FINAL TECHNICAL REPORT

Peanut allergy: routes of pre-natal and post-natal exposure

T07043

Dr Adam Fox
Prof Gideon Lack
Imperial College, London

Project Start Date: 6/9/2004

Project End Date: 2/9/2005

Report Date: 31/1/2006

Contents

Executive Summary

Over 90% of peanut allergic children react on their first known exposure. The route by which sensitisation occurs is unclear. Much work has focused on maternal consumption of allergen (during pregnancy or lactation) yet interventional studies have failed to demonstrate any benefit of dietary elimination. Recent data demonstrate that rashes and the topical application of peanut-oil containing preparations to the infant's skin are risk factors for the development of peanut allergy. This suggests that this low dose cutaneous exposure is a likely route of sensitization. However, consumption of peanut containing foods by household members, especially during the child's first year of life, is another important source of environmental peanut exposure.

Our study aimed to investigate the role of the infants' environmental peanut exposure in the later development of allergy. We designed a dietary questionnaire to retrospectively measure an individual's weekly peanut consumption. This was used in a cohort of children with peanut allergy and age-matched controls. We thus quantified peanut consumption by all household members during infancy as well as maternal peanut consumption during pregnancy and lactation. Details of numerous other possible risk factors for peanut allergy were also collected. Recall bias regarding peanut consumption by families whose child was known to be peanut allergic was avoided by obtaining data before such a diagnosis was suspected. This required administration of the questionnaire to children with difficult eczema or other food allergies who had not reacted to peanuts in the past. After information on peanut consumption had been obtained, the data was only utilised if later allergy testing to peanut returned values that were >95% positive predictive values for clinical allergy. Two groups of controls were recruited. A High Risk Control group included children with proven egg allergy (have a 30-50% chance of having peanut allergy) but who were not sensitised to peanut. A further group of Normal controls comprised of children attending General Paediatric Clinics with a non allergy related problem.

Median weekly household peanut consumption during the first year of life in the peanut allergic Cases (n=133) was 78.9g as compared with 29.1g in the Normal controls (n=150) and only 7.8g in the High Risk Controls (n=160). Pair-wise comparisons between the three groups each gave significant differences with a p-value <0.0001. Similar effects were noted when consumption was considered in terms of episodes of peanut consumption or only peanut butter consumption. Differences in maternal peanut consumption during pregnancy and lactation were less significant and become non-significant after adjusting for other dietary factors. The form that peanut was consumed in also appeared to be important, with peanut butter consumption leading to the greatest risk of peanut allergy.

Some infants in the high risk control group, who were not peanut sensitised, were found to have high peanut consuming households. However, these infants differed from other High Risk controls, in that they were significantly more likely to have consumed peanut themselves. This highlights the critical

importance of the route by which allergen exposure occurs and implies that early oral exposure may induce tolerance, thus protecting children from potential sensitisation by low dose environmental exposure.

We also investigated the awareness and uptake of Department of Health Guidance aimed at preventing peanut allergy. We found that a combination of lack of awareness, misunderstanding of their relevance, lack of will or difficulty in following the DoH guidance has resulted in only 17% of the target mothers successfully adhering to it. However, the greater proportion of mothers adhering to the advice in the High Risk controls, relative to the peanut allergic Cases ($p=0.025$), suggests that the guidance did have some efficacy in preventing peanut allergy. However, this may not be due to the mechanistic theories upon which the advice was based.

Investigation of a number of other possible risk factors for peanut allergy failed to reveal any other specific influences. This included the application of peanut or soya containing creams, which were not found to be a specific risk factor for peanut allergy, although a marked decrease in the level of usage of peanut containing creams is the most likely explanation for our failure to replicate previous findings.

Comparison of all the food allergic children to non food allergic controls revealed higher rates of eczema, asthma, wheeze, use of soya milk, family history of atopy and mixed ethnicity amongst the food allergic group but a lower proportion of Caucasians, prematurity and dog ownership.

In conclusion, these results suggest that in susceptible individuals, increased exposure to environmental peanut promotes allergy whilst low levels may be protective. This supports our hypothesis that peanut sensitization occurs as a result of environmental exposure through cutaneous or inhalational routes rather than from maternal or infant allergen consumption. Our data also raise the possibility of early oral exposure playing an important role in the development of tolerance. If sensitization is indeed occurring through environmental exposure, this has important implications for public health policy. Future strategies to prevent allergy might include measures to reduce the levels of environmental peanut in the infant's milieu or the introduction of peanut orally in early infancy to induce tolerance. Both of these approaches need careful assessment in prospective studies before they could be recommended.

