LUPIN EXPOSURE STUDY

Anja Carina Süssmann

(305268945)

Royal Prince Alfred Hospital – Allergy Unit

Dr Robert Loblay, Dr Velencia Soutter, Dr Anne Swain

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Table of Contents

Appendices

Lupin Exposure Study:

Dietary Differences in Lupin sensitised Individuals

Anja C. Süssmann, Anne Swain PhD, Velencia Soutter MD, Robert Loblay MD

ABSTRACT

Purpose

This study explored whether there exist any correlations between lupin sensitisation and dietary intake in the presence and absence of sensitisation to other foods, especially peanut.

Hypothesis

There are dietary differences between lupin sensitised and non-sensitised individuals, in particular regarding the intake of high fibre and lupin breads, which are consumed more frequently in lupin sensitised subjects.

Methods

100 patients who underwent skin prick testing for investigation of possible food allergies at the RPAH Allergy Unit completed a food frequency questionnaire designed to elicit information about lupin intake and exposure. Responses were analysed (SPSS 15.0) for any significant differences after categorisation of participants into three groups depending on whether they were sensitised to lupin and/or peanut, and age groups younger and older than five years. 30 subjects were lupin and peanut sensitised (L+P+), 14 were sensitised to peanut but not lupin (L-P+), and 56 were neither sensitised to lupin nor peanut (L-P-). No participants were sensitised to lupin but not peanut (L+P-). SPSS was also used to assess any relationship between sensitisation to lupin/peanut and treenuts, sesame and almond.

Results

No significant differences in food intake were found in relation to lupin sensitisation, when peanut was excluded as a confounding factor.However there was a non-significant trend in the over five years age group which could be examined further with a larger sample size. This included an increased intake of nuts and seeds, legumes (except lentils), and chocolate confectionery in lupin positive/peanut positive individuals as opposed to lupin negative/peanut positive individuals.

Regarding sensitisation to other foods in subjects older than five years, more lupin positive/peanut positive individuals than lupin negative/peanut positive individuals were sensitised to almond and other treenuts. Most interestingly, lupin sensitised individuals were found to have a significantly higher rate of sensitisation tp sesame.

Conclusion

Trends indicating dietary differences between lupin sensitised and non-sensitised individuals exist. These need to be reassessed during the continuation of this study as the sample size is increasing. At this point in time no correlation between high fibre and lupin bread intake and lupin sensitisation could be detected, however this hypothesis cannot be rejected.

BACKGROUND

Lupin (*Lupinus spp*) is a plant belonging to the legume family [1] whose seeds have been used as a food source for humans and animals alike for over 2000 years [2]. Consumption had been limited by high levels of toxic alkaloids within the bean [3], necessitating laborious processing techniques to render it safe to eat [4]. Through the processes of selective breeding and genetic engineering (hybridization) [5] strains of lupin have been cultivated for human and animal consumption [6] that require little or no processing as they are low in toxic alkaloids [7]. As a result, four of 450 species of lupin are now used for consumption [6], namely *Lupinus angustifolia* (blue or narrow-leaf Lupin), *L. luteus* (yellow Lupin), *L.mutabilis* (pearl or tarwin Lupin), and *L. albus* (white Lupin) [8], the most widely used species [9].


L. angustifolius


L. albus


L. luteus


L. mutabilis

These non-toxic strains of lupin are an attractive source of food in the diet for numerous dietetic, economical and food technological reasons. Lupins contain high levels of fibre and protein, including essential amino acids [10], with levels higher than those found in soy [11]. Lupin flour and bran are currently being used to enrich European breads, pastas, cakes and biscuits with these nutrients [1]. Lupins also contain much less lipids than soy and are therefore lower in energy content [11]. Some micronutrients such as B vitamins, magnesium, iron phosphorus, copper and calcium are found in high quantities in lupin ingredients [11]. In addition, lupins are, compared to soy and other legumes, low in or even devoid of certain anti-nutritive factors, including lectins (phytohaemagglutinins, i.e.

can contribute to blood clotting), trypsin inhibitors (can cause indigestion), as well as phytate and tannins (can lower the bioavailability of proteins, iron, zinc and calcium by binding to them in the digestive tract) [3, 12].

	Lupin flour and grits		Soy flour	
Protein	41.0 g		34.5 g	
Carbohydrates (no fibre)	10.5 g		25.6 g	
Fibre	31.0 g		9.6 g	
Lipids	10.2 g		20.6 g	
SFA % of TFA	1.5 g	14.2%	2.3 g	11.2%
MUFA % of TFA	6.0 g	59.2%	4.6 g	22.3%
PUFA % of TFA	2.7 g	26.6%	11.7 g	56.8%
Omega 6	1.4 g		10.3 g	
Omega 3	0.8 g		1.4 g	
Ratio Omega 6/Omega 3	1.8		7.4	
Energy value	297.8 kcal		425.8 kcal	

SFA = saturated fatty acids; MUFA = mono unsaturated fatty acids; PUFA = poly unsaturated fatty acids, TFA = total fatty acids.

Table 1. Nutritional values (per 100 g) of lupin flour and grits compared with soy flour.

Taken from Knauf U, Seger A, Bagger C, Bez J: **Lupin food ingredients and lupin-based food products**. Grain Legumes Portal (March 2007) [11].

Some functional properties of lupins encourage its use in food products. Lupin acts as an emulsifier, colouring agent (yellow), taste enhancer and it has a high water-binding capacity, all of which are desirable characteristics in the manufacturing of many baked products, including gluten free varieties [11]. As a result of its emulsifying properties, lupin may also be added to processed meat products [13]. Furthermore, lupins can be used as a substitute for animal proteins such as egg and casein (found in dairy), enabling the production of 100% plant based foods that are no different from conventional products in texture and taste [11], meeting demands of the vegetarian consumer. Examples of lupin products include lupin milk, which can also be used as a base for vegetable drinks, yoghurts and creams, as well as egg-free, milk-free baked products such as lupin muffins [11]. Lupin is also gluten-free and therefore a good candidate for products aimed at gluten-intolerant and coeliac population [14, 10].

The use of lupins in foods is also favourable from an economical perspective. Lupin as an ingredient is an inexpensive source of protein [13] and gives higher yields of fermented products such as miso, tempeh and tofu, a major reason for its increased use in Asia [3].

Not surprisingly, lupin is being increasingly used in food products that are an integral part of many people's diets [4], often in place of soy [15] as an alternative to genetically modified ingredients [16]. Genetically modified (GM) lupins, such as high sulphur lupins, do exist, and in Australia they were first released in a limited and controlled manner in 1995. However, these varieties are supposedly not used for human consumption but for stock feeds and have been observed to improve wool growth in sheep. "There have been no reports of adverse effects on human health or the environment resulting from these releases" [17].

In France, adding lupin to wheat flour was officially authorized in 1999 [9], and Australia followed suit in 2001 [13], but introducing lupin to bread and cookies was first considered over 20 years ago [18, 19]. Lupins are also used in some cosmetics and pet foods, further increasing the market for and exposure of individuals to the crop.

Australia is now the biggest international sweet (non-toxic) lupin producer [20], after *L.angustifolius* had first been introduced to Western Australia in the 1950s [7]. Australia has produced about 85% of the world lupin over the past ten years, with lupin accounting for half of Australia's legume production, most of which (80%) is grown in Western Australia, followed by South Australia and New South Wales. On average, this amounts to 1.2 million tons of lupin a year. 41% of this is exported, expected to increase to 50% by 2012, and 90% of the exported harvest goes to the EU, Japan and Korea, resulting in an annual income of nearly $100 million, 2% of the total value "of Australian exports of grains and oilseeds" [21].

To date, little scientific literature exists about the potential risks of such exposure to lupin [4]. However, there are increasing reports suggesting that lupins can cause allergic reactions [22],

including anaphylaxis [23], in hypersensitised people. Since “an allergic reaction is a pathological response to an allergen to which the person has been previously exposed” [4] and to which he or she has developed antibodies [24], it is possible that the increasing rate of lupin allergy is a direct result of an increased exposure to lupin. The first case of lupin allergy was reported in the USA in 1994 [25], and three severe cases have been reported in Australia [9]. These individuals may have been exposed to lupin in their diet, their pets’ food, cosmetics, and/or their occupational environment (inhalation of lupin dust at mills, for example) [26].

Food Standards Australia New Zealand requires listing lupin on packaged foods containing lupin as an ingredient in the ingredients list. However, as lupin is as yet not recognized as a major allergen it does not need to be labelled as such [27]. Thus, unpackaged products and those containing traces of lupin will not display lupin on ingredient lists, resulting in its inadvertent consumption. In the EU, lupin has been recognised as a food allergen since December 2005, and it is now also included in the EU food allergens list [28].

There also seems to be a potential for cross reactivity between lupins and other legumes, especially peanuts in peanut-sensitised individuals, and the majority of allergic reactions to lupin have occurred in people allergic to other legumes, mostly in those allergic to peanut [16]. The first reported case of lupin allergy in 1994 in the USA, mentioned above, is an example of such cross reactivity. A five year old girl who had a known peanut allergy presented with an allergic reaction after ingesting pasta that contained lupin [4].

