

## ORIGINAL ARTICLE

## Sensitization to lupine flour: is it clinically relevant?

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## Clinical &amp; Experimental Allergy

## Summary

**Background** *Lupinus angustifolius* (blue lupine) is used for human and animal consumption. Currently, the lupine content in bread varies from 0% to 10% and from 0.5% to 3% in pastry. Although lupine flour is present in many products, anaphylaxis on lupine flour is rarely seen. **Objective** The aim of our study was to determine the clinical relevance of sensitization to lupine flour.

**Methods** From October 2004 until October 2005, we performed skin prick tests (SPT) with lupine flour, peanut and soy extracts in consecutive patients attending our allergy clinic with a suspected food allergy. In patients sensitized to lupine flour, double-blind placebo-controlled food challenges (DBPCFC) were performed and specific IgE was measured.

**Results** We tested 372 patients. SPTs with peanut, soy and lupine flour were positive in 135, 58 and 22 patients, respectively. Nine patients with sensitization to lupine flour underwent DBPCFC, which was negative in eight cases. In contrast, one patient experienced significant symptoms. Four of these nine patients suspected lupine by history. Two other patients with a positive history to lupine declined from challenges. In these patients, a 3-day dietary record showed that they could consume lupine without symptoms. Specific IgE in the serum was positive for *L. angustifolius*, peanut and soy in all nine patients.

**Conclusion** These results demonstrate that clinical lupine allergy is very uncommon, even in the presence of sensitization to lupine flour. The estimated prevalence of lupine allergy, among patients with a suspected food allergy, referred to a tertiary allergy centre in the Netherlands is 0.27–0.81%. In most, although not all cases, sensitization is not clinically relevant and is most likely caused by cross-sensitization to peanut. In selected cases, eliciting doses are low, making significant reactions possible.

**Keywords** allergenic foods, double-blind, food allergy, legumes, lupine flour, placebo-controlled food challenge

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## Introduction

Lupines are members of the legume family (*Fabaceae*). As far back as Roman times, lupine seeds have been used for human and animal consumption. Currently, these seeds are used for different foods e.g. vegan sausages, lupine-tofu and as a baking enhancer in baked goods.

The species of lupine used in food are *Lupinus albus* (white lupine), *Lupinus luteus* (yellow lupine) and *Lupinus angustifolius* (blue lupine). In the Netherlands, mostly *L. angustifolius* is consumed, whereas *L. albus* is used more in France.

In order to process the seeds to flour, they are cleaned, toasted, de-hulled, ground, crushed and added as an

ingredient to wheat flour for its nutritional and food-processing qualities. Lupine flour is characterized by a high percentage of protein (36%), a low fat content (8%) and the absence of gluten. Lupine flour is mainly present in bread, but also in pancakes, doughnuts, pasta, pizza dough, biscuits waffle batter, brioche and flaky pastry.

The lupine content in bread varies from 0% to 10% and from 0.5% to 3% in pastry, and currently, approximately 0.11 kg lupine flour and 5.5 kg lupine flour-containing products are consumed per person per year in the Netherlands. (Fa. L.I. Frank, Twello, the Netherlands, <http://www.lifrank.nl/>). Since the introduction of lupine flour as an ingredient in wheat flour in Europe in the 1990s, lupine

consumption has become more widespread and the amount consumed per person per year has increased considerably. Ever since its introduction, allergic reactions and even in some cases anaphylactic reactions [1–15] to lupine have been reported. Sensitization to lupine has been reported not only to occur by consumption but also by inhalation [15–19] of lupine flour by bakers. The frequency of sensitization and allergic reactions to lupine in the general population is unknown. The population sensitized to peanut, which represents about 0.7–1.5% of the European population, is the population at risk of developing sensitization to lupine, as peanut and lupine belong to the same legume family [1, 7, 9, 20–24].

In 2008, lupine was added to the list of foods that must be labelled in pre-packaged foods as advised by the European Food Safety Authority (EFSA) [25]. Although lupine flour is present in many products, anaphylaxis on lupine flour is rarely seen. We therefore undertook this study to estimate the prevalence of sensitization and clinical reactivity to lupine flour products in a population presenting to a tertiary referral centre allergy clinic.