Glossary

COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
DBPCFC	Double Blind Placebo Controlled Food Challenge
DoH	Department of Health
ETS	Environmental Tobacco Smoke
FFQ	Food Frequency Questionnaire
FSA	Food Standards Agency
HPC	Household Peanut Consumption
ICS	Inhaled Corticosteroids
IFN	Interferon
IgE	Immunoglobulin E
OVA	Ovalbumin
PA	Peanut Allergy
PPV	Positive Predictive Value
RSV	Respiratory Syncytial Virus
SPT	Skin Prick Test
SpIgE	Specific IgE

Aims & Objectives

Introduction

Peanut allergy (PA) is one of the most serious of the food hypersensitivities both in terms of persistence and severity. Most presentations of peanut allergy occur on the first known contact the child has had with peanut[1,2]. However, all type 1 hypersensitivity reactions require prior sensitization to the allergen before such an allergic reaction can occur. The mechanism by which this sensitization occurs remains unclear. The possibilities are that sensitization is occurring in utero, via breast milk or via indirect low dose environmental exposure. The latter may result from cutaneous contact or vapour inhalation of allergen.

A review of the available literature reveals no convincing evidence of sensitization via lactation or the in utero route[3]. Despite research interest, there has been no single randomised interventional study that has shown an effect on preventing the development of peanut allergy by avoiding ingestion during gestation, lactation or infancy[4,5]. Recent data is supportive of the possibility of sensitization through low dose cutaneous exposure as a result of the application of arachis oil containing creams to inflamed skin[6].

This study aims to quantify the exposure to environmental allergen during the peanut allergic child's infancy, prior to diagnosis. Environmental peanut exposure can occur through a variety of ways as well as the application of peanut containing creams. Other important environmental components include the peanut consumption of all household members and the cutaneous contact and vapour inhalation that can result from this. A literature search confirms that the investigation of overall environmental peanut exposure coupled with maternal consumption is novel.

We hypothesise that if environmental contact is indeed the route of sensitization then levels of such exposure would be significantly higher in those who developed peanut allergy than in appropriate controls. Furthermore, if the in utero or lactation routes are not the source of sensitization, then maternal peanut consumption would not be higher amongst

the mothers of peanut allergic children, unless this was merely a surrogate marker of a higher household consumption.

Current literature regarding possible routes of sensitization in peanut allergy

The route by which sensitization to peanut occurs in children with PA remains unclear. However, an understanding of the routes of exposure that leads to either allergic sensitization or immunological tolerance is crucial for the development of effective prevention strategies. South African data[7] from a case control study suggested that peanut sensitization was related to maternal ingestion of peanut during pregnancy and early introduction of peanut into the infant's diet. The effect of maternal consumption was not significant and controls were not adequately matched. Two prospective birth cohort studies show no association between maternal consumption and the later development of peanut allergy[6,8]. Prenatal sensitization is also unlikely given that specific IgE has not been detected in the cord blood of children in the ALSPAC cohort who later developed peanut allergy[6]. Importantly, total IgE was identifiable in the cord blood indicating that the absence of specific IgE to peanut was not due to IgE degradation or an inability to detect IgE.

It is recognised that some food allergens may be transmitted to the neonate during lactation[9] and indeed, peanut protein can be found in breast milk[10]. However, a study which excluded mother and child dietary dairy, egg and peanut through late pregnancy, lactation and infancy failed to show a reduction in peanut sensitization compared to families with no dietary interventions[4,5].

Recently, we showed that exposure to preparations containing arachis oil was a risk factor for the development of peanut allergy[6]. Almost 91% of the children with peanut allergy had been exposed topically to creams containing arachis oil in the first 6 months of life. Moreover, comparison of levels of exposure showed that children in the peanut allergy group were exposed to significantly more preparations containing arachis oil than controls. The route of exposure was critical as maternal application of arachis oil containing

breast creams was not associated with later allergy. Eczema was also identified as a risk factor which points to the possibility that exposure to low doses of peanut antigen through inflamed skin causes allergic sensitization.
However, high incidence of peanut allergy is still found in countries where there is not such high usage of peanut containing creams. This discrepancy could be explained if sensitisation is occurring through the application of other creams containing cross-reactive proteins such as tree nut or soya. Alternatively, creams may represent only one component of environmental exposure. Another form of environmental exposure includes exposure to allergen that is generally distributed in the environment. This includes food allergens, which can be measured in dust samples from household environments. Allergens such as egg, milk and fish have been shown to be well distributed around the home, rather than simply present in kitchens[11]. Levels of ovomucoid in a further series of dust samples were as high as 6300ng/g dust[12]. It has been shown that in house dust mite (a known environmental allergen) that 2mg D.Pteronyssius/g dust is enough to cause sensitization[13], suggesting that the concentration of food allergen in dust may be sufficient to cause sensitization by the environmental route.

Another further potential source of environmental exposure is that which may occur when a tolerant household member eats allergen containing food and then touches or kisses somebody else who is naïve to that allergen. Perry et al [14] demonstrated that after peanut butter has been consumed, there is often residual detectable Arachis hypogaea allergen 1 (Ara h 1; range of detection, 30-2000 ng/mL) on hands despite hand washing with plain water and antibacterial hand sanitizer. There are thus many opportunities for an infant to experience such cutaneous allergen exposure in households where peanut containing foods are being consumed.