A likely explanation for cross reactivity between lupin and peanut is the genetic likeness of these legumes, with both peanut and lupin belonging to the Fabacae family (Pea family) according to taxonomic classification. A study conducted in France showed that the major allergens found in lupin flour and peanut share the same molecular mass of 43kd [6], and highly significant amino acid sequences [29]. Another study showed that the dose necessary to elicit an allergic reaction to lupin

flour is very similar to the eliciting dose of peanut [30]. Studies such as that conducted by Moneret-Vautrin in 1999 suggest that approximately 30% of individuals allergic to peanut will react to lupin flour [6], but another study found reactions in 68% of peanut allergic individuals [31].

Nonetheless, “lupin allergy can occur as a separate entity, without evidence of clinical or laboratory cross-reactivity to other legumes” [16].

Interestingly, a recently published Norwegian study by Holden at al., using a new enzyme-linked immunosorbent assay (ELISA) technique, could not detect any cross-reactivity between lupin and peanut, or lupin and other legumes including soybean, yellow pea, green lentil, as well as some nut and milk proteins, however, they did detect significant cross-reactivity with almond, and pumpkin seeds, amongst others [32]. This was unexpected and suggests that peanut-sensitised individuals are not the only ones at risk of allergic reactions as a result of lupin ingestion.

Trials studying this cross reactivity have so far mostly relied on skin prick tests (SPTs) and radioallergosorbent tests (RAST), which reliably measure the presence of IgE antibodies, but not necessarily clinical allergy [4]. For instance, an individual who is known to be clinically allergic to peanut is not necessarily allergic to lupin, even if a SPT is positive for lupin in that individual. The only way of determining clinically important food allergies is oral food challenges [33]. Double blind, placebo controlled food challenges (DBPCFC) employed to test cross reactivity across different species of legumes revealed that only 5% of positive SPTs were clinically significant [4]. Also, using cases of allergic reactions to lupin in individuals who have a known allergy to a legume other than lupin, and who state that they have not had lupin before, cannot be used as evidence for cross reactivity, as the individual may have been unknowingly exposed to lupin prior to the allergic reaction.

It becomes apparent that there is a need for further studies into the correlation between lupin exposure and lupin allergy and cross reactivity amongst legume allergies using oral food challenges.

The RPAH Allergy Clinic recognized this need and is currently involved in such trials. It has routinely recorded over 2500 SPTs to peanut, lupin and soybean over the past two years, and is continuing to do so. Currently, individuals who have tested positive to lupin are being recruited and given oral food challenges of lupin containing foods to ascertain clinically important allergenicity. The results will show how many subjects are allergic to peanut only, lupin only, or to both peanut and lupin.

Peanut allergy is the most common cause of food-related anaphylaxis and usually persists through to adulthood [34]. Whether it might be rivalled in severity and persistence by lupin allergy [16] is unknown. If so, replacing soy with lupin may not be as attractive as initially believed. Results of such studies as conducted at the RPAH Allergy Unit will have numerous implications, most notably for food labelling regulations, so problem foods can be avoided by individuals allergic to lupin and/or other legumes, especially to peanut.

Future studies may also investigate whether the rise in peanut allergy may be associated with a rise in lupin exposure.

HYPOTHESIS

Diets between lupin sensitised and non-sensitised individuals does differ, especially in intake of high fibre/lupin breads, with a higher intake in lupin sensitised subjects, as some fibre rich breads in Australia are known to contain lupin and breads have been one of the first foods to contain lupin [18,19]. (see Appendix for a list of lupin containing foods available in Australia)

AIMS

To determine whether there is a correlation between lupin exposure in foods and food ingredients and lupin sensitisation in patients with and without existing food allergies and sensitisations.

To identify any other dietary differences among the study groups.

To identify areas of improvement of the food frequency questionnaire as a study tool for the successful continuation of the Lupin Exposure Study.

METHODS

Recruitment

The Lupin Exposure Study consisted of the design, distribution and analysis of a food frequency questionnaire (FFQ) (see Appendix) - adapted from a previous study, the Women's Health Study - to identify any dietary differences among patients of the RPAH Allergy Unit who present with lupin and/or other food sensitisations, in particular peanut.

The FFQ, enquired about the subjects' environment, i.e. potential environmental exposure to lupin, participants' knowledge about the crop and which foods might contain it, as well as whether there are any existing food allergies or intolerances, including usual symptoms, in the subjects or any other persons in their household. It also determined factors that might influence food purchasing such as

an existing food allergy, food intolerance, or heart disease/diabetes, as well as other variables such as fat/salt/cholesterol contents of foods, for instance, or health claims listed on packaging. The participant also indicated whether he/she is a vegetarian, and whether fish/seafood, chicken, eggs, dairy and soy are consumed.

Subjects needed to state how often they consume a food, specifying frequency per day, week or month, rarely (less than once a month), or never within the last six months. This was indicated by using the following key: xD, xW, xM (where x is the number of times a food is eaten per day, week or month, respectively. Eg, 2D = twice a day, 4M= four times a month); L indicated consumption less than once a month. The list of food products included breakfast cereals, grains and flours, pasta and flours, crisp breads, sweet biscuits, cakes/buns/pastry and muffins, pancakes/pikelets, breads, breakfast and snack bars, vegetable sauces and soups, dairy and special nutrition drinks, ice creams, dairy desserts, deli/processed meats, sauces, mayonnaise and salad dressings, butter and margarines, snack foods, spreads, confectionery, chocolates, cold drinks, flavoured milk drinks, as well as cosmetics and creams/moisturizers. For each category, subjects could also mention products used that are not included in the current list. Completion of the FFQ took approximately 30 minutes.

Subjects were recruited in two ways. All patients coming into the Allergy Unit for food allergy skin prick testing between 13 August and 28 September 2007 (7 week period, 23 clinic days) were asked to participate in the study and given a questionnaire to fill in, irrespective of whether they have been skin prick tested positive or negative for lupin or any other food. Patients who have been skin prick tested in the past, and who came to the clinic for follow up consultations and/or desensitisation injections were also asked to participate. If the subject was a child, the parent/guardian was asked to fill in the FFQ on their child's behalf. Participants were asked to complete the FFQs on site if possible and to hand them in before they left the premises, but the option was given to take the FFQ home with them along with a reply paid envelope and to post it back to the Allergy Unit.

The FFQ was also mailed out to the 54 individuals who had been previously challenged with lupin at the RPAH Allergy Unit due to positive results to skin prick tests (SPTs).

Candidates were approached either by the dietetic student in the Allergy Unit's waiting rooms or asked to participate by the consulting doctor or nurse. FFQs were also available at both reception desks. Additionally, the dietetic student phoned patients that had been at the unit for SPTs for food allergies within the data collection period to ask whether they had received a questionnaire. Questionnaires were mailed to those individuals who could be contacted and who had not received one at the Unit.

Apart from having had a food allergy skin prick test, there were no other inclusion criteria for this study. Excluded from the study were infants who were breastfed or who received formula.

The study has been approved by the Ethics Review Committee (RPAH Zone) of the Sydney South West Area Health Service.

Data Entry and Analysis

Data from completed FFQs was entered into Excel spreadsheets and correlated to results of SPTs, as listed on the Allergy Unit's database, and to clinical reactions to oral lupin challenges. Subjects were divided into one of three groups according to the presence of lupin and/or peanut sensitisation:

- Lupin SPT positive, Peanut SPT positive (L+P+)
- Lupin SPT negative, Peanut SPT positive (L-P+)
- Lupin SPT negative, Peanut SPT negative (L-P-)

No subjects were identified as being lupin positive but peanut negative (L+P-).

Although sensitisation is usually defined as a SPT ≥3x3mm, in the present study any SPT ≥1x1 was considered to be a biomarker of exposure. Defining a relationship with clinical allergy would require oral challenges which were beyond the scope of the present study.

In addition to categorising subjects according to the presence or absence of lupin and peanut sensitisations, each of these groups was further subdivided into age groups younger and older than five years. This was done as food consumption in one to four year olds differs from that in individuals that are able to feed themselves and that can communicate likes and dislikes. Furthermore, children younger than five years are more likely to have multiple food allergies/sensitisations that they will grow out of as they grow older. This would also have an impact on food intake.

For each of the food categories analysed (see below), numbers of serves of a food eaten were translated into a fraction reflecting the number of serves eaten per day. For example, 3M (three times a month) was translated into 3/30, or 0.1, and 2W (twice a week) into 2/7, or 0.3. L (less than once a month) was translated into “once every 2 months” or 1/60, or 0.02. This allowed for categorising certain foods together and calculating total serves eaten per day. For example, in addition to analysing consumption of individual legumes such as chickpeas, lentils, kidney beans, etc. a number for consumption of total legumes could be generated, allowing for comparison of entire food groups between study groups.

