
Epinephrine	1 (2%)	1 (<1%)

NA=not applicable. *Available for 45 patients, one dose per day.

Table 3: Adverse events during oral immunotherapy

IQR 95–2000) and between those who remained hazelnut allergic (median 100 mg, 20–480; $p=0.37$). Given that patients in the control group did not show a significant reduction in sensitisation to cross-reactive nuts and given their persistent walnut allergy, oral food challenges to cross-reacting nuts were not repeated in this group. We then examined immunological changes in the 34 people with co-allergy to either hazelnut ($n=15$) or cashew ($n=19$). IgE:IgG4 ratio and BAT decreased significantly ($p=0.001$) and IgG4 increased significantly ($p=0.001$) to the co-allergenic nut (hazelnut or cashew) after walnut oral immunotherapy ($n=34$), whereas SPT did not change significantly (appendix). IgE also decreased significantly ($p=0.019$).
After completing walnut oral immunotherapy, 36 (64%) of 56 patients were desensitised to all tested tree nuts (walnut only, $n=9$; walnut and pecan, $n=18$; and walnut, pecan, and hazelnut or cashew, $n=9$) and 20 (36%) of 56 patients remained allergic to at least one of these nuts. Quality-of-life questionnaires, completed at the start and end of the study, were available from 52 patients (34 who were desensitised to all tested nuts and 18 who remained allergic). Patients who were desensitised to all tested nuts had a clinically significant improvement (ie, a difference of >0.5) in emotional impact (from 4.0 to 2.1; $p=0.001$), food

	Pecan (n=46)		Hazelnut (n=15)		Cashew (n=19)	
	Patients at baseline	Patients desensitised*	Patients at baseline	Patients desensitised* or responding to treatment†	Patients at baseline	Patients desensitised* or responding to treatment†
Anaphylaxis‡	5 (11%)	5 (100%)	2 (13%)	2 (100%)	6 (32%)	0
<20 mg	4 (9%)	4 (100%)	1 (7%)	1 (100%)	2 (10%)	0
20–99 mg	4 (9%)	4 (100%)	3 (20%)	3 (100%)	4 (21%)	2 (50%)
100–249 mg	5 (11%)	5 (100%)	4 (27%)	4 (100%)	3 (16%)	1 (33%)
250–499 mg	7 (15%)	7 (100%)	2 (13%)	1 (50%)	2 (10%)	1 (50%)
500–1249 mg	12 (26%)	12 (100%)	1 (7%)	1 (100%)	0	NA
≥1250 mg	9 (20%)	9 (100%)	2 (13%)	2 (100%)	2 (10%)	1 (50%)

NA=not applicable. *Desensitisation was defined as the ability to consume 2500 mg of pecan, 4000 mg of cashew, or 4000 mg of hazelnut protein. †Response to treatment was defined as at least a 10 times increase in reaction dose or a tolerated dose of at least 1000 mg, but which was less than the maximal dose of the corresponding nut. ‡Recent anaphylaxis was an immediate (within 2 h of exposure) reaction (cutaneous, gastrointestinal, respiratory, or systemic symptoms) to pecan, hazelnut, or cashew in the past year.

Table 4: Baseline pecan, hazelnut, and cashew reaction dose distributions and cross-desensitisation after walnut oral immunotherapy

anxiety (from 4.0 to 2.0; $p=0.001$), and social and dietary limitation (from 3.0 to 1.0; $p<0.0001$) subscales, as well as improvement in the total score (from 3.3 to 2.0; $p<0.0001$) of the FAQLQ following treatment. By contrast, in patients who remained allergic to at least one of the nuts, a statistically (but not clinically) significant improvement was noted only for the social and dietary limitation subscale (from 3.0 to 2.6; $p=0.034$), but not for emotional impact (from 3.6 to 3.1; $p=0.28$), food anxiety (from 4.6 to 3.9; $p=0.11$), or total score (from 3.7 to 3.0; $p=0.13$; appendix).

Of the 56 patients who were desensitised to walnut, 45 (80%) were followed up for at least 6 months after the end of treatment, and were consuming a daily dose of 1200 mg walnut protein (figure 1). 11 (24%) of 45 patients described mild allergic reactions (local rash, sneezing, and mild abdominal pain) that were treated with antihistamines only during the maintenance period (table 3). One patient required intramuscular epinephrine during that time, but continued treatment. After 6 months, a supervised oral food challenge consisting of a single dose of 4000 mg walnut protein was done. All 45 patients successfully consumed 4000 mg walnut protein without adverse reactions. There were no further reports of adverse reactions during pecan or hazelnut consumption among participants who were cross-desensitised to these nuts during the maintenance period.

Discussion

Our study shows that walnut oral immunotherapy is effective (89%) in desensitising patients with walnut allergy and is associated with the development of markers associated with immunological tolerance. In addition, all patients who had co-allergy to pecan before walnut oral immunotherapy were also desensitised to pecan following walnut desensitisation. Pecan desensitisation was associated with pecan-specific markers of immune tolerance. Most patients (60%) who were desensitised to walnut and who had a co-allergy to hazelnut or cashew at baseline were either desensitised or responded to walnut