There is considerable scientific evidence underlying the hypothesis that sensitization may occur by environmental exposure. Immunological sensitization by cutaneous routes is well described in contact dermatitis. For example, sensitisation to Nickel is increasingly likely, as cutaneous exposure to Nickel is increased. As a result, guidelines to reduce Nickel exposure

amongst Danish schoolgirls successfully resulted in a significant decrease in rates of Nickel sensitisation[15]. Rodent models show that sensitization to ovalbumin (OVA) can occur preferentially through the skin[16] or lungs[17]. More recently, Hsieh et al[18] demonstrated definitively that food allergy can be induced by epicutaneous allergen exposure. BALB/c mice were shaved on the back, and a patch impregnated with 100 mg of ovalbumin was applied to the dorsal skin for a 1-week period and then removed. After three courses of sensitization, OVA-specific antibodies in sera were measured, and then mice were orally challenged with 50mg of OVA. Epicutaneous sensitization of mice to OVA induced a high level of OVA-specific IgE. Subsequent oral challenge with OVA resulted in symptoms of systemic anaphylaxis with elevated levels of plasma histamine as well as histological changes in both intestines and lungs. In the presence of anti-IL-4 antibodies, epicutaneous sensitization failed to provoke an IgE response, but still induced a Th2-predominant cellular immune response in lungs after oral challenge. Strid et al[19] have further examined the possible role of epicutaneous exposure to peanut in sensitization using animal models. This work investigated the immune responses obtained by skin exposure to common high molecular-weight protein antigens. The stratum corneum was disrupted in these experiments, by gentle removal with adhesive tape, to mimic the desquamated skin of atopic dermatitis and other inflammatory skin conditions. Application of OVA or partially purified peanut protein to skin after removal of the stratum corneum elicited a potent systemic immune response. Both the cell mediated- and antibody responses obtained were predominantly Th2, as indicated by increased IL-4 and reduced IFN-γ production by T cells from draining lymph nodes, and by high levels of IgG1 and IgE antibody and little or no IgG2a. In contrast, the response elicited by subcutaneous immunization was predominantly Th1.

In food allergic children, reactions have been demonstrated after vapour inhalation and cutaneous contact[20], confirming that allergen in the environment is immunoreactive. In the occupational setting, where 1% of all adult asthma is due to food allergens[21], sensitization to a number of allergens may occur by inhalation or cutaneous routes[22,23,24]. The best known example

is baker's asthma – an inhalant allergy to flour. It has been estimated that 10-30% of unselected bakers may develop occupational asthma[25] There are also reports of an allergy to inhaled egg material in egg-processing workers[22]. In peanut allergic patients, allergen specific T cells to peanut have been discovered in the skin[26]. In one case report, a previously non-allergic patient suffered anaphylaxis to peanut after receiving a liver and kidney transplant from a peanut allergic donor[27]. Chimerism was only detected in the skin of the recipient suggesting that allergen specific T cells had a homing commitment to the site of sensitization.

Justification of research

Knowledge of the route of sensitization to peanut has critical implications for public health policy. In June 1998 the Department of Health (DoH) published the following recommendations[28] aimed at halting the rising incidence of peanut allergy[29].

'Pregnant women who are atopic (have eczema, asthma, hayfever or food allergies) or have an atopic partner may wish to avoid eating peanuts during pregnancy and lactation. Infants with a family history of atopy should be exclusively breastfed for 4-6 months and should avoid peanuts until 3 years.'

This guidance was based on the conclusion that peanut sensitization occurring as a result of exposure in utero or via lactation was mechanistically possible. However, contemporary data was inconclusive, despite decades of work into the pathogenesis of childhood food allergy. The possibility that sensitization is occurring through environmental exposure, rather than ingestion, was largely ignored.

If sensitization to peanuts is occurring by environmental routes, the current guidance will have little or no influence on the incidence of peanut allergy. Further to this, the guidance could potentially be harmful[30]. The report also recommends that children avoid peanut consumption until they are 3 years of age. This measure removes a cheap source of protein from the child's diet and ensures that children are subject to only very low levels of exposure to peanut antigen. This could paradoxically increase the likelihood of allergic sensitization. Animal models suggest that early high dose oral exposure tends to lead to tolerance[31] and is supported by the observation that in certain cultures (Israel, Southern Africa and China) where childhood peanut consumption is high, peanut allergy is less prevalent[7,32]. This implies that avoiding ingestion may actually be preventing the development of tolerance.
If sensitization to peanut is indeed occurring as a result of environmental exposure then very different and more extensive measures for allergen avoidance will need to be considered.

