Original Article

Threshold Dose Distribution in Walnut Allergy

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What is already known about this topic? Eliciting doses (EDs) of foods on a population level can improve risk management and labeling strategies for the food industry and regulatory authorities. Previously, data available for walnut were unsuitable to determine EDs.

What does this article add to our knowledge? Population EDs for walnut were established and were slightly higher compared with those previously found for peanut and hazelnut allergy.

How does this study impact current management guidelines? Current data indicate that the ED values for hazelnut could be used as a conservative temporary placeholder when implementing risk management strategies for other tree nuts where little or no food challenge data are available.

BACKGROUND: In food allergy, eliciting doses (EDs) of foods on a population level can improve risk management and labeling strategies for the food industry and regulatory authorities. Previously, data available for walnut were unsuitable to determine EDs.

OBJECTIVE: The objective of this study was to determine EDs for walnut allergic adults and to compare with previously established threshold data for peanut and tree nuts.

METHODS: Prospectively, adult subjects with a suspected walnut allergy underwent a low-dose double-blind, placebo-controlled food challenge. Individual no observed and lowest observed adverse effect levels were determined and log-normal, log-logistic, and Weibull models were fit to the data. Estimated ED values were calculated for the ED_5, ED_{10}, and ED_{50}, the dose respectively predicted to provoke an allergic reaction in 5%, 10%, and 50% of the walnut allergic population.

RESULTS: Fifty-seven subjects were challenged and 33 subjects were confirmed to be walnut allergic. Objective symptoms occurred in 20 of the positive challenges (61%). The cumulative EDs in the distribution models ranged from 3.1 to 4.1 mg for the ED_{05}, from 10.6 to 14.6 mg walnut protein for the ED_{10}, and from 590 to 625 mg of walnut protein for the ED_{50}.

CONCLUSIONS: Our data indicate that population EDs for walnut are slightly higher compared with those for peanut and hazelnut allergy. Currently available data indicate that the ED values for hazelnut could be used as a conservative temporary placeholder when implementing risk management strategies for other tree nuts where little or no food challenge data are available. **(J Allergy Clin Immunol Pract 2017;5:376-80)**

Key words: *Food allergy; Walnut; Allergen thresholds; Threshold dose distributions; Eliciting doses*

The English or common walnut (*Juglans regia*) is a frequently consumed tree nut with high nutritional value and claimed health benefits.[1] In walnut allergic individuals, however, symptoms on ingestion of walnut can vary from oral allergy symptoms to anaphylaxis.[2,3] Walnut allergy is the most reported tree nut allergy in the United States.[4] In Europe, walnut sensitization was demonstrated in 2% to 3% of the population, a number very similar to peanut.[5,6]

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Abbreviations used
DBPCFC- Double-blind, placebo-controlled food challenge
ED- Eliciting doses
ICSA- Interval-Censoring Survival Analysis
LOAEL- Lowest observed adverse effect level
NOAEL- No observed adverse effect level
sIgE- Specific IgE
SPT- Skin prick test
VITAL- Voluntary Incidental Trace Allergen Labeling

Walnut, together with other tree nuts, has to be declared when used as an ingredient in prepackaged foods in the European Union, the United States, Australia, and New Zealand, amongst other countries.[7] This, however, does not rule out the unintended presence of allergens in foods. Uncertainty on possible cross-contamination of foods during the production and packaging process has triggered the food industry to increasingly add precautionary labels to food products (eg, "may contain tree nuts"). These precautionary labels are voluntarily applied by the industry in a nonstandardized fashion. Studies have found that precautionary labels poorly correspond with the presence of an allergen, therefore unnecessarily reducing the choice of foods for allergic consumers, although a small risk of the unintended presence of allergens does remain.[8,9] On the other hand, the absence of precautionary labeling might provide a false sense of safety. In some products without this type of labeling, traces of, for example, peanut and hazelnut are still found.[10] In the end, current labeling strategies might have contributed to the fact that precautionary labeling is often ignored by allergic consumers.[9]

Assessment of the eliciting doses (EDs) of a specific food on a population level can provide scientific data to improve risk management and labeling strategies for the food industry and regulatory authorities.[11] To acquire EDs, data from cohorts of subjects challenged in a (low-dose) food challenge are used.[12] EDs have been established for several foods, including peanut, hazelnut, cashew, cow's milk, and hen's egg.[13-16] For tree nuts, most data are available for hazelnut, with individual data on 202 challenges with objective symptoms in both children and adults.[15,16] Previously, data available for walnut were unsuitable to determine EDs.[13]

The aim of this study was to acquire EDs for objective symptoms in walnut allergy from a cohort of subjects with a confirmed walnut allergy based on low-dose double-blind, placebo-controlled food challenges (DBPCFCs). In addition, we compared these EDs with previously established threshold data in other foods.