Analysis also included identifying any lupin containing foods (if present) in the diet in correlation with existing allergies. A list of known lupin-containing foods, previously compiled and regularly updated by the RPA Allergy Unit, was used for this purpose (Appendix). This information was used to

identify a correlation between an increased exposure to lupin in the diet and increased likelihood of developing lupin sensitisation and/or allergy.

Once all information was entered into Excel spreadsheets, consumption of individual foods and food categories among the three subject groups listed above was statistically compared using SPSS 15.0. A *P* value of 0.05 or less was considered statistically significant.

The following categories were analysed:

- Nuts and Seeds
- Lupin bread
- High Fibre Bread
- Legumes (individual varieties and total)
- Curry
- Confectionery (chocolate and non-chocolate varieties)
- Sweet baked Products

(For further discussion of each of these groups, please refer to the Appendix)

For each of the categories age/sensitisation crosstabulations were performed to generate Chi-Square tests for independence. Categorical variables were used to indicate the sensitisation and age ("<5" or ">5") groups an individual belongs to, frequency of consumption of a food (usually less or or more frequently than once a month), as well as the presence of another other food sensitisation (treenut, sesame, almond).

Sensitisation groups compared were

L+P+/L-P+,

L+P+/L-P-,

L-P+/L-P-,

As well as L+/L- and P+/P- in some cases, where L- was derived by combining groups L-P+ and L-P-, and P+ is derived by combing groups L+P+ and L-P+. No L+P- subjects participated.

Food consumption was specified as either less than once per month (0.03 and below; 0.03*30=0.9, i.e. 0.9 times per month) and greater than once per month (>0.03). This cut-off was chosen as a food eaten less frequently than once a month was thought to be unlikely to cause food sensitisation and thus positive SPT results. Foods not consumed were included in this group. The only exception to this was made with sweet baked products as these were consumed the most frequent of all foods listed. Consumption of sweet baked products was thus categorised into eaten less or more frequently than 10 times per month (or < or >0.33; 0.33*30=9.9, i.e. 9.9 times per month; this also equals to 2.3 times per week).

In addition to the statistical analyses of the seven food groups listed above, SPSS was used to assess any relationship between lupin/peanut sensitisation and food sensitisations to treenuts, sesame and almond. The same statistical test as for foods was performed, but food sensitisation could be grouped into simple yes/no categories.

In cases where the sample size was too small (e.g. less than 5 individuals of a certain age group consumed the food analysed), the *P* value generated by the Fisher's Exact Test was used to determine significance of variability in consumption or sensitisation between groups.

RESULTS

Participation

Of 100 participants, 30 had lupin and peanut sensitisation (L+P+), 14 were sensitised to peanut but not lupin (L-P+), and 56 were neither sensitised to lupin nor peanut (L-P-). No participants were sensitised to lupin but not peanut (L+P-). Of the 56 L-P- individuals, 13 (23%) had some other food sensitisation, and 43 (77%) had no other existing food sensitisation. 18 of the 100 perticipants were younger than five years, and 82 were older than five years. The average ages of groups L+P+ and L-P+ are similar at 15 years and 10 years respectively. L-P- individuals were generally older, with the average age being 30 years. *Figures 1* and *2* summarise the results.

Figure 1: Study population of the Lupin Exposure Study.

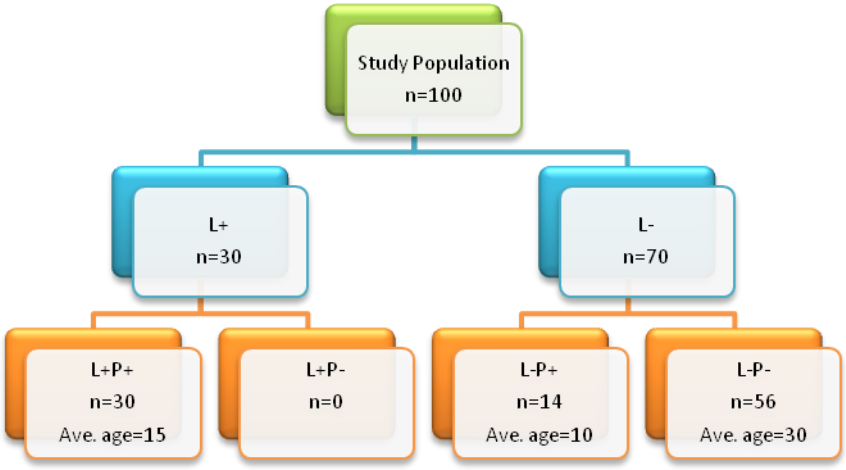
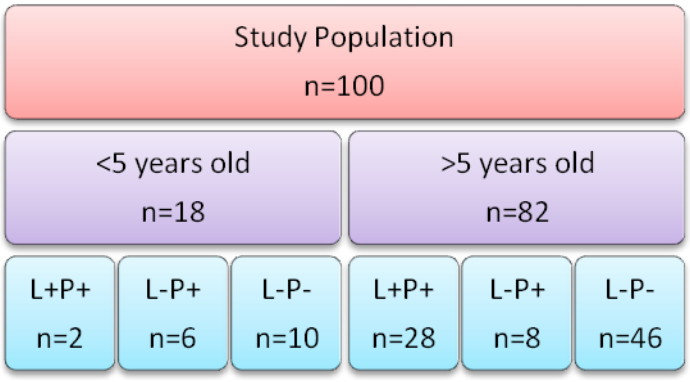


Figure 2: Study population stratified by age.



Foods

Nuts and Seeds

Under 5 year olds

Chi-Square tests for independence revealed no relationship between consumption of nuts and seeds and the three sensitisation groups (L+P+, L-P+, and L-P-) in subjects under the age of five years. However, a general trend towards increased consumption of nuts and seeds could be observed with decreased sensitisation; lowest intake occurs in the L+P+ group (0%; n=0), moderate intake in the L-P+ group (33.3%; n=2), and highest intake in the L-P- group (70%; n=7).

Over 5 year olds

More interesting results were observed with the over five year old age group. Highest consumption of nuts and seeds more often than once a month occured in group L-P- (89.1%; n=41), followed by L+P+ (35.7%; n=10) then L-P+ (25.0%; n=2). Highly significant differences in nuts and seeds consumption have been found between groups L+P+ and L-P- (p =.000) and L-P+ and L-P- (p =.000), which can be attributed to the difference in peanut sensitisation between the groups. Statistical comparison of peanut positive individuals consuming nuts and seeds more often than once a month (33.3%; n=12) to peanut non-sensitised individuals (89.1%; n=41) revealed a significant difference (p=.000). A Fisher's Exact Test indicated no significant association between lupin sensitisation and nut/seed intake for L+P+/L-P+ (p=.69).

Lupin containing bread

Under 5 year olds

No lupin containing bread was consumed by subjects under the age of 5 years.

Over 5 year olds

No significant differences in consumption of lupin containing breads were observed between groups of subjects over five years of age. Only one subject each within the L+P+ group (3.6%; n=1) and L-P+ group (12.5%; n=1), and three subjects within group L-P- (6.5%; n=3) consumed lupin bread (Appendix, Figures 4&5).

High Fibre Breads

Under 5 year olds

No significant differences in high fibre bread consumption were found between groups of subjects less than five years of age. No high fibre bread was consumed within group L+P+, while half of the L-P+ individuals (n=3) and 60% of L-P- individuals (n=6) ate high fibre bread more than once a month.

Over 5 year olds

As with the <5 years subpopulation, Chi-Square analysis revealed no significant relationship between lupin or peanut sensitisation and consumption of high fibre bread. 57.1% of L+P+ (n=16), 75.0% of L-P+ (n=6), and 56.5% of L-P- (n=26), consumed high fibre breads more than once a month (Appendix, Figures 6&7, Table 7).

Legumes

Under 5 year olds

Legumes consumed by the under five years age group included broadbeans, chickpeas, lentils, tofu, yellow split peas and green peas. No statistically significant relationship could be determined

between legume consumption and lupin sensitisation, however there was a general trend of increased consumption of legumes with decreasing severity of sensitisation, i.e. least consumption occured in the L+P+ group, highest consumption in the L-P- group (Appendix, Table8).

Over 5 year olds

In addition to the legumes consumed by subjects less than five years of age, subjects older than five years also listed dhal, lupini, miso, mung beans, red kidney beans, green beans, sensitisations beans and black eyed beans. However, only broadbeans, chickpeas, dhal, lentils, miso, mung beans, tofu and red kidney beans were consumed by more than one person. Significant differences in legume consumption between groups were found for lentils, chickpeas, dhal, tofu, broadbeans and legumes total. These differences were found between groups L+P+/L-P-, L+/L-, and P+/P-. In all cases, the lupin *and* peanut negative groups consumed more legumes than the lupin *and* peanut sensitised groups (Appendix, Table 9).

No significant difference in legume consumption was found between groups L+P+ and L-P+, however a general trend of *increased* consumption of legumes (> 1 per month) could be observed among L+P+ individuals as opposed to L-P+ individuals (Appendix, Table 10).