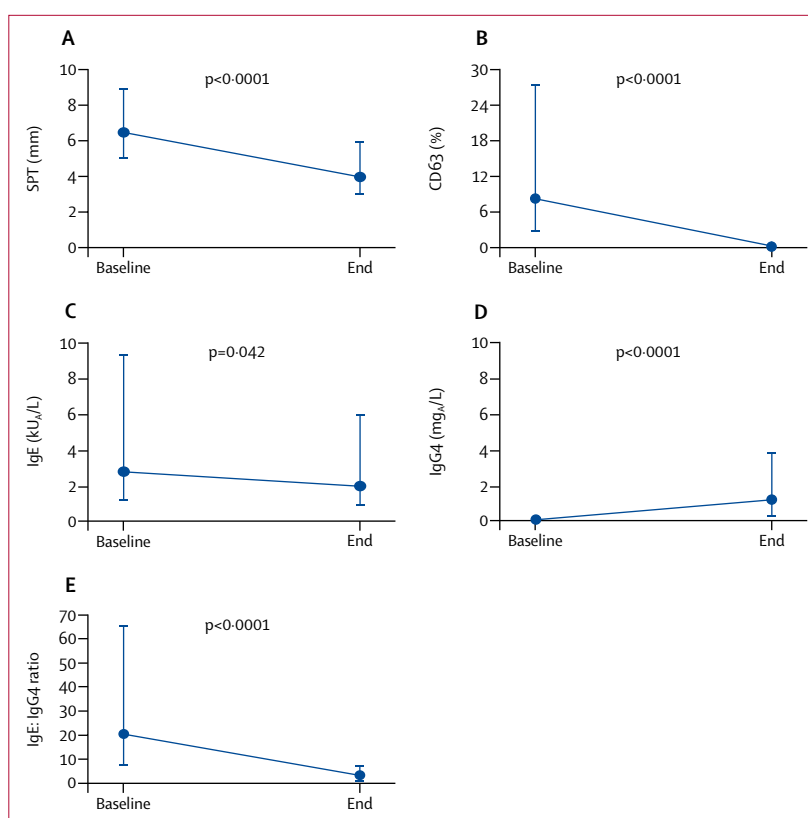


Figure 3: Immunological correlates of pecan allergy before and after walnut oral immunotherapy
Medians (IQR) of pecan SPT (A), basophil activation (CD63 expression; B), IgE (C), IgG4 (D), and IgE:IgG4 ratio (E) before and after walnut oral immunotherapy. Data for SPT and BAT are available for 42 patients and data for IgE, IgG4, and IgE:IgG4 ratio for 38 patients. BAT=basophil activation test. SPT=skin prick test.

oral immunotherapy. This effect was primarily evident for hazelnut, occurring in 14 (93%) of 15 patients. Patients who were desensitised to all tested nuts had a considerable improvement in quality of life compared with those who remained allergic. To our knowledge, this is the first controlled study to show the efficacy of single-nut oral

immunotherapy in desensitising patients to both the treated nut and to additional, botanically related tree nuts.

Oral immunotherapy has gained considerable interest as an alternative treatment to elimination diet in patients who are allergic to specific foods. Efficacy has been shown for the treatment of milk, egg, and peanut allergy, with desensitisation ranging from 55% to 90%.³⁰ However, no study to date has investigated oral immunotherapy in patients who are allergic to walnut. This study, which included individuals at high risk, such as patients with asthma and those with a very low initial tolerated dose (as low as 1 mg of walnut protein), suggests that walnut oral immunotherapy is effective, with 89% of the patients becoming desensitised to 4000 mg by the end of the study.

One of the main obstacles in managing tree nut allergy is the cross-reactivity between different tree nuts.^{21,31} Patients with tree nut allergy are often required to eliminate a wide range of foods, and even nuts to which they are not allergic are often avoided because of anxiety.³² Sequential oral immunotherapy treatments for individual nuts in a patient who is allergic to several tree nuts is time and resource consuming. Simultaneous oral immunotherapy for several nuts was successful in patients allergic to walnuts only when done in conjunction with omalizumab treatment.¹⁹ This result was probably due to an increase in adverse events during multiple food oral immunotherapy, which could be reduced with omalizumab.¹⁹ In our previous study,⁷ we found that all patients allergic to pecans were also allergic to walnuts (ie, no patient who was allergic only to pecans was identified). This finding suggested that walnut desensitisation might enable desensitisation to pecan as well. Indeed, among patients desensitised to walnut, all 46 patients who also had a pecan allergy were desensitised to pecan. In the case of hazelnut and cashew, we previously found that almost 80% of patients allergic to hazelnut and 50% of those allergic to cashew were co-allergic to walnut, suggesting a more complex interaction between these nuts. The effect of walnut oral immunotherapy on hazelnut and cashew allergy was noteworthy, with 60% of patients becoming either cross-desensitised or responding to treatment. The main effect, occurring in 93% of patients, appears to be for hazelnut, although this preliminary result requires further study. This finding emphasises that with single-nut oral immunotherapy, two to three tree nut allergies can be treated, making walnut oral immunotherapy an attractive treatment even for patients with allergies to several nuts. Reaction dose before oral immunotherapy did not seem to predict the likelihood for desensitisation for hazelnut or cashew following walnut oral immunotherapy, but the numbers in our study are too small to draw firm conclusions. Cross-desensitisation also seems to have had a substantial effect on patient quality of life, although this part of the analysis was underpowered and should therefore be studied in a larger group of patients.

Oral immunotherapy is typically associated with immunological shifts from allergy to tolerance. These changes include a decrease in mast cell sensitivity as reflected in reduced wheal size on SPT, a reduction in basophil reactivity, and an increase in food-specific IgG4.^{10–14} Similar changes were seen in the patients of the present study after successful walnut desensitisation, both for walnut (as established by SPT, IgE and IgG4 levels and ratio, and basophil activation) and its components (as established by IgE and IgG4 levels and ratio). Immunological changes for additional non-treated allergenic foods following single-food oral immunotherapy have not been previously shown.¹⁷ We show for the first time an immunological shift from allergy to a state of desensitisation in a food that is different from the one treated. A reduction in SPT, IgE, IgE:IgG4 ratio, and basophil reactivity, and an increase in IgG4 for pecan in patients desensitised to walnuts was noted. These changes paralleled the clinical cross-desensitisation that was observed in all patients who were co-allergic to pecan following walnut desensitisation. The immunological changes in patients allergic to hazelnut and cashew following walnut desensitisation were substantial for BAT, IgG4, IgE, and IgE:IgG4 ratio, but not for SPT, probably reflecting the less uniform clinical effect of these nuts than with pecan. Further studies, perhaps using component-resolved diagnostics for hazelnut and cashew, are required to identify which patients who are co-allergic to hazelnut or cashew would benefit from walnut oral immunotherapy.