MATERIALS AND METHODS

Study design

A prospective study was conducted. Patient selection, study protocol, and DBPCFC procedures were previously described.[3] Briefly, adult outpatients with a suspected walnut allergy based on patient history, assessed by a trained physician or dietician, were included. We chose to include subjects suspected of walnut allergy to avoid further selection based on the skin prick test (SPT) or specific IgE (sIgE) results and in this way kept the cohort as similar to the general population as possible. Assessment of sIgE to walnut extract and walnut component rJug r 1 was performed using ImmunoCAP (ThermoFisher, Uppsala, Sweden), according to the manufacturer's instructions. A positive test was defined using the criteria of $\geq$3 mm for SPT and $\geq$0.35 kUA/L for ImmunoCAP.

A low-dose DBPCFC with raw walnut was performed over 2 days, in accordance with the PRACTALL consensus document.[17] The lowest dose was set to 3 μg of protein, corresponding with 20 μg of walnut, in line with the general Europrevall challenge protocol.[18] The following doses were 200 μg, 2 mg, 20 mg, and 216 mg of walnut. An additional open challenge with 3 doses of unmasked walnut flour of 615 mg, 2 g, and 6.6 g walnut was conducted subsequently on the second day in case the blinded doses did not result in symptoms matching the stopping criteria. Walnuts from 2 different suppliers were used, because of availability issues. Protein content of the walnut material was determined for both batches (14.1% and 11.9%). A challenge was considered positive in case of objective or repeated subjective symptoms suggestive of a type I allergic reaction on the verum day or during the open doses. In case of only subjective symptoms, no or clearly less pronounced symptoms had to occur on the placebo day. Ethical approval was given by the Medical Ethics Review Committee of the University Medical Center Utrecht, the Netherlands.

Sample size calculation

A binomial distribution was used to determine the sample size for the study. Previously, others have demonstrated that if 29 subjects are challenged and a dose is identified where 0/29 subjects react, then there is 95% confidence that 90% of walnut individuals will not react to this amount of walnut protein or less.[19] However, the sample size should also be sufficient to fit threshold distributions based on individual no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL). The fitting was done using a parametric model on interval censored failure time data. Klein Entink et al[20] conducted a simulation study and demonstrated that 29 subjects are sufficient. On the basis of experience from our allergy clinic, approximately 50% of all patients with a suspected food allergy undergoing a diagnostic challenge have a positive DBPCFC. Therefore, to acquire 29 subjects with a positive challenge we expected that at least 60 subjects needed to be challenged.

Symptom grading and threshold data

Symptoms during the challenge were graded as the most severe symptom according to the adapted Mueller classification, as previously described[21,22]: grade 0 for oropharyngeal symptoms, grade 1 for symptoms of skin and mucous membranes, grade 2 for gastrointestinal symptoms, grade 3 for respiratory symptoms, and grade 4 for cardiovascular symptoms. Furthermore, symptoms reported during the challenges were categorized as either subjective or objective in accordance with previously defined criteria.[11,16] The highest cumulative dose of walnut protein that did not elicit an allergic reaction (NOAEL) and the threshold dose for objective symptoms (LOAEL) were established by food challenge for each individual subject. As the exact ED is not known, but it is known to fall into a particular interval, an Interval-Censoring Survival Analysis (ICSA) approach was used.[23] In case a patient reacts to the second dose of a challenge, with ICSA the threshold lies somewhere between the first and second dose. If a subject reacted to the first dose, the NOAEL was set to zero and the LOAEL to the first dose (left-censoring). If a subject only had subjective symptoms up to the highest dose, the NOAEL was considered the last dose and the LOAEL was set to infinity (right-censoring).