Hypotheses and Objectives

Hypothesis 1:

Sensitization to peanut may occur via environmental exposure when other household members eat peanut and peanut containing foods. Overall household consumption of peanut will be significantly higher in the infant milieu of those who develop peanut allergy than in those of controls.

Objectives:

01a) Compare the overall household peanut consumption between peanut allergic Cases (n=133) and High Risk (n=160) and Normal controls (n=150), during pregnancy, breast feeding and infancy. This will also include comparison of maternal and infant consumption during these periods. This will allow us to demonstrate:

- that peanut consumption is higher in the households of children who develop peanut allergy and that this is independent of maternal peanut consumption during pregnancy and lactation.
- that low household peanut consumption can act as a protective factor against developing peanut allergy in children at high risk (egg allergic) controls.

01b) To compare Cases (n=133) and High Risk Controls (n=160) with regards to their prior knowledge of the DoH guidance and, where appropriate, if they adhered to it. This will demonstrate whether knowledge of, and adherence to, DoH guidelines influences later development of peanut allergy.

Hypothesis 2:

Sensitization to peanut allergen may occur through the direct application of preparations containing peanut or soya oil to inflamed skin. Sensitisation via this route is more likely if the rash presents earlier and is more severe.

Objectives:

02a) To compare the use of preparations containing peanut oil applied to the skin during infancy between peanut allergic Cases (n=133) and High Risk (n=160) and Normal controls (n=150).

02b) To compare the use of preparations containing soya oil applied to the skin during infancy between peanut allergic Cases (n=133) and High Risk (n=160) and Normal controls (n=150).

02c) To compare the use of all other preparations applied to the skin during infancy between peanut allergic Cases (n=133) and High Risk (n=160) and Normal controls (n=150). By comparing data obtained form Objectives 02a), b) &c), we may determine whether any increase in the use of peanut, tree nut or soya oil containing preparations is a selective phenomena.

02d) To compare the presence of oozing or crusting rashes during infancy, as well as their onset and severity between peanut allergic Cases (n=133) and High Risk (n=160) and Normal controls (n=150). We may thus determine whether application of peanut oil containing cream to the skin where there is earlier onset or increasing severity of rash, increase the risk of peanut sensitization.

Hypothesis 3:

Maternal consumption of peanut during pregnancy or lactation is merely a marker of overall household consumption and does not cause allergen sensitisation per se.

Objective:

03a) Compare maternal peanut consumption during pregnancy, lactation and infancy with that of other family members to determine whether maternal consumption of peanut correlates with family consumption. This will be done amongst both cases (n=133) and the 2 control groups (n=310). Using a regression analysis, any links between maternal consumption of peanut and peanut allergy may be explained by an association with family consumption.

Hypothesis 4:

There are other risk factors or protective factors for the development of PA.

Objective:

Until recently there has been little data on risk factors for the development of peanut allergy. Established risk factors include a family history of peanut allergy and the presence of atopy[33,34]. Other work has suggested early infant consumption of peanut[35] and soy consumption[6] as risk factors. A number of other factors such as presence of cat and dog in household during infancy [36], socio-economic status[37] and RSV bronchiolitis[38] have been established as protective or risk factors for other atopic conditions yet they have not been investigated specifically in relation to peanut allergy. Through FSA funded work, a further group of risk factors and protective factors for sensitization to foods has been identified. Risk factors include non-caucasian ethnicity and 'wheeziness' by 6 months of age. Wheeze in the first 6 months of life points towards early respiratory viral infections. Protective factors include a greater number of siblings, passive smoke exposure and increasing gestational age.

04a) To compare cases (n=150) with egg allergic (n=150) and normal controls (n=150) with regard to potential risk factors and protective factors. These will include :

- Socio-economic status
- Ethnicity of infant based on parental ethnicity
- Nationality of infant based on parental nationality
- Age of initial peanut consumption
- Soy consumption in infancy
- Prematurity
- Presence of cat and dog in household during infancy
- Bronchiolitis in infancy
- Passive smoking
- Family history of allergy
- Breastfeeding (this information was collected for Objective 1) & results are presented with Objective 3.

Experimental Procedures

The ideal study design to observe factors in the environment that may influence the development of peanut allergy would be prospective. Unfortunately, the relatively low prevalence of the condition (1-2%) would require a huge cohort to be observed for many years in order to obtain a large enough sample of peanut allergic children to provide meaningful data. A retrospective study design allows the inclusion of a large number of cases of peanut allergy, in a fraction of the time period with fewer resource implications. A further consideration of limitations of the retrospective study design is included in the Discussion section.