Curry

Under 5 year olds

No significant differences in curry consumption could be detected among sensitisation groups in the under five year old subpopulation. A Fisher's Exact Test for groups P+ vs P- revealed borderline significance (2-sided p=.066; 1-sided p=.057), where only 12.5% of peanut sensitised individuals consume curry more than once a month, as opposed to 60% of peanut non-sensitised individuals. 0% of L+P+ (n=0), 16.7% of L-P+ (n=1), and 60% (n=6) of L-P- subjects consumed curry.

Over 5 year olds

Chi-Square analyses revealed significant differences in curry consumption among all sensitisation groups except between groups L+P+ and L-P+ (p=1.00) (Appendix, Table 11). 14.3% of group L+P+ (n=4), 12.5% of group L-P+ (n=1), and 52.2% of group L-P- (n=24) consumed curry more than once per month, i.e. most curry was consumed within the lupin and peanut non-sensitised group.

Chocolate containing Confectionery

Under 5 year olds

No significant relationships between lupin sensitisation and consumption of chocolate containing confectionery could be observed with subjects under the age of 5 years. Proportionally, most chocolate was consumed in groups L+P+ and L-P+ (100% within each group), followed by group L-P- (80%). In absolute terms, however, chocolate consumption seemed to increase with decreasing numbers of food sensitisation, with 2 L+P+ subjects, 6 L-P+ subjects, and 8 L-P- subjects consuming chocolate more than once a month.

Over 5 year olds

As with the <5 year old subjects, no significant relationships between lupin sensitisation and consumption of chocolate containing confectionery could be observed among subjects older than 5 years of age. However, interestingly chocolate consumption seemed greater in lupin sensitised individuals than lupin non-sensitised ones as 64.3% of L+P+ (n=18), and 50% of L-P+ (n=4) individuals ate chocolate containing confectionery more than once a month.

Chocolate free Confectionery

Under 5 year olds

No significant relationships between lupin sensitisation and consumption of chocolate free confectionery could be observed with subjects under the age of 5 years. A trend of increasing

consumption with decreasing number of sensitisations was observed. Within groups L+P+, L-P+, and L-P-, 0% (n=0), 33.3% (n=2), and 60% (n=6) consumed chocolate free confectionery.

Over 5 year olds

As with the under 5 year old subpopulation, no significant relationships between lupin sensitisation and consumption of chocolate free confectionery were found with subjects over the age of 5 years. 5 subjects within group L+P+ (17.9%), 1 subject within group L-P+ (12.5%), and 12 subjects within group L-P- (26.1%) consumed chocolate free confectionery more than once a month. As with chocolate, consumption of chocolate free confectionery seemed higher in the lupin and peanut sensitised group (L+P+) than in the lupin non-sensitised, peanut sensitised group (L-P+).

Sweet Baked Products

Under 5 year olds

For this food group, frequency of consumption was classified as either less often (n=8) or more often (n=10) than 10 times per month (or 2.3 times per week).

Except for peanut sensitised (P+) versus peanut non-sensitised (P-) (Fisher's Exact Test, p=.025), Chi-Square analyses revealed no significant differences in consumption of sweet baked products between the various sensitisation groups. 7 of 8 peanut sensitised individuals (87.5%) consumed foods in this category > than 10 times per month opposed to 3 of 10 peanut negative individuals (30%).

Within groups L+P+, L-P+, and L-P-, 100 % (n=2), 83.3% (n=5), and 30% (n=3), respectively, consumed sweet baked products.

Over 5 year olds

Of 82 individuals, 35 consumed sweet baked products <10 times per month, and 47 consumed them > 10 times per month. Chi-Square analyses revealed no significant differences in consumption between the various sensitisation groups. Comparing groups L+P+ and L-P+ revealed that a smaller

proportion (53.6%) of the lupin sensitised group ate sweet baked products, as opposed to the lupin non-sensitised group (62.5%). In absolute terms, however, more lupin sensitised individuals claimed to consume sweet baked products (n=15) than lupin non-sensitised individuals (n=5) (Appendix, Figure 11).

Treenut Sensitisation

Table2: Frequencies of treenut sensitisation in subjects younger and older than 5 years.

Age			Frequency	Percent
<5	Valid	nonsensitised	12	66.7
		sensitised	6	33.3
		Total	18	100.0
>5	Valid	nonsensitised	53	64.6
		sensitised	29	35.4
		Total	82	100.0

Under 5 year olds

No significant associations between lupin and treenut sensitisation in subjects younger than five years could be determined using Fisher's Exact Test analyses. 0% of group L+P+ (n=0), 50% of group L-P+ (n=3), and 30% of group L-P- (n=3) had one or more treenut sensitisation.

Over 5 year olds

A significant relationship between peanut and treenut sensitisations was observed in the older than five years subpopulation (P+ versus P-; p=.000), but not between lupin and treenut sensitisations. Nonetheless, most treenut sensitised individuals were found in the L+P+ group (n=21), some in the L-P+ group (n=6), and two treenut positive subjects had neither a lupin nor peanut sensitisation (L-P-, n=2).

Almond Sensitisation

Table3: Frequencies of almond sensitisation in subjects younger and older than 5 years.

Age			Frequency	Percent
<5	Valid	not sensitised	14	77.8
		sensitised	4	22.2
		Total	18	100.0
>5	Valid	not sensitised	63	76.8
		sensitised	19	23.2
		Total	82	100.0

Under 5 year olds

Significant differences in the presence of almond sensitisation were found between the following groups: L+P+/L-P-, L+/L-, and P+/P-. 100% of L+P+ subjects (n=2 of 2), 33% of L-P+ subjects (n=2 of 6), and 0% of L-P- subjects (n=0 of 10) had an almond sensitisation. 50% of peanut positive individuals (n=4 of 8), and none of the peanut negative individuals (n=0 of 10) had an almond sensitisation.

Over 5 year olds

In the over five year old population, comparisons of all sensitisation groups revealed a significant difference in prevalence of almond sensitisation, with the exception of L+P+ versus L-P+. Prevalence of almond sensitisation was highest among L+P+ (50%; n=14) and L-P+ (50%; n=4) groups, and lowest in group L-P- (2.2%; n=1). Peanut sensitised individuals were much more likely to have an almond sensitisation (p=.000). In this study population, 50% (n=18) of peanut positive, and 2.2% (n=1) of peanut negative subjects had an almond sensitisation.

Although there was no significant difference in almond sensitisation between groups L+P+ and L-P+ (p=1.00), more individuals were sensitised to almond when they were also sensitised to lupin (n=14), than when they were not sensitised to lupin (n=4).

Sesame Sensitisation

Table4: Frequencies of sesame sensitisation in subjects younger and older than 5 years.

Age			Frequency	Percent
<5	Valid	nonsensitised	14	77.8
		sensitised	4	22.2
		Total	18	100.0
>5	Valid	nonsensitised	70	85.4
		sensitised	12	14.6
		Total	82	100.0

Under 5 year olds

No significant associations between lupin and sesame sensitisation in subjects younder than five years could be determined using Fisher's Exact Test analyses. 50% of the L+P+ group (n=1), 33.3% of the L-P+ group (n=2), and 10% of the L-P- group (n=1) had a sesame sensitisation.

Over 5 year olds

Significant relationships were found among *all* sensitisation groups, including between groups L+P+ and L-P+ (p=.033), except between groups L-P+ and L-P-, as neither group contained any individuals sensitised to sesame. 42.9% of the L+P+ group (n=12) had a sesame sensitisation. There was also a significant difference in sesame sensitisation when comparing sesame sensitisation between peanut positive (33.3%, n=12) and peanut negative (0%, n=0) subjects (p=.000) (Appendix, Figures 15&16, Table 14).

DISCUSSION

Due to limited information available, exposure to lupin through occupation, place of work/home, pet food, and cosmetics was not considered in this study. Emphasis was solely placed on dietary factors. Co-sensitisations to other nuts and seeds were also explored. Results are discussed below.

Foods

For the purpose of this study, comparison of food intake and other variables between the groups L+P+ and L-P+ was the most interesting, as this comparison controlled for a confounding effect by peanut sensitisation. Any difference between the two groups was likely to be related to the absence or presence of lupin sensitisation, rather than peanut sensitisation/allergy, which—due to its notorious severity—is bound to have an influence on dietary practices.

While there are also cases of lupin sensitisation in the absence of peanut sensitisation (L+P-), no such individuals participated in this study, and comparison of this group to L-P- was not possible.

Nuts and Seeds

Over 5 year olds

The main finding within this food category was a difference in intake depending on peanut sensitisation. Peanut sensitised individuals consumed less nuts and seeds than non-sensitised individuals. Comparison of nuts and seeds consumption between groups L+P+ (35.7%) and L-P+ (25.0%) – i.e. controlling for peanut sensitisation as a confounding factor – showed that lupin sensitised individuals may consume more nuts and seeds than those not sensitised to lupin (Appendix, Figure 3 & Table 6). A larger sample size and comparison of groups L+P+ versus L+P- are needed to establish any significant trend. A correlation between intake of nuts and seeds and lupin sensitisation may be due to the avoidance of all or most nuts, as well as traces of nuts, in the presence of a peanut allergy.