## Methods

### Study design

All consecutive patients who were referred to the outpatient allergy clinic of the Erasmus Medical Centre in Rotterdam from October 2004 until October 2005 with a suspected food allergy underwent (additional) skin prick tests (SPTs) with extracts of lupine flour and peanut produced in house and a commercially available extract of soy (1 : 20 w/v) (ALK/Abello, Horsholm, Denmark).

Patients sensitized to lupine flour in SPT were asked to participate in this study. Consenting patients were asked whether they had any symptoms after consuming products possibly containing lupine flour. Patients also underwent an additional SPT with inhalant allergen extracts (*Dermatophagoides pteronyssinus*, tree mix, grass mix; ALK/Abello). Furthermore, they were asked to undergo a double-blind placebo-controlled food challenge (DBPCFC) with lupine flour to determine the clinical relevance of this sensitization. Sensitization to other legumes was ascertained by determining specific IgE levels for *L. angustifolius*, peanut and soy. Finally, the patients with a negative DBPCFC were asked to undergo open challenges with two waffles, containing 2300 mg lupine flour each. Patients with a positive history (suspected) who declined from participation in DBPCFCs were asked to keep a 3-day dietary record, which was analysed for the presence of lupine in their diets by a dietician with knowledge of food allergy.

The local ethics committee approved the study and all subjects gave written informed consent before entering the study.

### Skin tests

Fifteen minutes after performing the skin tests, the contours of the weal were encircled with a fine-tip pen and transferred to a record sheet by means of a translucent tape. Besides measuring the mean weal of the surface within the encircled area, histamine-equivalent prick (HEP) was calculated by dividing the area of the SPT of the allergen by the area of the mean of two histamines of the SPT using a flat bed scanner (Hewlett Packard 2400c, Houston, TX, USA) and software that was earlier developed in our hospital. Calculation of the surface of the weal in this way is more accurate and reproducible than manually determining the mean weal diameter [26–29]. With this technique, a cut-off of 0.21 corresponds to a weal size of 3 mm, the internationally accepted cut-off level for SPT. This classification is a modification of the grading system described by Niemeijer et al. [30] In addition, reactions had to have a mean weal diameter of 3 mm or more to be considered as positive. The skin test had to be positive using both methods.

### Preparation of in-house food extracts

In-house food extracts were prepared as described earlier [31]. We used *L. angustifolius* as it is the most frequently consumed species in the Netherlands.

Fresh, unroasted peanuts were homogenized mechanically, ground with a mortar, defatted by ether extraction and subsequently air-dried. This pre-treated material was stored at  $-20^{\circ}\text{C}$  until use for further preparation. To make a 10% extract, the pre-treated peanuts and lupine flour (Fa. L.I.frank,) were mixed with PBS (phosphate-buffered saline pH 7.4), containing 0.03% human serum albumin and 0.5% phenol. All extracts were subsequently centrifuged for 10 min at 2000 g, and the supernatants were passed through a 0.22  $\mu\text{m}$  filter (Millex GS, Millipore, Amsterdam, the Netherlands). Aliquots were stored at  $-20^{\circ}\text{C}$ . The extracts were thawed 1 h before performance of the skin tests and mixed by Vortex. The protein concentration of each in-house extract was determined using the method of Watanabe et al. [32] The protein concentration of the lupine and peanut extract was 1.82 and 1.22 g/L, respectively. Further characterization of the homemade extracts was performed by SDS-PAGE (Wageningen University, the Netherlands). SDS-PAGE, under denaturing conditions on 10% Bis-Tris gels, were performed as described by Werten and de Wolf [33]. After electrophoresis, gels were stained with Coomassie Simply-Blue SafeStain (Invitrogen, Leek, the Netherlands).

Three non-atopic volunteers had a negative SPT with both extracts.