The design of this study is a retrospective questionnaire based case control study. Families of children with peanut allergy and their controls were asked detailed questions about the consumption of peanut by all household members during the child's first year of life as well as many other questions relating to the objectives listed above. A copy of the questionnaire can be found in Appendix A. To limit recall bias, data was obtained from families before a diagnosis of PA was made. Only data from children whose later allergy testing led to a diagnosis of PA were included in the study. Questionnaire data was also obtained from 2 groups of controls. The first group were children with egg allergy **who are not sensitised to peanut**. These represent a group of High Risk Controls as a large proportion of children with egg allergy are also sensitised to peanut. The second group of controls (Normal Controls) were children attending general paediatric clinics, with a non allergic complaint. Figure 1 illustrates the basic study design.

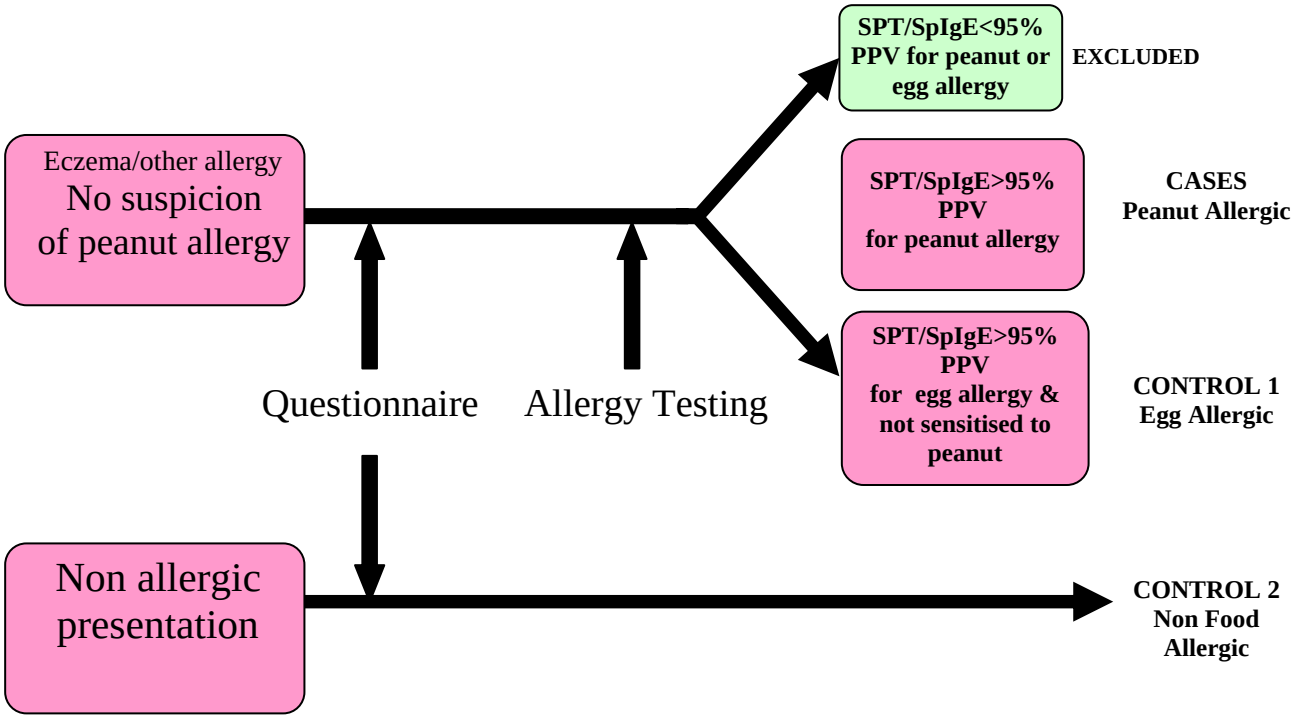


Figure 1 Study Design

Detailed study methodology will be considered in sections as follows:

- Questionnaire Design
- Questionnaire Validation
- Phenotypic characterisation of cases and 2 control groups
- Initial Pilot Study
- Obtaining Information from Appropriate Cases and Controls
- Entry of raw data into computer
- Analysis of results and their implications

Questionnaire Design

For study purposes, the required data was obtained by means of a study questionnaire. This questionnaire contained questions relating to each family member, regarding past peanut consumption as well as all the other risk factors of interest.

In order to obtain the required data on environmental peanut exposure, details were required on:

- Maternal peanut consumption during pregnancy
- Maternal peanut consumption during lactation
- Maternal peanut consumption after lactation during child's first year of life
- Breastfeeding and it's duration during infancy
- Paternal peanut consumption during child's first year of life
- Sibling peanut consumption during child's first year of life
- Index child's peanut consumption during first year of life
- Peanut consumption of any other person living in the household during child's first year of life
- Application of preparations containing peanut protein to the skin during first year of life

Data was also collected with regard to demographics and a number of secondary end points relating to other objectives:

Objectives 01b):

- Knowledge, relevance and application of DoH guidance regarding peanut avoidance during pregnancy, lactation and early infancy

Objectives 02b):

- Application of preparations containing tree nut or soya protein to the skin during first year of life

Objectives 02c):

- Application of all preparations to the skin during first year of life

Objectives 02d):