Lupin containing Bread

Over 5 year olds

Results do not confirm the hypothesis that lupin sensitised individuals consume more lupin containing foods, especially bread, than individuals not sensitised to lupin. However, due to limitations of the FFQ and study design (discussed below), and due to the fact that lupin does not need to be labelled as an ingredient when present in small quantities [27], it is likely that consumption has been under estimated. Also, the sample size was too small to make any definitive conclusions.

High fibre Bread

Over 5 year olds

While a larger proportion of the L-P- group consumed high fibre bread (>once per month) than of the L+P+ group, in absolute terms a larger number of L+P+ subjects (n=16) consumed high fibre bread than L-P+ subjects (n=6) (Appendix, Figures 6 and 7). This is an important finding and should be reassessed in the continuation of this study as the sample size is growing, as any significant difference would support the hypothesis that lupin sensitised individuals are consuming more fibre rich breads than lupin non-sensitised individuals.

Under 5 year olds

As stated in the results section, least consumption of legumes occured in the L+P+ group, highest consumption in the L-P- group, and moderate consumption in the L-P+ group. The reason for this is unknown and worth investigating if a trend can be ascertained with a larger sample size.

Legumes

Over 5 year olds

As mentioned before, for the purpose of this study, comparison of food intake and other variables between the groups L+P+ and L-P+ was of particular interest in this study. Even though no significant difference in legume consumption was found between these two groups, a general trend of an *increased* consumption of legumes (> 1 per month) could be observed among L+P+ individuals as opposed to L-P+ individuals (Appendix, Figure 8), lentil consumption being the only exception (0% in L+P+ group). Greater consumption of legumes in lupin sensitised individuals is an important finding and should be considered in the continuation of this study as the sample size is growing, as an increased exposure to other legumes may play a role in lupin sensitisation.

Curry

Over 5 year olds

No significant difference in curry consumption was found between groups L+P+ (14.3%; n=4) and L-P+ (12.5%; n=1), but intake was marginally higher in the lupin sensitised group. During the course of this study, a possible association between fenugreek and lupin sensitisation was identified. Fenugreek is a common ingredient in curry pastes and powders, and future research should include further investigations into this matter, as an increased exposure to fenugreek in curry or otherwise may increase risk of developing lupin sensitisation.

Chocolate containing Confectionery

Over 5 year olds

Chocolate consumption seemed larger in the lupin sensitised population than in the lupin non-sensitised population, though a larger sample size is needed to determine any definitive trend (Appendix, Figures 9&10*)*. In contrast, peanut sensitised individuals seemed to eat less chocolate containing confectionery. This was expected, as chocolate is often avoided by peanut allergic individuals, due to the high risk of contamination by peanut in production lines. It is unknown whether any chocolate confectionery in Australia contains lupin as an ingredient or contaminant.

Sweet baked Products

Over 5 year olds

A larger sample size is needed to ascertain whether the trend of greater consumption of sweet baked products among lupin sensitised individuals (n=15) than lupin non-sensitised individuals (n=5) holds any significance. As discussed in the background section, lupin has many desirable characteristics for the production of baked products [11], and this category may significantly contribute to the lupin exposure.

Nut and Seed Sensitisations

The presence/absence of nut and seed sensitisation was included in the analysis. This was done as correlations between nut and seed allergies and lupin allergies, such as cross-reactivity between lupin and almond and pumpkin seeds [32], have been cited in the literature. Also, nut and/or seed sensitisations/allergies may cause changes in food intake that may affect an individual's exposure to lupin; this may be investigated further in the continuation of this study.

Treenut Sensitisation

No statistically significant difference could be observed between groups L+P+ and L-P+ (p=1.00) (Appendix, Table 12). Nonetheless, more individuals seemed to be sensitised to treenut in the presence of lupin sensitisation. 75% of peanut sensitised individuals are sensitised to one or more treenuts, as opposed to 4.3% of peanut non-sensitised individuals (Appendix, Figures 12&13).

Almond Sensitisation

Under 5 year olds

While there was no statistically significant difference in almond sensitisation between groups L+P+ and L-P+, proportionally, there is a larger prevalence of almond sensitisation in the lupin sensitised group (Appendix, Figure 14 & Table 13). However, a larger sample size is required to determine any significant trend.

Sesame Sensitisation

Over 5 year olds

A significant difference between groups L+P+ and L-P+ (p=.033) indicates that lupin sensitisation increases the likelihood of also having a sesame sensitisation (and vice versa), irrespective of whether there is a peanut sensitisation. This is important finding and the relationship between these two sensitisations should be researched further to explore the possibility of cross-reactivity between lupin and sesame.

The Food Frequency Questionnaire

While a FFQ is a good tool to collect a large amount of information across a broad population, common problems with this form of data collection include over and under estimations of food consumption due to false recollection of intake, not understanding instructions on how to answer a question, dishonesty or lack of interest on the part of the participant. Often questions were answered incompletely.

Incomplete and inaccurate FFQs may also have been returned due to the questionnaire's length. It includes extensive lists of foods and food categories and, combined with the method of specifying frequency of consumption, completion became laborious and time consuming. Parents with young children suffering from some form of sensitisation or who are irritated by the skin prick test often felt overwhelmed by the additional task of completing a questionnaire.

All participants were patients of the RPAH Allergy Unit, thus there may have been selection bias. For instance, lupin and peanut negative individuals (L-P-) may not be a true reflection of non-allergic individuals in the general population, as patients are likely to have some other health concern such as food intolerance, which may have been the reason they sought professional advice at the Allergy Unit. Food intolerances, in turn, are likely to impact a person's diet, as some foods may be avoided.

Furthermore, no participants were positive to lupin but negative to peanut. It is likely that individuals who are allergic to peanut (or their parents) were more likely to participate due to a greater awareness of the potential danger of anaphylaxis upon ingestion of lupin.

Not all participants were skin prick tested on the day they were given the questionnaire. Some participants had done the SPT within a few weeks, sometimes months, before given a questionnaire. This creates the possibility that they may have changed their diets in response to a positive SPT result. As a result, answeres to questions within the FFQ may not have reflected their usual diet (and thus exposure to lupin).

In light of the shortcomings listed above, some recommendations can be made regarding the improvement of the study in the future:

i) The FFQ should be shortened to the most relevant foods consumed. This compromises the quantity of information collected, but may improve the quality of data and provide a more accurate reflection of the association of diet and lupin sensitisation. For instance, instead of providing an extensive list of breads, it may be more practical to let participants state how often they consume white, wholemeal, or wholegrain breads (stating name, brand, baked) in addition to breads known to contain lupin.

ii) Instead of specifying how often a food is consumed per day, week or month, this process could be simplified to daily, weekly, monthly, rarely and never. This would be sufficient to assess exposure to lupin in the diet and would make completion of the FFQ a less complicated task.

iii) Participants should be receiving the FFQ on the day of their SPT, or should not have been skin prick tested more than a few weeks prior to that date. In the latter case, participants must be notified that they should provide information about their usual diet if they have made any recent changes. The date of the SPT should also be specified.

CONCLUSION

Results need to be interpreted with caution as the sample size was very small. This report forms the basis for further data collection and is not meant to draw definite conclusions.

No significant differences in food intake were found in relation to lupin sensitisation, when peanut sensitisation was controlled for as a confounding factor; however the data should be re-analysed with a larger sample size during the continuation of this study. Observations are summarized in the table below.

Table5: Main findings relating to lupin sensitisation and diet in > 5 year olds.

Food Category	Consumption
Nuts and Seeds	**increased**
Lupin Bread	inconclusive
High Fibre Bread	inconclusive
Legumes Total	**Increased (except lentils)**
Curry	inconclusive
Chocolate containing confectionery	**increased**
Chocolate free confectionery	inconclusive
Sweet baked products	inconclusive

Observations made in the <5 years population are not listed here as sample size is too small to draw any conclusions.

Significant differences in food intake were mainly found in subjects over the age of five years in relation to peanut sensitisation (nuts and seeds, lentils, chickpeas, dhal, tofu, broadbeans and legumes total, curry), and also in the intake of sweet baked products in children younger than five years old. Less nuts and seeds, and less legumes, and more sweet baked products were consumed in the presence of a peanut sensitisation.

The hypothesis of an increased consumption of high fibre and lupin containg breads could not be supported in this study, but should also not be rejected until further investigations have been conducted.

Regarding other food sensitisations in subjects older than five years, more lupin positive/peanut positive individuals seemed to have another treenut sensitisation than lupin negative/peanut positive individuals. The same was observed for almond sensitisation. Most interestingly, lupin sensitised individuals were found to have a significantly higher rate of sesame sensitisation than lupin non-sensitised individuals.