One important, yet unanswered question in the treatment of food allergy is the identification of markers that would distinguish desensitisation (requiring maintenance consumption) from tolerance.^{10,11,33,34} In the absence of such markers, prolonged consumption of the allergenic food will probably be required. We previously found that 1200 mg peanut protein consumption is effective in maintaining peanut desensitisation and is well tolerated by patients.²⁴ In our study, we show that daily consumption of 1200 mg walnut protein (equivalent to 1.5 walnuts) is well tolerated by patients and maintains walnut (as well as pecan and additional nuts) de-sensitisation. This low maintenance dose is likely to improve both long-term compliance in patients following oral immunotherapy and their quality of life.

This study is limited by the absence of randomisation or masking. However, the objective assessment of patients with oral food challenges at the beginning and the end of the study, together with the associated immunological changes, provide strong support for our findings. Another limitation is the absence of repeat oral food challenges for cross-reactive nuts in the control group. However, given the persistence of oral food challenge-proven walnut allergy, the short interval of observation (median 7.1 months, IQR 3.8–9.2), the absence of significant reduction in sensitisation to the cross-allergenic nuts, and the known low proportion (<10%) of patients who outgrow

tree-nut allergy, we found that re-challenging these patients for each cross-reacting nut was unethical.

In terms of the safety of walnut oral immunotherapy, adverse events were not infrequent and 15% of patients required injectable epinephrine for home dosing. This finding partly reflects our encouragement to patients to liberally use epinephrine at home for reactions whenever in doubt. Despite a similar proportion of patients with adverse events, the proportion of patients who did not complete oral immunotherapy in our study was low compared with previous studies.^{10,12–13,35} Furthermore, only one patient of those who had reactions requiring epinephrine discontinued treatment, which might reflect the ongoing communication (via a web reporting system) we maintain with our patients during the more challenging home treatment phase. The fact that adverse reactions during the 6 months of follow-up were rare and mostly mild is reassuring.

In summary, to our knowledge, this study is the first to show not only the efficacy of walnut oral immunotherapy in desensitising patients who are allergic to walnut, but also its efficacy in cross-desensitising to additional nuts, primarily pecan and hazelnut. In addition, a low daily walnut dose is sufficient to maintain desensitisation to these nuts. Our findings suggest that walnut oral immunotherapy might be an attractive therapeutic option, even for patients who are allergic to additional nuts. Whether the cross-desensitisation shown here is limited to nuts derived from the same botanical order or whether a more general desensitised state is induced in some patients requires further investigation.

Contributors

AE contributed to the conception and design of the study, acquisition of data, data analysis and interpretation, and drafting of the manuscript. MYA contributed to the performance and analysis of the basophil activation tests and drafting of the manuscript. LN, MBL, and NER contributed to the acquisition and interpretation of the data and to the drafting of the manuscript. BP and JL contributed to the performance of IgE and IgG4 measurements and to the drafting of the manuscript. MRG contributed to the design of the study, preparation of the skin prick test extract, interpretation of the data, and drafting of the manuscript.

Declaration of interests

MRG is funded by a Kamea grant from the Ministry of Health, Israel. BP and JL are employees of Thermo Fisher Scientific. All other authors declare no competing interests.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Elizur A, Appel MY, Nachshon L, et al. Walnut oral immunotherapy for desensitisation of walnut and additional tree nut allergies (Nut CRACKER): a single-centre, prospective cohort study. *Lancet Child Adolesc Health* 2019; published online March 26. [http://dx.doi.org/10.1016/S2352-4642\(19\)30029-X](http://dx.doi.org/10.1016/S2352-4642(19)30029-X).

SUPPLEMENTARY FILES

Supplementary materials and methods

Supplementary tables – 3

Supplementary figures - 6

SUPPLEMENTARY MATERIALS AND METHODS

Standardized skin prick tests

Tree-nut flour (walnut, Wellbees, Monsey, NY and hazelnut, Holmquist Hazelnuts, Lynden, Washington) or tree-nuts (pecan) were finely ground in a coffee grinder until a homogenous paste was generated. A different grinder was dedicated for the preparation of SPT material for each tree-nut. This was then diluted to 10 mg/ml with mineral water pH 7.9 and frozen at -20 °C. Fresh aliquots were thawed weekly and stored at 4 °C for daily use. SPTs were performed on the volar surface of the forearm after application of the respective tree-nut solutions with single peak lancets (Heinz Herenz, Hamburg, Germany), as previously described (7). Mean wheal diameter was measured after 15 minutes. Histamine 1 mg/mL (ALK-Abello) and saline were used as positive and negative controls, respectively. A positive SPT was defined as a mean wheal diameter ≥ 3 mm greater than the negative control.

Oral food challenges

Open OFCs were performed according to international guidelines (22, 23). The challenge was terminated if objective cutaneous (urticarial rash), respiratory (rhinitis, cough or wheeze) or gastrointestinal (persistent abdominal pain or vomiting) symptoms occurred within 2 hours of exposure. If no reaction occurred for at least 2 hours following the final dose, the OFC was deemed negative.

Walnut dose preparation

Walnut doses up to 120 mg were prepared from walnut flour (Wellbees, Monsey, NY). Walnut flour was finely ground in a coffee grinder until a homogenous paste was generated. A total of 65.65 grams of flour was ground and placed in a total of 400 ml of mineral water pH 7.9 to yield

a walnut protein composition of 25 mg/ml. This was then used or frozen in aliquots at -20⁰ C for future use. Walnut doses of ≥ 150 mg protein were given as regular walnuts.