- Presence of an oozing or crusting rash during first year of life
- Time of onset of any oozing or crusting rash and use of different strength steroid creams as a measure of severity

Objectives 04a):

- Soy consumption in infancy and beyond
- Socio-economic status as defined by the Standard Occupational Classification (SOC2000)
- Ethnicity and nationality
- Prematurity
- Presence of cat and dog in household during infancy
- Bronchiolitis in infancy
- Passive smoking
- Family history of allergy

Data relating to peanut consumption will be based on individual's recall of diet at a time in the distant past. Therefore an accurate method was required for retrospectively assessing peanut consumption that takes errors of memory, conceptualization and portion sizes into account, between the initial intake and the attempted measure up to 3 years later[40].

There is evidence that the best estimate of a diet from several years in the past may be derived directly from a retrospective dietary history which focuses on that past period of time rather than simply using current diet and drawing inference from that[41].

Semiquantative food frequency questionnaires (FFQs) were considered to be the most appropriate tool to use for the retrospective assessment of peanut consumption[42]. They represent an appropriate measure in a study involving relatively large numbers of subjects, where comparative consumption between groups is of greater importance than accurate absolute intakes of peanut protein in individuals. The study questionnaire includes the same FFQ

(figure 2) for completion by each different family member as well as 3 specifically for the mother to cover the periods of pregnancy, breast feeding and the period during the child's first year of life when they were not breastfeeding. The FFQ provides data on how many times the specific food is eaten per week and how much was eaten on each occasion. As precise peanut content of all of products on the questionnaire has been obtained directly from manufacturers (figure 3), the data on food consumption allows the calculation of the quantity of actual peanut in grams, consumed per week by each household member as well as the number of peanut eating episodes.

Try and remember back to when you were pregnant with your child.
How many times did you eat the foods listed in a normal week and how much of the food did you eat each time?

Example:

If you ate 1 snickers bar on 2 occasions in a normal week then you would fill in:
Snickers 2 times 1 bars

and if you ate 2 slices of bread & peanut butter on 4 occasions during a normal week:
Peanut butter 4 times 2 slices

A sandwich of 2 slices of bread filled with peanut butter would only count as 1 slice of peanut butter.

Type of food	Times eaten in a week	Amount eaten each time
Peanut butter		slices
Snickers		bars
Peanut M&Ms		packs
Whole peanuts		handfuls
Crunchy Nut Cornflakes		bowls
Crunchy Nut Cornflakes Red		bowls
Revels		bars
Tracker Roasted nut		bars
Rowntree's Lion Bar		bars
Cadbury's Star Bar		bars
Cadbury's Fuse		bars
Cadbury's Picnic		bars
Reese's Peanut Butter Cups		cups
Satay sauce		servings
Peanut soup		servings
Bamba snack		packs
Hazelnut spread eg Nutella		slices

Please write down any other sources of peanuts you may have eaten, and how much per week:

Figure 2 Excerpt from FFQ

A major barrier to the conduct and interpretation of retrospective studies linking dietary consumption to disease in later life has been the uncertainty about the reliability of retrospective assessments of diets from the distant past. SFFQs have been demonstrated to be a reliable method of assessing consumption of both individual nutrients as well as food components[42]. In addition, there is also good evidence of a strong correlation between retrospective and contemporaneous estimates of food intake using SFFQs[43] with reasonable reproducibility. Despite this, any new FFQ used to estimate the previous diet of the mothers of children with peanut allergy as well as their controls, needs to be assessed, within the population of interest, for the accuracy of recall over the time frame that recall would be required in the study setting.

SFFQ design

In the absence of a previous validated SFFQ looking at peanut consumption, it was necessary to generate a new food list. Ideally, this list needed to include all the commonly consumed foods within our target population's (mothers of peanut allergic children) diet that contained significant quantities of peanut protein. Normally foods included in a food frequency questionnaire are taken from a 7 day food history from the target population. This method was not used due to parents not always being aware of which foods do or do not contain peanuts rather than tree nuts. The researchers were thus concerned that many of the commonly consumed peanut containing foods would not be included in the final SFFQ. Therefore both the paediatric dietitians' peanut avoidance diet sheets, with common peanut-containing foods (in use in our own tertiary allergy clinic for the past 5 years) as well as the Anaphylaxis Campaign (a charitable organisation supporting families of children with allergies) food lists were used when developing the FFQ. In addition to the above, any other foods that contained peanut, as stipulated by the European Labelling Law, were also included in the list. Given that peanut oils contain no protein (or tiny quantities)[44] and that it is peanut protein which is implicated in allergic sensitisation, foods containing only peanut oil were not included. Similarly, items that listed peanut either as a trace ingredient or as a

possible contaminant were not included as they were considered unlikely to contribute significantly to overall peanut consumption.