### CAP fluorescent enzyme immunoassay

Specific IgE to *L. angustifolius*, peanut and soy was determined using the ImmunoCAP system [CAP-fluorescent

enzyme immunoassay (FEIA), Phadia B.V., Nieuwegein, the Netherlands]. CAP-FEIA test results of  $\geq 0.35$  kU/L were considered positive.

### Food challenges

Patients with sensitization for lupine flour were asked to undergo DBPCFCs [34–36]. DBPCFCs were performed with baked cookies, with and without lupine flour, which was obtained from Fa. L.I. Frank. Recipes were developed and validated with hospital volunteers at University Medical Centre Groningen (UMCG) using the methodology described previously [35]. The placebo test food recipe and active food recipe contained whole-wheat flour, wheat flour, dairy-free margarine, cane sugar, vanilla and salt. In the active test food, lupine flour was added and in the placebo test food, custard was added. From the material, cookies were baked and weighed afterwards in the correct quantities. Briefly, placebo and active challenges were administered in a random order, and were administered on separate days with at least a 2-week interval in between. The time interval between any two challenge doses was 30 min. The challenge procedure included a six-step incremental design containing 5, 10, 39, 194, 972 and 4861 mg lupine flour (total 6081 mg lupine flour, containing 2190 mg lupine protein), respectively. As the protein content of the product is 36%, the total protein equivalent is comparable with the amount of protein masked in recipes used in earlier studies for DBPCFC [36]. Patients with negative DBPCFCs were asked to undergo open challenges with waffles containing 4600 mg lupine flour.

The challenge was discontinued when objective allergic symptoms occurred; symptoms were scored as described earlier. [36] Immediate symptoms were defined as symptoms that occurred during the challenge session. Forty-eight hours after the second challenge session, the code was broken and the outcome of the DBPCFC was assessed.

Patients with a positive history (suspected) who declined from participation in DBPCFCs were asked to keep a 3-day dietary record, to assess the total amount of lupine in their diets, by a dietician with knowledge of food allergy [34, 35]. The calculation is made by summing up the total amount of consumed lupine flour in e.g. bread, croissants and lasagne, using given percentages of lupine flour on the labels of the products and information form Fa L.I. Frank. For example, lasagne contains 0.5–3% lupine flour. When the patient consumed 100 g of lasagne, we calculated from this figure 500–3000 mg consumed lupine flour.

## Results

### Sensitization to lupine flour and other allergens

Among 1040 consecutive patients who were referred to the outpatient allergy clinic, 372 were suspected of having

food allergy. All 372 patients underwent additional skin tests, and no patients were excluded. Their mean age was 28.3 years (range 3–75 years). 22 (6%) patients (F/M 18/4; mean age 31.4; range 10–70) were sensitized to lupine flour. (Table 1) Sensitization to peanut and soy was found in 135 (36%) and 58 (16%) of these patients, respectively. SDS-PAGE of the lupine flour and peanut extracts are shown in Fig. 1. In 6/22 subjects sensitized to lupine flour, further skin tests were not performed. Of the other 16 patients, 15 were sensitized to at least one inhalant allergen, 14 patients were sensitized to birch pollen, 13 to grass pollen and 10 to house dust mite. One patient was not co-sensitized to any inhalant allergen.

### CAP fluorescent enzyme immunoassay

The mean levels and range of specific IgE in patients who underwent DBPCFC ( $N=9$ ) were 3.18 (0.98–6.68), 40.24 (3.13–> 100) and 5.78 (0.88–16.2) kUA/L, for *L. angustifolius*, peanut and soy respectively. Table 1

### Dietary history

Most people could not accurately evaluate whether they were allergic to lupine flour, not being aware that lupine is present in many baked products and sausages, and they did not generally read the ingredient list.

This was the case in 12/22 patients. Four patients were certain they could tolerate lupine flour and six patients were suspected to be allergic to lupine flour. They reported symptoms such as oral allergy syndrome, urticaria and rhinoconjunctivitis after ingestion of food containing lupine flour.