Walnut OIT Protocol

Anticipatory home treatment guidance to patients and parents was given. Each patient received antihistamines, bronchodilators and two up-to-date Epi-Pen® with instructions to administer at any severe reaction (severe abdominal pain, dyspnea, or apathy). A staff physician was on call 24/7 for consultation and patients were instructed to send a daily report of their home dose consumption, including any experienced reactions and any treatments provided, via e-mail or a web-based reporting system (26). Patients were instructed to take the home dose under parental supervision for 1.5 hours, to avoid physical activity 30 minutes before and 2 hours after the dose, and to not take the dose on an empty stomach. A 30% reduction in the daily dose and antihistamine for 5 days was recommended if mild reactions occurred and/or during viral infections. Dose reduction by 30% until the next up-dosing in clinic was performed in cases of repeated or severe reactions requiring adrenaline. Home treatment periods were followed by monthly cycles in clinic (lasting between 1-4 days) with up to 4-fold dose escalations in the first two rounds, 3-fold in the third round and 2-fold in the 4th and fifth round. Additional monthly 50% increases were performed under observation until the goal of 4000 mg walnut protein was achieved.

Basophil Activation Test

The basophil activation test was performed as previously described (NUTCRACKER study) (7). To generate solubilized extract for stimulation, tree nut mixtures (from the same stock as used for SPT) were centrifuged at 14,000 RCF for 15 minutes. The supernatants were collected and their protein concentration measured (DC Protein Assay, Bio-Rad, Berkely, CA). 1 mg/ml stock

tubes were prepared and stored at -20°C. Whole blood was collected in EDTA-containing tubes and processed within four hours. Whole blood was stimulated for 18 minutes at 37 °C with 1ng/ml recombinant human IL3 (R&D Systems, Minneapolis, MN) with or without each of the tree nut protein soluble extracts (1µg/ml) diluted in RPMI 1640 (Biological Industries, Beit HaEmek, Israel). Stimulation with 1 µg/ml polyclonal rabbit anti human IgE (Bethyl Laboratories, Montgomery, TX) was used as positive controls. Cells were incubated with fluorescent antibodies (Fluorescein isothiocyanate -anti human CD123 (clone 6H6), R-**Phycoerythrin**-Cy7 anti human HLA-DR (clone LN3), R-**Phycoerythrin** anti human CD203c (clone NP4D6) (All from eBioscience) and Allophycocyanin anti human CD63 (clone H5C6) (BioLegend, San Diego, CA)) during the stimulation, and then PBS/EDTA added for an additional 10 minutes on ice. Red blood cells were subsequently lysed at room temperature for 15 minutes (eBioscience RBC lysis buffer). Cells were centrifuged (500 G, 4 °C, 8 minutes), and were re-suspended in phosphate buffered saline (PBS) with 1% bovine serum albumin and 2 mM EDTA. Cells were acquired on a Beckman FC-500 or Navios flow cytometer. Basophils were identified as CD123+, HLA-DR- cells with approximately 500 basophils counted per sample. Analysis was performed with Flowing Software 2.5.1 (Turku Centre for Biotechnology, Finland).

eTable 1: First week walnut oral immunotherapy protocol

	Dose	Amount (mg walnut protein)	Interval (min)
Day 1	1	0.1	0
	2	0.5	10
	3	1.0	10
	4	2.5	20
	5	5	30
	6	10	30
	7	20	30
	8	40	30
	--	Observation	90
Day 2	1	40	0
	2	60	30
	3	80	30
	4	120	30
	5	150	30
	6	225	30
	7	300	30
	--	Observation	90
Day 3	1	A dose below the SHTD	0
	2	SHTD	90
	3	SHTD	90
	--	Observation	90
Day 4	1	SHTD	0
	2	SHTD	90
	--	Observation	90

^a If no reaction occurred on day 1 the protocol continued to day 2. Once a reaction occurred, the following days were used to determine the maximal dose that could be safely consumed by the patient. Patients with a reaction dose >300 mg walnut protein began treatment with a dose of 300 mg. SHTD, Single highest tolerated dose

eTable 2: Hazelnut reaction dose before and after walnut OIT

Patient	Hazelnut reaction dose (mg)		
	Pre-walnut OIT	Post walnut OIT	Desensitized or treatment responders *
1	Recent anaphylaxis **	Passed OFC	Yes
2	Recent anaphylaxis	Passed OFC	Yes
3	12	4000	Yes
4	20	1800	Yes
5	20	4000	Yes
6	20	Passed OFC	Yes
7	100	4000	Yes
8	120	Passed OFC	Yes
9	120	Passed OFC	Yes
10	180	Passed OFC	Yes
11	480	1800	Yes
12	480	600	No
13	600	1800	Yes
14	2000	Passed OFC	Yes
15	2000	Passed OFC	Yes

* Treatment responders refers to a significant increase (≥ 10 fold) in RD or an increased RD to ≥ 1000 mg but < 4000 mg hazelnut protein. Desensitized patients were able to consume 4000 mg hazelnut protein