Once a list of commonly consumed peanut containing foods had been compiled, foods were categorized to form the SFFQ. Different brands of the same food, such as peanut butter were simply grouped as the generic item as this would have considerably lengthened the food list yet added little information given the similar peanut content in different peanut butters. Peanut containing foods were then grouped according to their presentation: smears, bars, sauces, snacks

Once the FFQ food list was completed, it was piloted on a group of 50 mothers, from different ethnic backgrounds, in our food allergy clinic. This is the same clinic from where our later study population would be drawn. This first pilot study was aimed at evaluating the list of foods in the FFQ. In addition to completing the FFQ, respondents were also asked to name any other foods that contain peanut (with portion sizes) that they had consumed, which were not listed in the current FFQ. Foods that were brought to our attention by the open ended questions were only included in the FFQ if they were listed as containing peanut ingredients as stipulated by the European Labelling Law. Members of ethnic groups were also further interviewed to obtain a clearer understanding of peanut consumption within their culture, which enabled the researchers to add two further foods commonly consumed in these groups.

The revised FFQ was then piloted again on a further randomly selected sample of 50 parents from our allergy clinics. In addition to assessing the foods listed in the FFQ, this pilot was also aimed at confirming the portion sizes consumed. Foods were converted into a standard portion sizes, which were translated into household measurements. This was considered important in light of evidence that individuals have difficulty in estimating portion size when reporting what they have consumed[45]. For foods which come pre-packaged in standard sizes such as chocolate bars, the amount consumed was requested in terms of this standard unit. Standard portion sizes were obtained for other food items by using the Ministry of Agriculture's

Food Portion Sizes. The actual peanut protein content in each product on the SFFQ was obtained from the manufacturers directly. Consumption frequency was measured with reference to weekly intake (Figure 2). The most commonly and frequently eaten foods were listed at the top of the FFQ, as there is evidence to suggest that accuracy of responses may decline through boredom and fatigue towards the end of questionnaires[46].

Questions regarding frequency and amount consumed were kept in a closed format to reduce coding time, transcription errors and minimize the number of peanut-containing-foods having to be rejected due to difficulty interpreting answers or incomplete responses. A final pilot study enabled us to ensure that the final questionnaire could be completed in a reasonable time frame and was easy to understand. Feedback also suggested that a simple worked example should be included in the instructions for completing the FFQ and this was included in the final version.

Food	**Peanut composition**	**Amount of Peanut (g)**
Peanut butter	90%. 15g in typical serving per slice of bread	13.5g per slice
Snickers	23.8% peanut in standard 64.5g bar	15.4g per bar
Peanut M&Ms	9.9g per 45g pack	9.9g per 45g pack
Whole peanuts	10g in typical handful (typical pack 50g)	10g in typical handful
Crunchy Nut Cornflakes	7% of 30g serving	2.1g per bowl
Crunchy Nut Cornflakes Red	4.5 % of 30 g serving	1.35g per bowl
Revels(Mars)	4.6% of standard 35g packs	1.61g per pack
Tracker Roasted nut (Mars)	17.4 % of 37g bars	6.44g per bar
Rowntree's Lion Bar	18% of 49g bar	8.89g per bar
Cadbury's Star Bar	20% of 54g bar	10.8g per bar
Cadbury's Fuse	7% of 49g bar	3.43g per bar
Cadbury's Picnic	9% of 48.4g bar	4.34g per bar
Reese's Peanut Butter Cups	30%peanut butter so 27% peanut. Standard cup is 45.36g.	12.25g per cup
Satay sauce	25g peanut in typical serving	25g peanut in typical serving
Peanut soup	54g peanut in typical serving	54g peanut in typical serving
Bamba Snack	55.5% of 25g bag	13.9g per bag

Table 1 – Peanut content of foods

Assessment of Recall Accuracy of FFQ

The FFQ was designed for interviewer administration, as this method has been shown to have a superior correlation coefficients between FFQs and reference measures than self administered questionnaires and also improved repeatability[46]. It was administered by a single researcher (AF), thus limiting inter-rater issues of reliability. Ideally the validation of a FFQ should include comparison to an independent measure, with a measurement error that differs from the FFQ. Unfortunately in the case of a peanut specific FFQ, a gold standard, such as a biochemical marker, as "truth reference" does not exist. Such validation was thus not undertaken. However, assessment of the accuracy of recall of peanut consumption using the FFQ could be performed through repetition of the same questionnaire, on the same sample population at a 2 year interval.

As this FFQ was to be used in most cases by mothers at an interval of about 2-3 years after the birth of their child, we require an assurance of the accuracy of maternal recall of her diet over this period. For the main study, only children under 4 years of age were eligible for inclusion and thus assessment of dietary recall over a longer period was not required.