### Food challenges and 3-day dietary records

Nine of the 22 patients sensitized to lupine agreed to DBPCFC with lupine flour. (Table 1; in bold). The reasons for not participating in DBPCFC were mostly lack of time or interest. No patient declined because of fear of severe reactions.

Eight patients did not react during the DBPCFCs. One patient experienced significant symptoms during a challenge with cookies containing lupine flour and not to placebo. Within a few minutes after ingestion of dose 3 (14 mg lupine protein), she complained of itching ears, itching and irritated eyes, itch and a metallic taste in the mouth. Symptoms intensified after dose 4 and dose 5 (70 and 350 mg lupine protein, respectively). After dose 4, she felt nauseous and her tongue started to tingle and after dose 5 she started to sneeze, developed urticaria, labial angio-oedema and malaise. She experienced no drop in blood pressure, difficulty in breathing or a need for epinephrine. The challenge test was discontinued and the patient was given an oral antihistamine, upon which the

**Table 1.** Characteristics of the patients, results of the SPT, CAP-FEIA, CH for lupine flour and peanut, DBPCFC and OP

| Age | M/F | HEP         |             |             |             |             |             | CAP-FEIA (kUA/L) |                 |             | CH      |                  | DBPCFC   | OP       |
|-----|-----|-------------|-------------|-------------|-------------|-------------|-------------|------------------|-----------------|-------------|---------|------------------|----------|----------|
|     |     | Lupine      | Peanut      | Soy         | HDM         | Tp          | Gp          | Lupine           | Peanut          | Soy         | Lupine  | Peanut           | Lupine   | Lupine   |
| 24  | F   | 1.23        | 2.11        | 0           | ND          | ND          | ND          | ND               | ND              | ND          | Suspect | anaph., e        | ND       | ND       |
| 29  | M   | <b>0.41</b> | <b>4</b>    | <b>0.27</b> | <b>0.88</b> | <b>1.42</b> | <b>1.86</b> | <b>3.55</b>      | <b>83.2</b>     | <b>4.78</b> | Suspect | oas, rhi, gi     | Negative | Negative |
| 12  | F   | <b>0.78</b> | <b>2.11</b> | <b>0.24</b> | <b>0.61</b> | <b>0.73</b> | <b>0.54</b> | <b>4.16</b>      | <b>25.9</b>     | <b>2.2</b>  | Unknown | urt, oas, dys    | Negative | ND       |
| 54  | F   | 0.39        | 0.63        | 0.36        | 0           | 1.27        | 0.43        | ND               | ND              | ND          | None    | None             | ND       | ND       |
| 26  | F   | 1.45        | 0.87        | 1.02        | ND          | ND          | ND          | ND               | ND              | ND          | Suspect | anaph            | ND       | ND       |
| 16  | F   | <b>0.61</b> | <b>0</b>    | <b>0.51</b> | <b>0</b>    | <b>0</b>    | <b>0</b>    | <b>4.27</b>      | <b>11.7</b>     | <b>12.9</b> | None    | None             | Negative | ND       |
| 27  | F   | 0.40        | 0           | 0           | 0.85        | 1.52        | 1.4         | ND               | ND              | ND          | Unknown | Unknown          | ND       | ND       |
| 70  | F   | 0.32        | 0.47        | 0.25        | ND          | ND          | ND          | ND               | ND              | ND          | Unknown | e, urt, dys      | ND       | ND       |
| 37  | F   | <b>0.36</b> | <b>2.41</b> | <b>0.96</b> | <b>0.78</b> | <b>2.05</b> | <b>0.76</b> | <b>0.23</b>      | <b>3.13</b>     | <b>3.34</b> | None    | anaph, e         | Negative | ND       |
| 33  | F   | <b>0.44</b> | <b>4.53</b> | <b>0.78</b> | <b>0.79</b> | <b>2.32</b> | <b>0.76</b> | <b>6.68</b>      | <b>64</b>       | <b>4.26</b> | Suspect | oas, urt, dys    | Negative | ND       |
| 10  | F   | <b>0.36</b> | <b>3.8</b>  | <b>0</b>    | <b>0.46</b> | <b>2.6</b>  | <b>1.85</b> | <b>0.98</b>      | <b>46.3</b>     | <b>4.03</b> | Suspect | anaph, oas       | Negative | ND       |
| 35  | F   | 0.27        | 0           | 0           | 2.8         | 1.01        | 0.9         | ND               | ND              | ND          | Unknown | Unknown          | ND       | ND       |
| 31  | F   | 0.36        | 1.91        | 0.41        | 0           | 3.05        | 1.08        | ND               | ND              | ND          | Unknown | oas              | ND       | ND       |
| 31  | F   | 0.21        | 0.22        | 0.24        | 3.6         | 3.91        | 1.9         | ND               | ND              | ND          | Unknown | Unknown          | ND       | NDND     |
| 21  | F   | <b>1.10</b> | <b>2.1</b>  | <b>0.3</b>  | <b>1.85</b> | <b>2.3</b>  | <b>3.19</b> | <b>3.93</b>      | <b>&gt; 100</b> | <b>16.2</b> | Unknown | oas, anaph., urt | Negative | ND       |
| 31  | F   | 0.26        | 0           | 0           | 0           | 0.81        | 1.08        | ND               | ND              | ND          | None    | None             | ND       | ND       |
| 27  | F   | 0.31        | 4.8         | 0.21        | 0           | 0.76        | 1           | ND               | ND              | ND          | Unknown | urt, dysp        | ND       | ND       |
| 61  | F   | 0.21        | 0.23        | 0.32        | ND          | ND          | ND          | ND               | ND              | ND          | Unknown | Unknown          | ND       | ND       |
| 12  | M   | 1.41        | 2.95        | 0           | 0.84        | 0           | 0           | ND               | ND              | ND          | Unknown | oas              | ND       | ND       |
| 41  | M   | 1.39        | 0           | 0           | ND          | ND          | ND          | ND               | ND              | ND          | Unknown | Unknown          | ND       | ND       |
| 43  | F   | <b>1.69</b> | <b>6.8</b>  | <b>0</b>    | ND          | ND          | ND          | <b>0.55</b>      | <b>35</b>       | <b>0.88</b> | Suspect | anaph, oas, urt  | Positive | ND       |
| 19  | M   | <b>2.54</b> | <b>6.21</b> | <b>0</b>    | <b>0</b>    | <b>2.15</b> | <b>3.32</b> | <b>4.24</b>      | <b>52.7</b>     | <b>3.47</b> | Unknown | anaph., oas, urt | Negative | Negative |