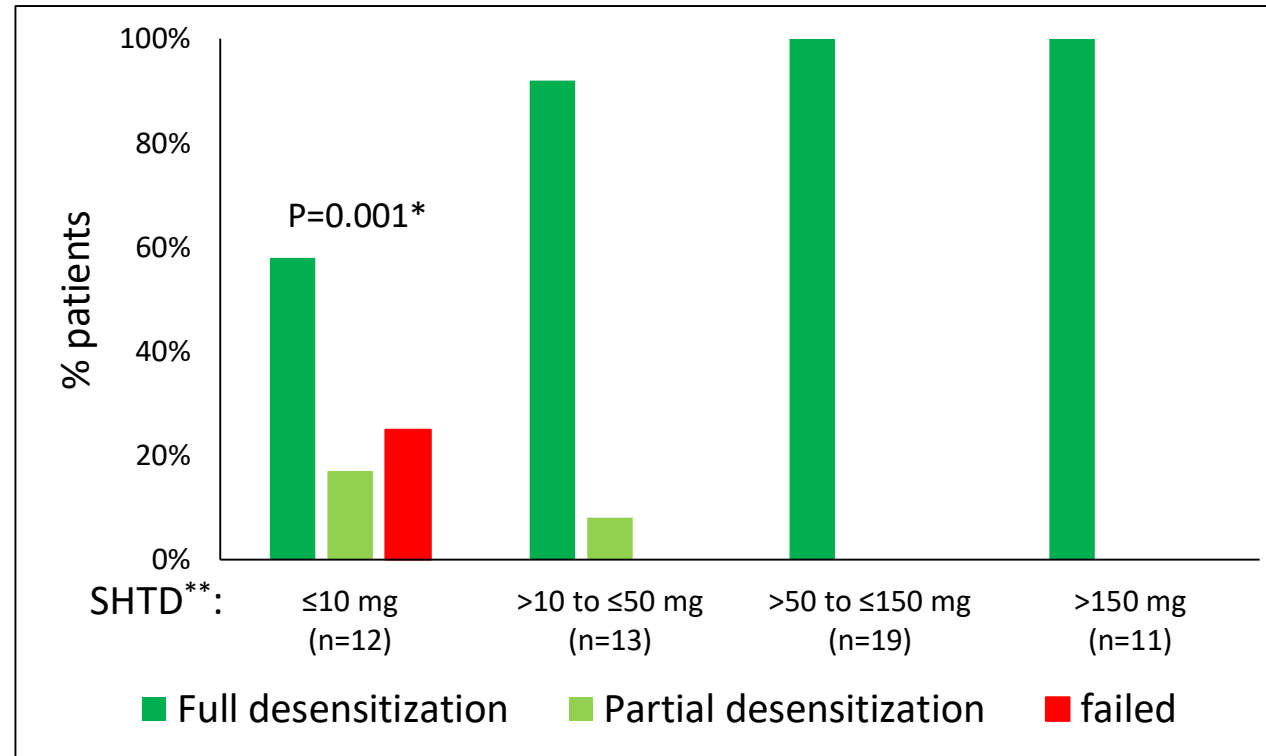
** Recent anaphylaxis – An immediate (within 2 hours of exposure) reaction (cutaneous, gastrointestinal, respiratory and/or systemic symptoms) to hazelnut in the past year

eTable 3: Cashew reaction dose before and after walnut OIT

Patient	Cashew reaction dose (mg)		
	Pre-walnut OIT	Post walnut OIT	Desensitized or treatment responders*
1	Recent anaphylaxis**	Recent anaphylaxis	No
2	Recent anaphylaxis	Refused	No
3	Recent anaphylaxis	20	No
4	Recent anaphylaxis	60	No
5	Recent anaphylaxis	60	No
6	Recent anaphylaxis	240	No
7	5	Refused	No
8	9	20	No
9	20	Passed OFC	Yes
10	20	60	No
11	50	100	No
12	50	720	Yes
13	100	720	No
14	100	Passed OFC	Yes
15	145	145	No
16	290	60	No
17	486	Passed OFC	Yes
18	4000	4000	No
19	4000	Passed OFC	Yes

* Treatment responders refers to patients with a significant increase (≥ 10 fold) in RD or an increased RD to ≥ 1000 mg but < 4000 mg cashew protein. Desensitized were patients able to consume 4000 mg cashew protein

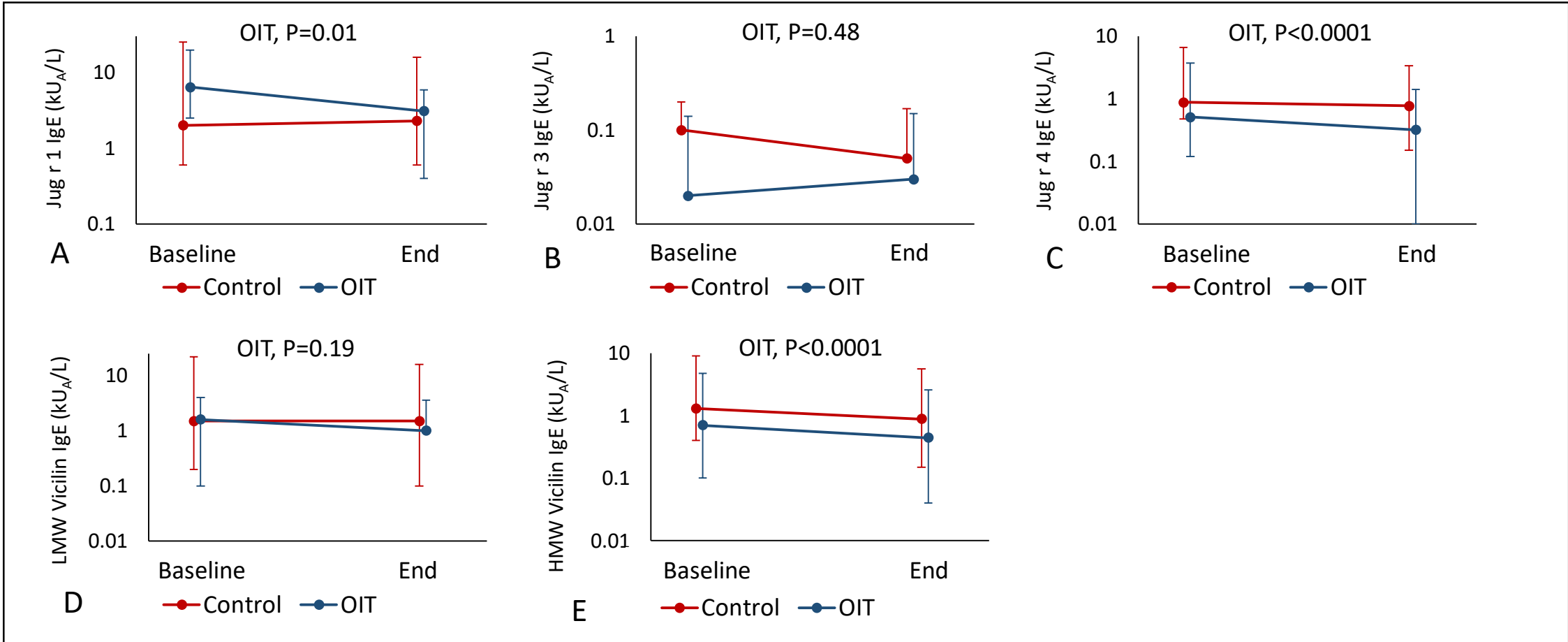
** Recent anaphylaxis – An immediate (within 2 hours of exposure) reaction (cutaneous, gastrointestinal, respiratory and/or systemic symptoms) to walnut in the past year