No significant relationship between lupin and treenut, almond or sesame sensitisations could be found in participants younger than five years of age, and sample sizes were too small to observe possible trends.

Further investigations in this and other studies may include correlating total fibre intake and lupin sensitisation, and also an exploration of any relationship between consumption and/or sensitisation of fenugreek and lupin.
Lastly, this study did not take into consideration any occupational or demographic exposure to lupin, an area that may be focused on in the future.

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APPENDICES

APPENDIX 1: CALCULATIONS OF FOOD CATEGORIES

Categories chosen from the FFQ for analysis included:

- Nuts and Seeds
- Lupin bread
- High Fibre Bread
- Legumes (individual varieties and total)
- Curry
- Confectionery (chocolate and non-chocolate varieties)
- Sweet Baked Products

1) Nuts and Seeds

This category included the following items: almonds, brazil nuts, cashews, fenugreek, hazelnuts, linseed, LSA, pine nuts, poppy seeds, sesame seeds, sunflower seeds, walnuts, and other nuts or seeds listed by the participants. It is likely that results obtained are an underestimation of true exposure to nuts and seeds, as these are found in many other foods such as breads, pastes, spreads and sauces (e.g. pesto), which participants may not have taken into consideration.

Fenugreek seeds were added into this category as, during the same time of this project, it was discovered at the RPAH Allergy Unit that a high proportion of lupin sensitive individuals is also sensitive to fenugreek. Once again it is likely that intake is underestimated as fenugreek is a common ingredient of curry pastes and powders, which many participants may not have known.

2) Lupin Bread

The RPAH Allergy Unit's list (see Appendix 3) of lupin containing products was used for this purpose. Baker's Delight Hi Fibre Tiger Loaf/Roll Bread, Bodhi's Nurture Bread, Bodhi's Rye with Omega 3 Mini Pack, Bodhi's Wupper with Omega 3, Coles Lekkerbrot, Coles Roggenbrot, Country Life Bakery Lupin Loaf, Lifestyle Bakery Lupin Loaf, as well as Defiance Bread Mixes (Lupin Bran) (listed in the Pasta&Flours section of the FFQ). Also included in this section should have been IGA ISB Lavash Bread White/Wholemeal, Bazaar Ciabatta Rolls/Ciabatta 520g, Bazaar Lavash Bread White/Wholemeal, Bodhi's Wupper with Omega 3, as these are now known to contain lupin aswell. Other breads may also contain lupin as an unlabelled ingredient, so it is possible that more subjects consume more lupin than can be estimated from the FFQ results.

3) High Fibre Bread

As lupin is often used as a source of fibre in a range a food products, especially in breads, but may not be stated as an ingredient, attention was given to the consumption of breads that are high in fibre. To calculate this category, serves given for 'Bread – Multibrain' and 'Bread – Wholemeal' were added to give a total value for these products. However, this method needed to be reconsidered as many participants did not state any serves of wholemeal or wholegrain bread consumed, even when they did specify serves of Vogel's Bread eaten, for instance. Thus, the difference of total serves eaten of 'Vogel's Bread', 'Burgen Bread' (all varieties), all Tip Top Grain breads, as well as any other brands where participants stated "wholemeal" or "wholegrain" was added to the total serve previously calculated. However, a very small proportion of participants actually stated brand name and type of bread, and it is assumed that consumption of breads high in fibre is underestimated.

4) **Legumes**

Legumes listed in the FFQ included broad beans/four bean mix, chickpeas, dhal, lentils, lupini, miso, mung beans, tempe, tofu, and yellow split peas. In addition to these, participants also recorded red kidney beans, green peas, green beans, baked beans and black eyed beans. As with nuts and seeds, legume consumption may have been under reported as legumes occur in other foods such as spreads, pastes and sauces. Soy, which occurs as a common ingredient across many food categories, was not included in this category.

5) **Curry**

Curry paste, sauce and powder were added to the FFQ as they often contain fenugreek as an ingredient. Inclusion of fenugreek into the study is discussed above (Nuts and Seeds).

6) **Confectionery (Chocolate-containing and chocolate-free varieties)**

All products listed in the 'Confectionery' and 'Chocolate' sections of the FFQ, which includes products added by participants, were grouped together and then divided according to whether or not they contain chocolate. Subsequently total consumption was then calculated per person per day.

7) **Sweet Baked Products**

Total consumption of this food category was obtained by combining the following food categories as listed in the FFQ: sweet biscuits; cakes, buns, pastry, muffins; and pancakes and pikelets.

Not all foods/food categories listed in the FFQ were included in the analysis phase. This was mainly due to time constraints and worth investigating in the continuation of the study.

APPENDIX 2: RESULTS – Graphs and Tables

Nuts and Seeds

Figure 3: Consumption of nuts and seeds in subjects >5 years.

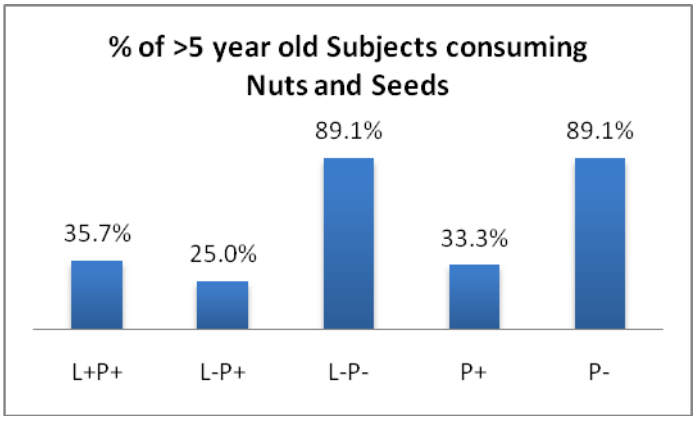
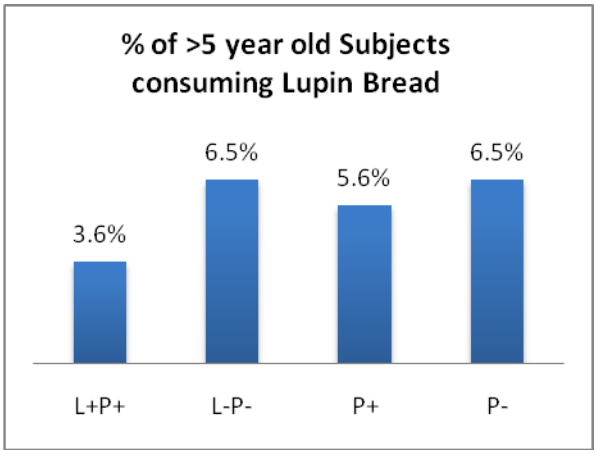
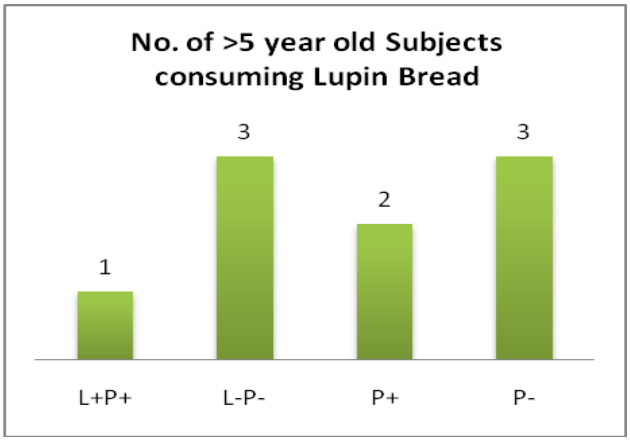


Table6: P values generated by Chi-Square tests among sensitivity groups of subjects consuming nuts and seeds in the >5years age group.

	L-P+	L-P-	P-
L+P+	p=.691	p=.000	
L-P-	p=.000		
P+			p=.000

Figures 4&5: Consumption of lupin containing breads in subjects >5 years.





High Fibre Bread

Figure 6

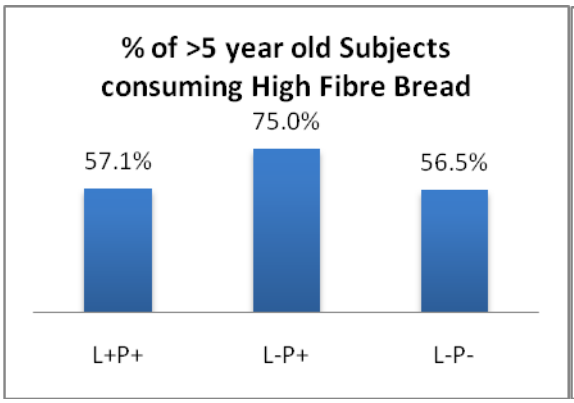


Figure 7

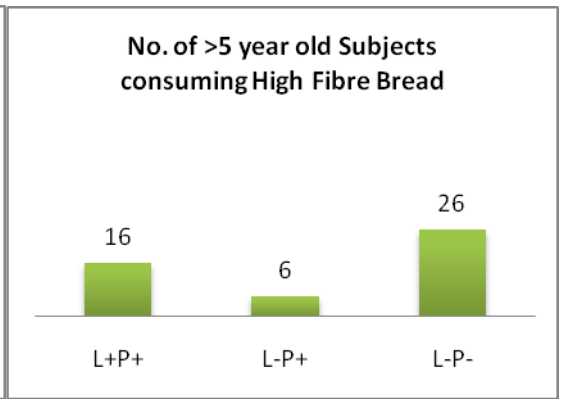


Table7: P values generated by Chi-Square tests among sensitivity groups of subjects consuming high fibre breads in the >5 year age group.