Nine of the 22 patients who agreed to DBPCFC with lupine flour in bold.

M/F, male/female; HEP, histamine equivalent prick indices; DBPCFC, double-blind placebo-controlled food challenge; CAP-FEIA, CAP-fluorescent enzyme immunoassay; CH, case history; OP, open provocation; HDM, house dust mite; Tp, tree pollen; Gp, grass pollen; ND, not done; oas, oral allergy symptoms; anaph., anaphylaxis; urt, urticaria; e, oedema; dys, dyspnoea, gi, gastro intestinal symptoms; rhi, rhinitis.

symptoms abated after 10 min. No reactions to placebos occurred. The estimated prevalence to lupine flour was established at 1/372 (0.27%) in this particular selected group of patients.

Four of the nine patients who participated in a DBPCFC had a positive history to lupine. Three of these patients did not react, while one reacted as just described. Analysis of the 3-day dietary records of the two patients who declined from challenge with a positive history revealed that they consumed approximately 5000–15 000 mg lupine flour daily in their diets without any symptoms.

### Open challenge

Only 2/8 patients who did not react to the DBPCFC with lupine flour agreed to undergo an open provocation with two waffles containing 2300 mg lupine flour each. Six patients did not agree to undergo another challenge because they were convinced that they were not allergic to lupine flour (negative DBPCFC).

### Discussion

Currently, lupine flour is used widely because of its high protein content, low fat content and good digestibility.

Although the estimated amount of lupine flour consumed each year per person in the Netherlands is relatively high compared with other potentially allergenic foods, anaphylaxis to lupine flour has only rarely been reported. This suggests that lupine may have a relatively low allergenic potential.