eFigure 1: Walnut treatment outcome according to the single highest tolerated dose. Treatment outcome (Desensitized = reaching 4000 mg walnut protein; partially desensitized = ≥ 10 -fold increase in the SHTD or an increased SHTD to ≥ 1000 mg but < 4000 mg walnut protein) at the end of the study is shown for groups categorized according to the SHTD at initial dose-escalation week.

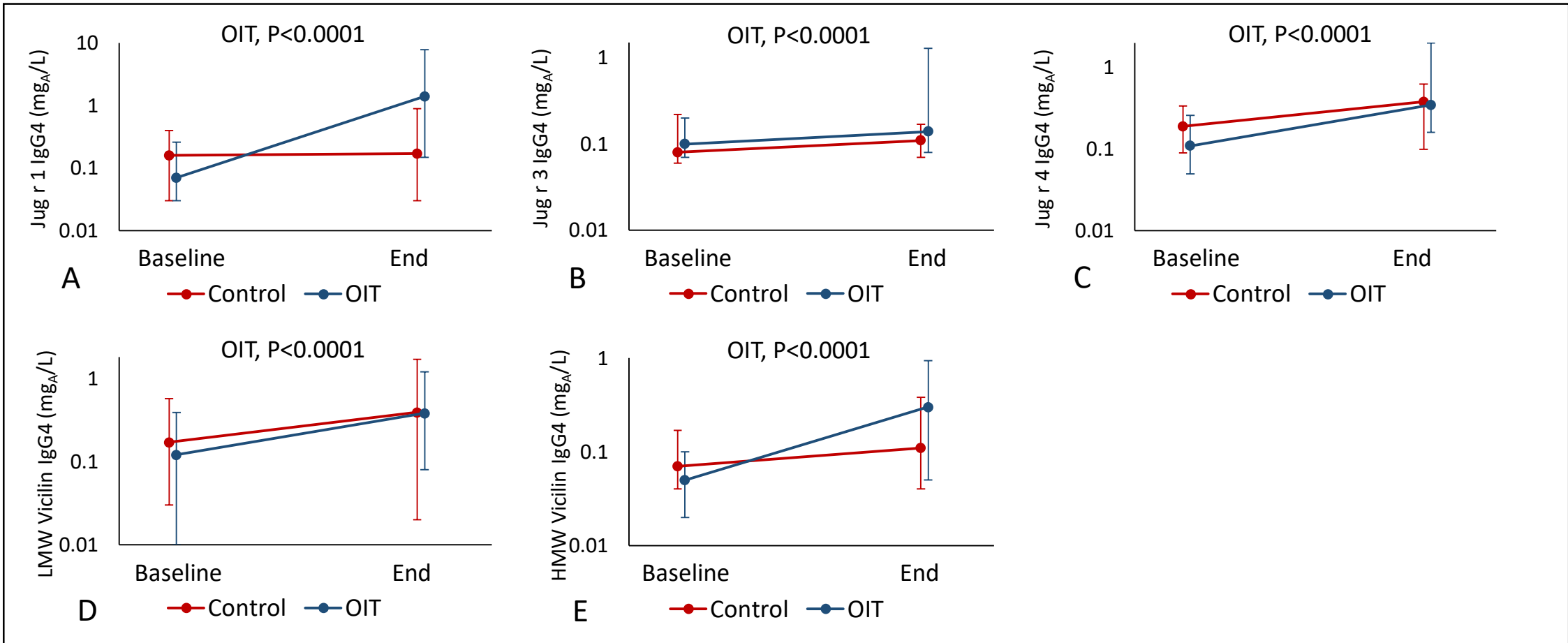
*Difference in desensitization rate between patients with SHTD ≤ 10 mg compared to those with SHTD > 10 mg at the beginning of treatment.