	L-P+	L-P-	L-	P-
L+P+	p=.44	p=1.00		
L-P-	p=.45			
L+			p=1.00	
P+				p=.82

Legumes

Table8: Number and Percentages of <5 year old subjects within each group consuming legumes >1 per month.

		Legumes total	Broadbeans	Chickpeas	Lentils	Tofu	Yellow split peas	Green Peas
L+P+	No. subjects consuming legume	1	0	1	0	0	0	0
	% Subjects consuming legume	50%	0%	50%	0%	0%	0%	0%
L-P+	No. subjects consuming legume	3	0	0	1	0	0	1
	% Subjects consuming legume	50%	0%	0%	16.70%	0%	0%	16.70%
L-P-	No. subjects consuming legume	6	2	3	4	1	1	1
	% Subjects consuming legume	60%	20%	30%	40%	10%	10%	10%

Table9: Proportions of individuals within their respective sensitivity groups consuming legumes >1 per month expressed as percentages, and P values generated by Chi-Square analyses for legume/sensitivity categories wherever significant associations could be determined.

	L+P+	L-P-	p-value	L+	L-	p-value	P+	P-	p-value
Lentils	0%	32.6%	0.001	0%	29.6%	0.001	2.8%	32.6%	0.001
Chickpeas	7.1%	37%	0.005	7.1%	31.5%	0.014	5.6%	37%	0.001
Dhal	0%	13%	.077 (.051)*	0%	11.1%	insig.	0%	13%	0.032
Tofu	3.6%	21.7%	0.044	3.6%	18.5%	.088 (.055)*	2.8%	21.7%	0.019
Broadbeans			insig.			insig.	2.8%	17.4%	.070 (.036)*
Legumes Total	28.6%	67.4%	0.002	28.6%	61.1%	0.01	27.8%	67.4%	0.001

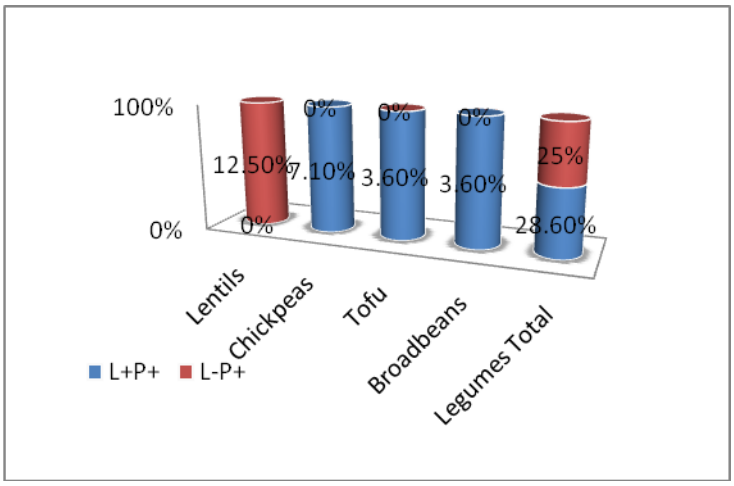
**Borderline significance. First number indicates 2-sided p-value, bracketed number indicates 1-sided p-value.*

Table10: Proportions of individuals within groups L+P+ and L-P+ consuming legumes >1 per month expressed as percentages and absolute numbers, and P values generated by Chi-Square analyses.

Column1	Column5	L+P+	L-P+	*P value*
Lentils	% within group*	0%	12.50%	.222
	No. of subjects within group*	0	1	
Chickpeas	% within group*	7.10%	0%	1.000
	No. of subjects within group*	2	0	
Tofu	% within group*	3.60%	0%	1.000
	No. of subjects within group*	1	0	
Broadbeans	% within group*	3.60%	0%	1.000
	No. of subjects within group*	1	0	
Legumes Total	% within group*	28.60%	25%	1.000
	No. of subjects within group*	8	2	

** legumes eaten >1 per month*

Figure 8: Increased consumption of most legumes in lupin sensitive individuals.



Curry

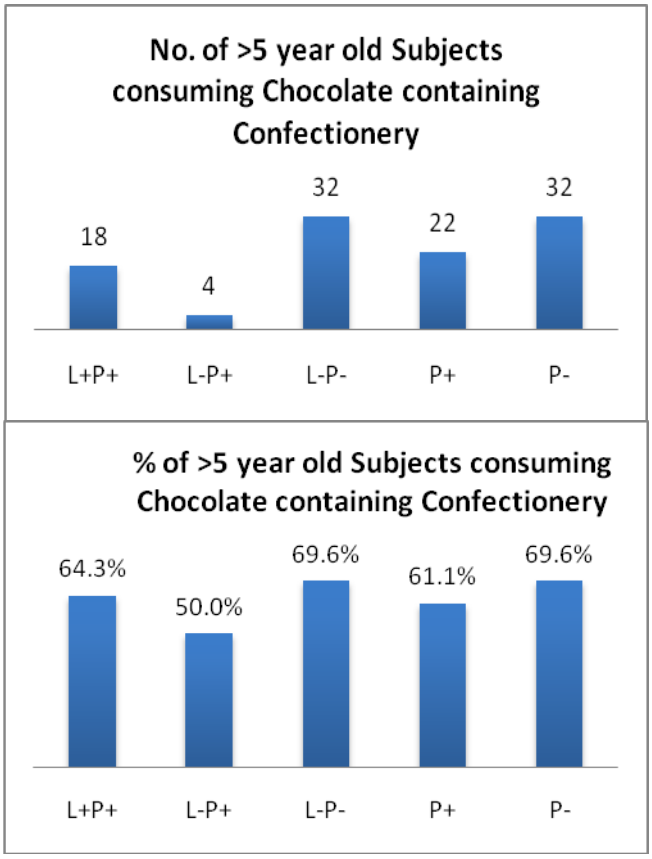
Table11: P values generated by Chi-Square tests among sensitivity groups of subjects consuming curry in the >5years age group.

	L-P+	L-P-	P-
L+P+	p=1.00	p=.001	
L-P-	p=.056 (.042)*		
P+			p=.000

**Borderline significance. First number indicates 2-sided p-value, bracketed number indicates 1-sided p-value.*

Chocolate containing confectionery

Figures 9&10: Consumption of chocolate containing confectionery in >5 year olds.

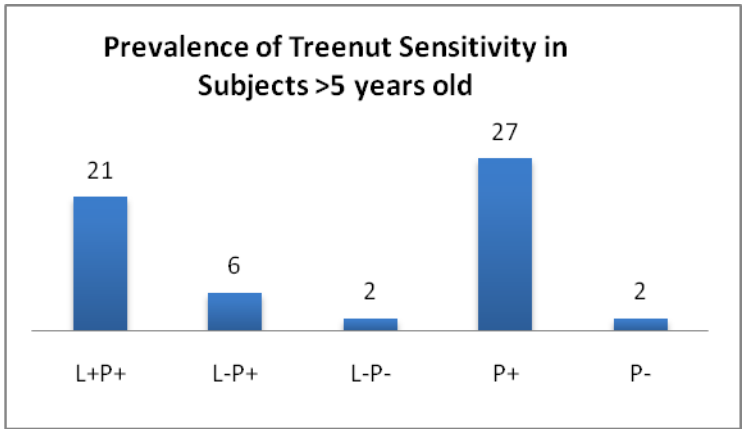


Sweet Baked Products

Figure 11: Consumption of sweet baked products (>10 times per month) by >5 years subpopulation.

Treenut Sensitivity

Figures 12&13: Prevalence of Treenut Sensitivity in the >5 years subpopulation.



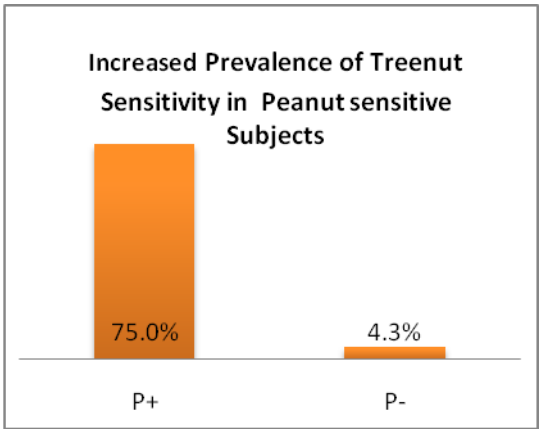


Table12: P values generated by Chi-Square tests between sensitivity groups of subjects with a treenut sensitivity in the >5years age group.