This is in agreement with our findings. In this study, 6% of patients with suspected food allergy being referred to a specialized University clinic appeared to be sensitized to *L. angustifolius*. This low percentage contrasted with the high number of patients sensitized to peanuts (36%). In only 1/372 patients (0.27%), a clinically relevant food allergy to lupine could be confirmed. However, two patients with a positive history to lupine declined from DBPCFC. Assuming that these two patients would have been clinically reactive to lupine, the estimated number of patients allergic to lupine would be 3/372 (0.81%) at most in this particular group of patients. The prevalence of clinical allergy to lupine flour found in this selected patient group does not represent the prevalence in the general population. It is, however, conceivable that the prevalence of food allergy at a tertiary Allergy centre is higher compared with the prevalence in the general population. Consequently, the prevalence of lupine allergy in the overall Dutch population might be even lower than the 0.27–0.81% we found.

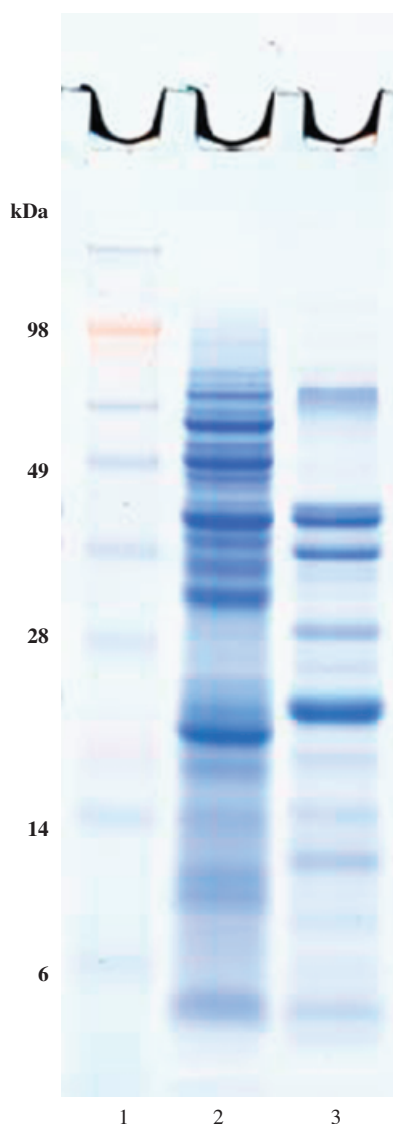


Fig. 1. SDS-PAGE home-made allergen extracts, 15 µg protein/lane. Molecular weight bands are shown to the left (kDa); molecular weight standard; lane (1) Lupine extract; lane (2) peanut extract; lane (3).

Our observation that only one patient reacted to lupine suggests that sensitization to lupine seldom leads to allergic symptoms. This is in agreement with other studies. Shaw et al. [3] also found this low outcome of positive DBPCFCs in peanut-allergic children with lupine sensitization. In his study, 2/9 had a positive reaction to DBPCFC. Furthermore, Lindvik et al. [4] found comparable results: 1/10 reacted positively on food challenges.

Nevertheless, the low eliciting dose of 70 mg lupine flour, which caused objective symptoms in the only one patient who reacted, indicates the significant allergenicity of lupine flour.

Peeters et al. [9] found six patients reacting to lupine. The most obvious reason for the difference between their study and ours is probably subject selection. In contrast to

the study by Peeters and colleagues, in which patients were selected because of a clear-cut clinical history of lupine allergy, we examined a much broader group of patients suspected of an allergy to a range of foods.

This study has a few limitations. First, 13/22 patients (59%) did not undergo food challenges. Against the background of a substantial presence of lupine flour in the normal Dutch diet, one might assume that patients without allergic symptoms after eating bread and other products with flour are not allergic to lupine. Moreover, most patients did not suspect lupine. We analysed the diet of two patients. It appeared that the daily intake of lupine amounted to 5000 mg in one patient and 15 000 mg in the other. This intake should elicit allergic symptoms if the patients were true reactors. There is no reason to believe that the diet of the other unchallenged patients is substantially different. Therefore, we assume that the patients are non-reactors.