**SHTD – Single highest tolerated dose.



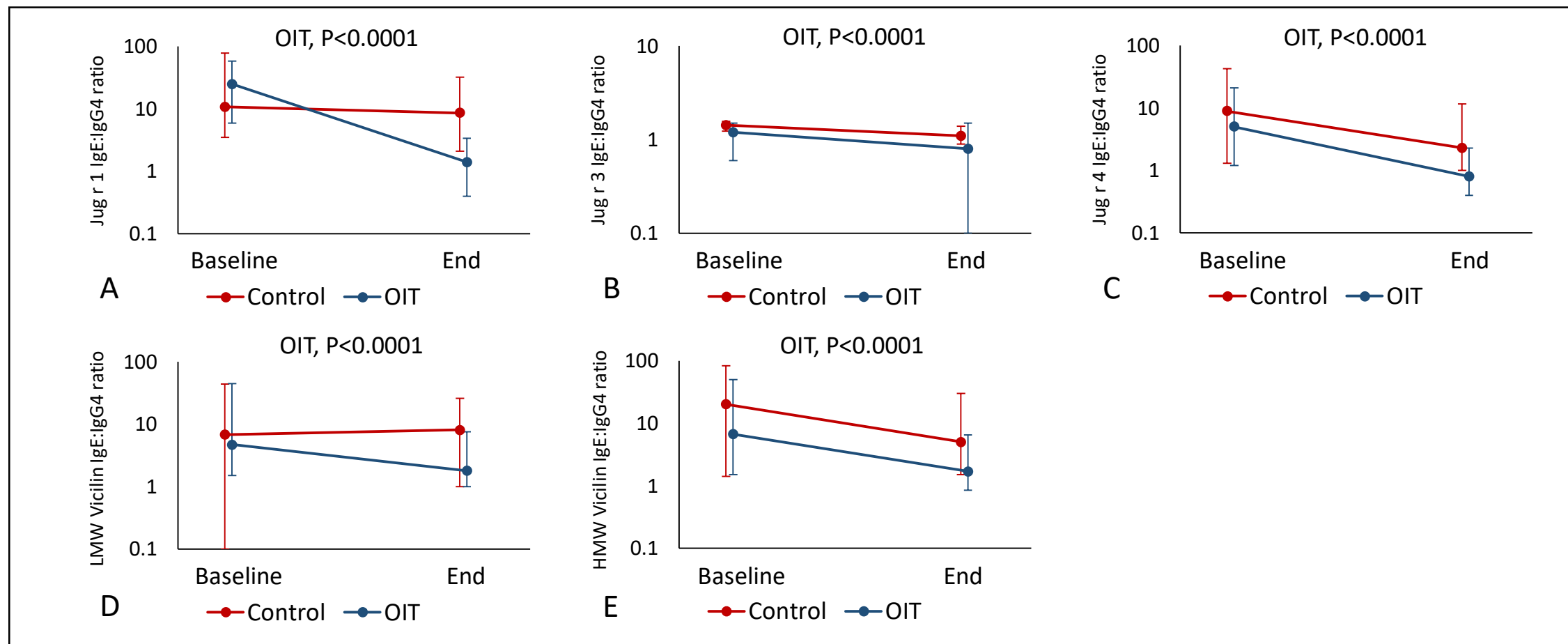
eFigure 2: IgE responses to walnut components in OIT patients versus controls

Changes in median (IQR) serum levels of IgE (kU_A/L) to walnut components (rJug r 1 (A), rJug r 3 (B), nJug r 4 (C), LMW vicilin (D), and HMW vicilin(E)) in OIT-treated walnut desensitized patients versus controls from study start to end. Data are available for 42 patients in the OIT group and in 18 control patients. p values for the difference between baseline and study end are shown for the OIT group only; none of the differences was significant in the control group. OIT=oral immunotherapy.



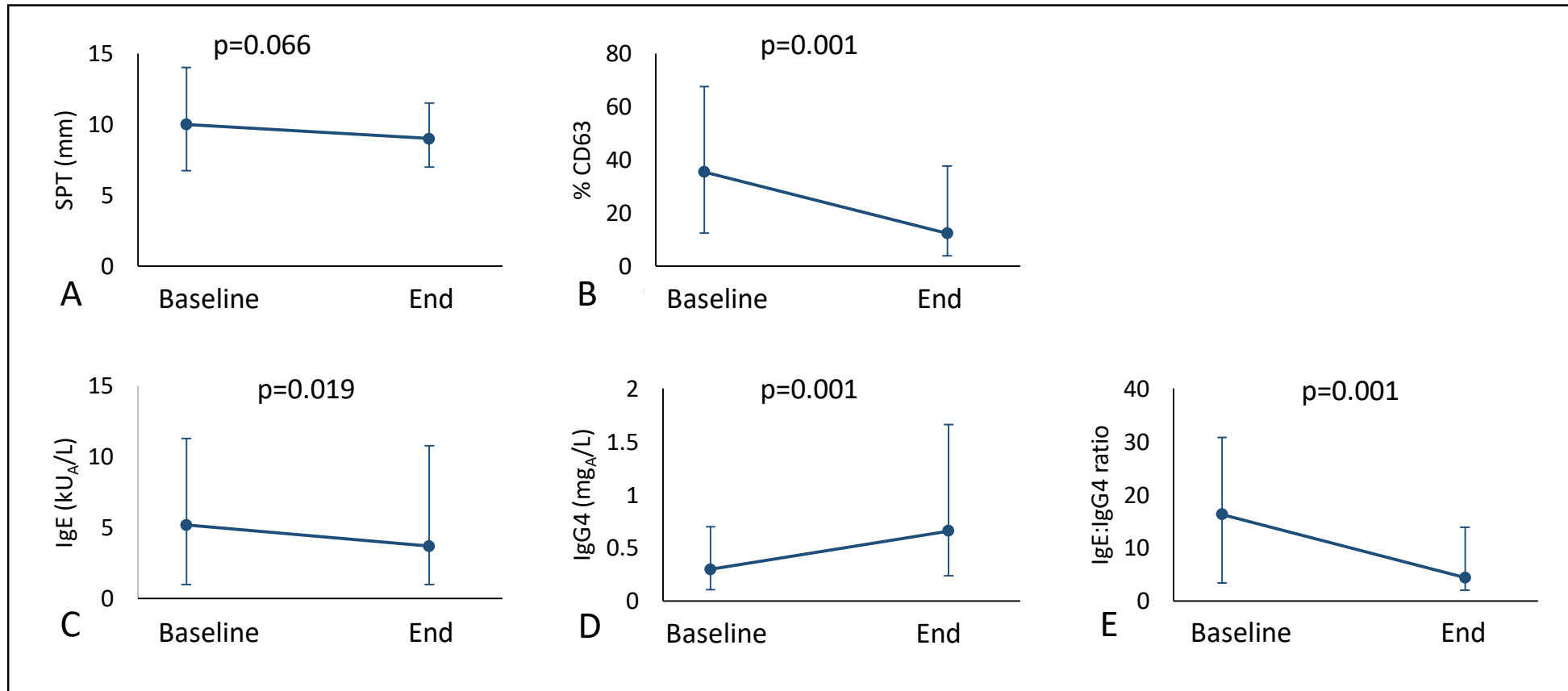
eFigure 3: IgG4 responses to walnut components in OIT patients versus controls

Changes in median (IQR) serum levels of IgG4 (mg_A/L) to walnut components (rJug r 1 (A), rJug r 3 (B), nJug r 4 (C), LMW vicilin (D), and HMW vicilin(E)) in OIT-treated walnut desensitized patients versus controls from study start to end. Data are available for 42 patients in the OIT group and in 17 control patients. p values for the difference between baseline and study end are shown for the OIT group only; A significant difference between baseline and study end in the control group was observed for Jug r 4 only (p=0.044). OIT=oral immunotherapy.