	L-P+	L-P-	L-	P-
L+P+	p=1.00	p=.000		
L-P-	p=.000			
L+			p=.000	
P+				p=.000

Almond Sensitivity (Under 5 year olds)

Figure 14

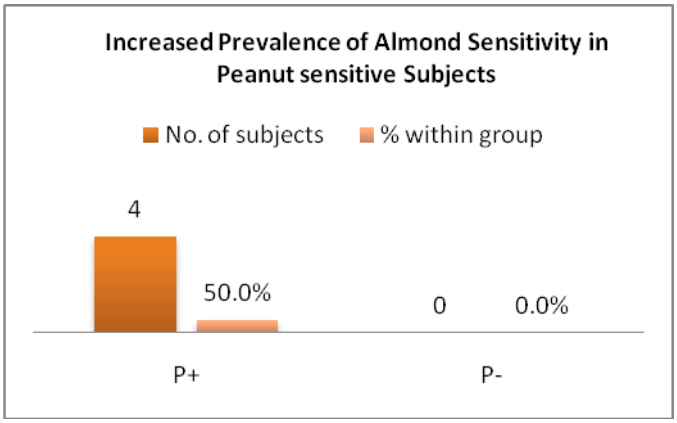
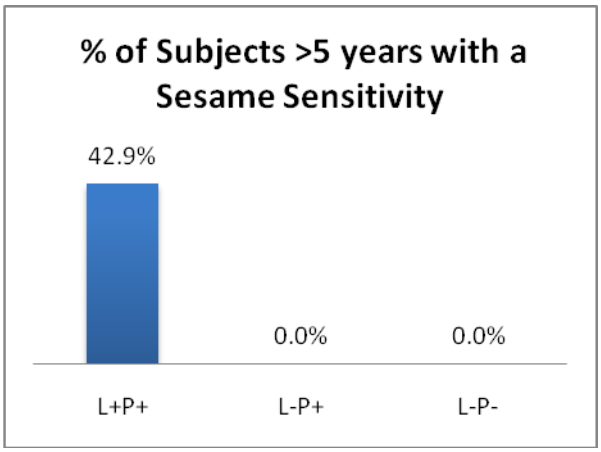


Table13: P values generated by Chi-Square tests between sensitivity groups of subjects with an almond sensitivity in the <5years age group.

	L-P+	L-P-	P-
L+P+	p=.429	p=.015	
L-P-	p=.125		
P+			p=.023

Sesame Sensitivity

Figures 15&16: Prevalence of Sesame Sensitivity in the >5 years subpopulation.



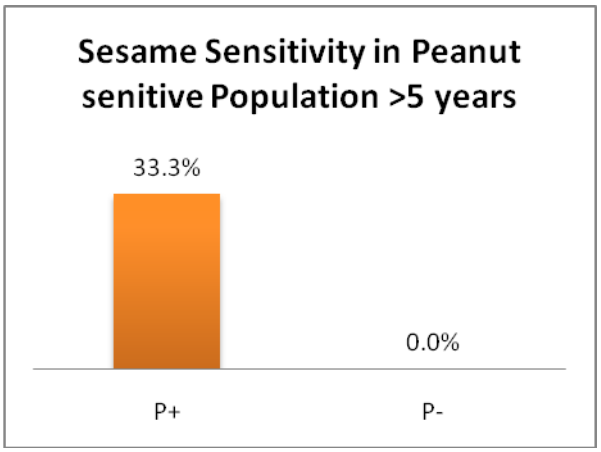


Table14: P values generated by Chi-Square tests among sensitivity groups of subjects with a sesame sensitivity in the >5years age group.

	L-P+	L-P-	L-	P-
L+P+	p=.033 (.024)*	p=.000		
L-P-	-			
L+			p=.000	
P+				p=.000

** First number indicates 2-sided p-value, bracketed number indicates 1-sided p-value.*

APPENDIX 3: Food list compiled by the Allergy Unit, Royal Prince Alfred Hospital
(correct at date of issue, and may be subject to change. Version 4, June 07)

Foods Containing Lupin

Product Name	Manufacturer
Baker's Delight Hi Fibre Tiger Loaf/Roll	Baker's Delight
Bazaar Ciabatta Rolls/Ciabatta 520g	George Weston
Bazaar Lavash Bread White/Wholemeal	George Weston
Bodhi's Nurture Bread	Bodhi's Bakehouse
Bodhi's Rye with Omega 3 Mini Pack	Bodhi's Bakehouse
Bodhi's Wupper with Omega 3	Bodhi's Bakehouse
Coles Lekkerbrot	Coles Supermarket
Coles Roggenbrot	Coles Supermarket
Country Life Bakery Lupin Loaf	Country Life Bakery
Hi Fibre Fruit/Nut Muffins	Muffin Break
IGA ISB Lavash Bread White/Wholemeal	George Weston
Jaka Confectionery (Rum Balls and various other flavours)	Jaka, Holland (imported, available at Coles Supermarkets)
Lifestyle Bakery Lupin Loaf	Lifestyle Bakery
Mrs Crocket's Lite Coleslaw	Mrs Crocket's (sold at Woolworths, Coles and independent supermarkets)

Foods Free of Lupin

Product/Manufacturer Name
Angas Park
Arnott's Biscuits
Ausfine Foods International (Dairy Products, Meat Products, Bakery Ingredients, Confectionery Ingredients, Ice Cream Ingredients, Manufactured Meat Ingredients, Nutraceutical Ingredients)
Barilla Pasta & Sauces
Bellamy's Organic Baby Food
Black Swan Foods (Dips, Yoghurts, Turnovers, Snacks, Antipasto) Poseidon Dips
Cadbury Schweppes (Cadbury, Schweppes, Dr Pepper, Halls, Trident, Gatorade, Natural Confectionery Company, Mother Earth)
Chris' Dips
Fawcett Brothers (Old Country, Stromboli, Aristocrat)
Fonterra (Perfect Italiano, Western Star, Mainland, Bodalla)
Freedom Foods
Frucor (G-Force, Mizone, H2Go, Evian, V, Bundaberg Ginger Beer, Danone)
George Weston Foods (Don Smallgoods, Tip Top, Burgen, Sunblest, Tip Top Up, Noble Rise)
Golden Circle
Goodman Fielder (Pampas, Meadow Lea Spreads, White Wings)
Heinz (Greenseas, Weight Watchers, Patak's, Imperial Garden, Hamper - corned beef, Tom Piper)
Ingham's Enterprises
Kelloggs
KFC
La Famiglia Fine Foods (Bread)
Devondale (Dairy Products)
Mayfair Quality Foods (Mayfair, Dandy, Presto)

McCain
McCormick Foods Australia (Herbs, Spices, Sauces, Mixes etc.,)
McDonalds (Restaurant & Café Range)
National Foods (Berri, Big M, Pura, Yogo, Farmers Union, Yoplait, Fruche, Divine Classic)
Paradise (biscuits, confectionery/muesli bars)
Pattie's Bakery (Herbert Adams, Four N' Twenty, Pattie's range, Nanna's)
Players Biscuits (made by Arnott's Biscuits)
Real Foods (Corn Thins, Rice Thins)
Specialty Cereals (Vogel's Cereal)
The Cookie Man
Vogel's Cereals
Woolworths "Homebrand" Products
Yum Restaurants International (KFC, Pizza Hut)
Poultry Processing & Smallgoods
Cavos Products
Green Star Enterprises Pty Ltd
Kennedy's Seafood, Meat & Game Products
Mavrikis Chicken
Meletis Foods
M & J Chickens
M & M Poultry
Poultry Traders International
Premium Poultry Products
Primo Smallgoods & Mayfair
Pronto Pollo
Quicken Chicken/Cordina Chicken
Red Lea Chickens
Sargent's Pty Ltd (Pies, Sausages)
Summertime Chicken
Supreme Poultry and Chicken
Bakery & Specialty Products
Baraka Delicacies & Wholesome Foods
Eagle Fine Foods
Brumby's Bakeries Limited
Flavour of Italy Pty Ltd
Hazelgrove's Homemade
Healthybake Breads
Heavenly Organic
Olive Green Organics
Goodman Fielder [Quality Bakers, Helga's, Mighty Soft, Vogel's Bread (Mixed Grain, Harvest Drye, Seven Seed, Soy & Linseed), La Famiglia (QLD), Wonder White, Atlantic Bread (VIC & SA), Mollenberg, Buttercup, Country Split, Taylor's Wholemeal (VIC)].

Possible Lupin Containing Products

- Dick Smith – not able to confirm if lupin is used in their products.
- Franklins "No Frills" – cannot confirm products are lupin free, due to possible ingredients changes.
- Sanitarium – lupin is not used as a major ingredient, however there is the possibility of cross contamination in crop growing and processing. Some soy products may possibly contain traces of lupin, hence the increased possibility of lupin cross contamination in their vegetarian products.

APPENDIX 4: Food Frequency Questionnaire