Nevertheless, we are aware that patients in the unchallenged group might have reacted to lupine. The rate of positive challenges was 11%. If we assume that the prevalence of positive reactions in this group is also 11%, the number of positive challenges will increase from one to two. Even then, the prevalence is low.

Six out of 22 patients sensitized to lupine were suspected to be allergic to lupine. Of these, 3/6 patients were not confirmed to be allergic to lupine by DBPCFCs, and only one subject reacted to challenge. The remaining two patients who declined from challenge with a positive history appeared to be tolerant to lupine as demonstrated by the dietary analysis.

Also, although the four monosensitized patients to lupine refused a challenge test, they had a negative history to lupine, which made suspicion of lupine allergy in these patients unlikely.

Second, a number of patients were also routinely tested to exclude food allergy. For instance, patients with chronic urticaria or gastro-intestinal problems were also included. This may partly explain the low percentage of sensitization to lupine flour in our study population. Our study focused on consecutive patients with a possible food allergy. Although the group is considerably small, the prevalence of peanut-sensitized patients in our group (12.8%) is comparable with other studies on peanut sensitization in Europe [37, 38].

The question arises as to whether sensitization to lupine flour is based on cross-reactivity to peanuts. The high number of patients sensitized to both peanuts and lupine in our study and studies from others [3, 8] suggests this. Apparently, in our group of patients, which represents the current population visiting our outpatient clinic, co-sensitization between lupine flour and peanut is high.

Concerning the clinical relevance of peanut sensitization in our selected group, the case history data for peanut suggest that most patients experienced allergic reactions

after consumption of peanut. In this study, we were particularly interested in the patterns of cross-sensitization. Lupine seeds contain several types of storage proteins called conglutins. Two major lupine globulins and candidate allergens are  $\alpha$ -conglutin (11S, legumin-like) and  $\beta$ -conglutin (7S, vicilin-like) [22].

Although these types of proteins are ubiquitously occurring in many nuts and seeds from variable biological origin, the chances of cross-reactions increase with a closer phylogenetic relationship. In this respect, it is important to note that both peanut and lupine belong to the legume family of plants (*Fabaceae*), which also includes soybean, common bean, lentil, garden pea and chickpea. Faeste *et al.* [7] found two lupine proteins of 38 and 15 kDa that were recognized by anti-peanut antibodies present in a patient's serum.

Fiocchi *et al.* [39] most recently reported cross-reactivity studies on lupine flour. He found  $\beta$ -conglutin to be the most important lupine allergen. This homologous *L. angustifolius* protein is now included in the list of allergens from plant origin as *Lup an 1* [40]. In his study, 2/12 peanut-allergic children reacted on lupine – enriched pasta. The second most important allergen was  $\alpha$ -conglutin, which shares homologue similarities to Ara-h-3. Guarneri *et al.* [24] found a highly significant sequence homology and molecular similarity between the  $\beta$ -conglutin and the Ara-h-1 (vicilin) allergen of peanut.

Furthermore, they reported significant similarities between Ara-h-8 and the pathogenesis-related protein PR-10 of lupine flour. This entire PR-10 family of proteins shares the amino acid sequence of Bet-v-1, the major pollen allergen of *Betula pendula*. Nearly all patients who were skin tested to birch pollen were sensitized (14/16). On this topic, more research should be carried out, and we hypothesize whether sensitivity to either Ara-h-1, Ara-h-3 or Ara-h-8 could predict the chance and the severity of clinical reactions on lupine flour.

In summary, we have demonstrated that despite the high amount of lupine intake in the Netherlands, clinical lupine allergy is very uncommon, even in the presence of sensitization to lupine flour. In most, although not all cases, sensitization is not clinically relevant and most likely caused by cross-sensitization to peanut or birch pollen. Nevertheless, although clinical lupine allergy is uncommon, in selected cases, eliciting doses are low, making significant reactions possible.

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