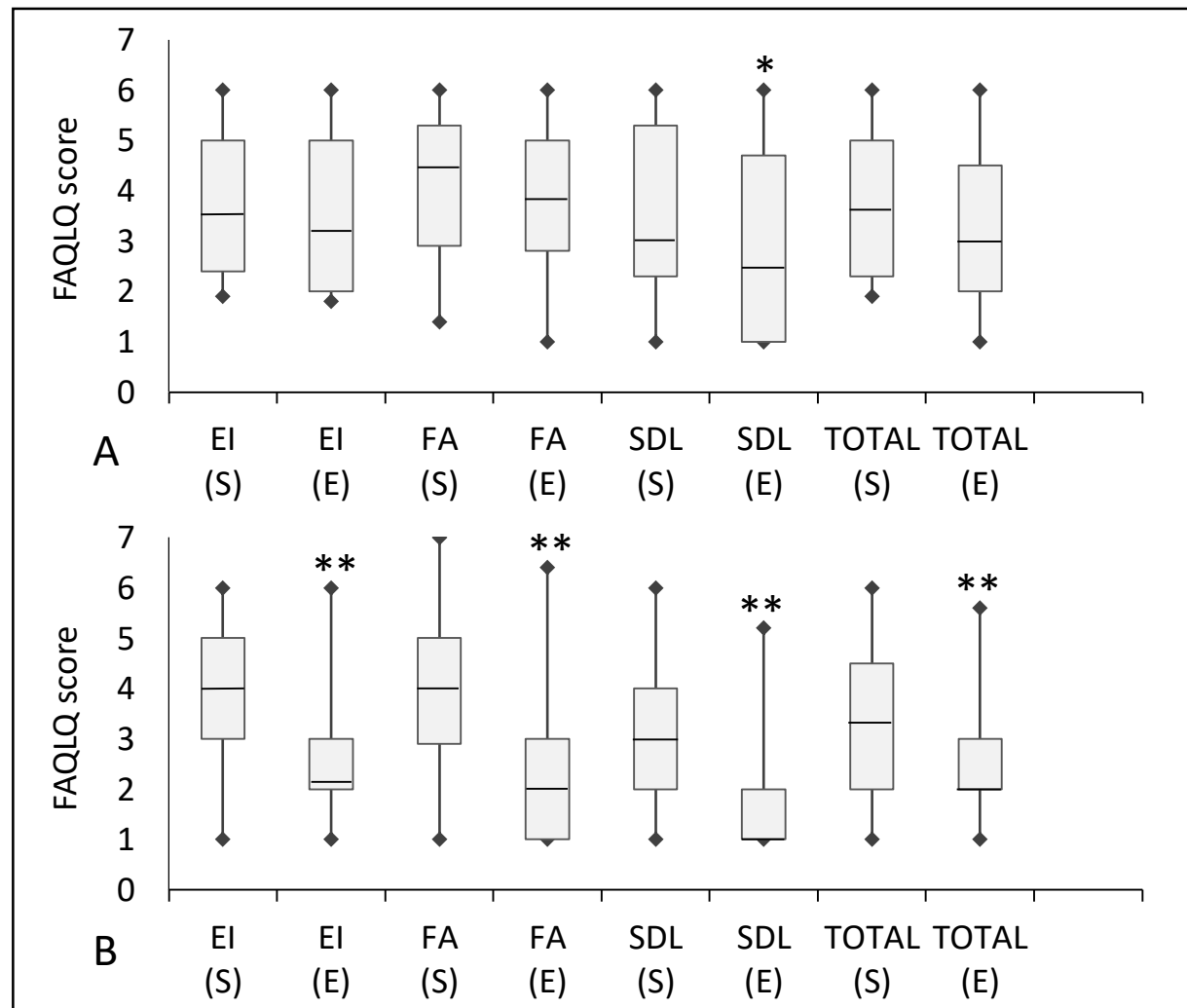
eFigure 4: IgE/IgG4 ratio responses to walnut components in OIT patients versus controls

Changes in median (IQR) IgE/IgG4 ratios to walnut components (rJug r 1 (A), rJug r 3 (B), nJug r 4 (C), LMW vicilin (D), and HMW vicilin(E)) in OIT-treated walnut desensitized patients versus controls from study start to end. Data are available for 42 patients in the OIT group and in 17 control patients. p values for the difference between baseline and study end are shown for the OIT group only; none of the differences was significant in the control group. OIT=oral immunotherapy.



eFigure 5: Immunological correlates of hazelnut or cashew allergy before and after walnut OIT

Medians (IQR) of hazelnut or cashew SPT (A), basophil activation (CD63 expression) (B), serum levels of IgE (C), and IgG4 (D) and IgE/IgG4 ratio (E) for co-allergic patients, before and after walnut OIT is presented. Data for SPT and BAT are available for 33 cases and for sIgE and sIgG4 for 30 cases. BAT=basophil activation test. SPT=skin prick test.



eFigure 6: Changes in food allergy quality of life questionnaire during OIT versus controls

Changes in the emotional impact (EI), food anxiety (FA) and Social and Dietary Limitation (SDL) sub-scales and in total score of the FAQLQ-PF from study start (S) and end (E), in walnut-desensitized patients who were desensitized to all tested allergenic nuts (A) versus those who remained allergic to at least one tested nut (B). Data are available for 34 patients desensitized to all tested nuts and 18 patients with a remaining tree nut allergy * $p < 0.05$ ** $p < 0.01$.