A group of 40 mothers attending routine antenatal appointments during the second trimester of pregnancy at St Mary's Hospital were approached and asked to fill in the revised FFQ with reference to their previous month's consumption (initial recall). Detailed contact information was taken but mothers were not informed that they would be asked to repeat the exercise at a later date. Two years after initial administration of the questionnaire, we attempted to contact the group of mothers and asked them to again complete the SFFQ with reference to the period of their pregnancy (follow up recall).

A further validation study of a very similar questionnaire is being conducted by another researcher in our department, with my assistance. In that study, the dietary period under study will be assessed more closely with a daily diet diary. The questionnaire is to be administered to a population of women

whose children are attending General Paediatric and Allergy Clinics. The study will be done prospectively. The women will initially be asked to keep records of their own and their child's daily diet on seven consecutive days, using a dietary record form where food items are listed. The dietary record form consists of a list of 18 peanut containing food items, as well as low allergenic foods: i.e. meat and vegetables. Six months later they will be contacted and requested to recall their food consumption during the index week, six months earlier. Recall will be recorded on a Food Frequency Questionnaire listing the same foods as the initial daily record forms. Quantification of peanut protein consumption will be achieved by using appropriate conversion charts and compared between the two time points. The results of this study are not yet available. This study will provide a validation of the FFQ in terms of accuracy relative to a 'truth' measure and, to a lesser extent, recall.

Phenotypic characterisation of cases and 2 control groups

At St Mary's Hospital, Paediatric Allergy clinics care for a population of children with a wide spectrum of allergic conditions. This includes large groups of infants and young children suffering from eczema and undiagnosed food allergies, who are referred primarily for assessment of the role of food allergies in their eczema. Recruitment of cases for this study focussed on this group of children. Previous data from our clinic population revealed that 30% of these children will suffer from egg and/or peanut allergy.

The main study group will consist of children with peanut allergy. There will be two control groups.

In a study such as this, it is important to accurately determine the allergic phenotype of the child. Therefore, diagnosis of allergy in our study required a SPT wheal diameter or specific IgE antibody level greater than the 95% positive predictive value (PPV) for clinical allergy or a positive DBPCFC. The threshold values we have chosen are based on validation of a >95% predictive value in a population of 14000 children (ALSPAC cohort) that have also been validated in our own tertiary clinic population. We did not want to compromise the study by enrolling patients with an uncertain diagnosis.

Cases – 'Peanut Allergy': describes children diagnosed with peanut allergy in our clinic either on the basis of DBPCFC, SPT or specific IgE levels. The children recruited to this group presented to our clinic primarily for an assessment of the role of food allergy in the course of their eczema. Relatively few of these children have had immediate hypersensitivity reactions to peanut in the past, as they are unlikely to have ever eaten peanut prior to evaluation. This, together with the exclusion of children with a confirmed diagnosis or parental perception of peanut allergy prior to clinic attendance, will substantially reduce the possibility recall bias.

Inclusion Criteria for Cases

- Age over 6 months but less than 4 years

AND (after questionnaires are completed)

- Skin prick test wheal >7mm using peanut (Soluprick, ALK-Abello)

OR

- Specific IgE>15kU to peanut(Pharmacia CAP, Pharmacia)

OR

- Positive DBPCFC to peanut

Exclusion Criteria for Cases

- Prior confirmed diagnosis of peanut allergy at presentation

OR

- Parental reporting of an allergic reaction to peanut

Control Group 1 – 'High Risk': describes children diagnosed with egg allergy in our clinic either on the basis of DBPCFC, SPT or specific IgE levels, but who are not sensitised to peanut. Egg allergic children are at high risk of coexisting peanut allergy with approximately 50% demonstrating sensitization to peanut on skin prick testing. This control group is of particular interest because despite their high risk for developing peanut allergy, these controls have not done so. This implies that they may have been subject to some protective factor against peanut sensitization.

Inclusion Criteria for Control Group 1

- Age over 6 months but less than 4 years

AND

- Skin prick test wheal >6mm using egg white, yolk (Soluprick, ALK-Abello) or raw egg

OR

- Specific IgE>6kU to egg(Pharmacia CAP, Pharmacia)

OR

- Positive DBPCFC to egg

Exclusion Criteria for Cases

- Prior confirmed diagnosis of peanut allergy at presentation

OR

- Parental reporting of an allergic reaction to peanut

OR

- Sensitization to peanut (SPT > 0mm or Specific IgE>0.35kU)

Control group 2 – 'Normal': describes children with neither known egg or peanut allergy. These children will be drawn from the general paediatric clinic attendees. They will act as a baseline for determining normal levels for parameters such as household peanut consumption. It is of note that although this control group excludes those with known egg or peanut allergy, we have not formally ruled out allergy to these foods by skin testing or food challenge. As a result, recent data on UK populations[39] suggest that around 1.5% of this control group could comprise of children with peanut or egg allergy. On specific IgE testing of this group, we would anticipate about 5% to be sensitised to peanut based on analysis of 700 randomly chosen blood samples from the ALSPAC cohort (age 2-3 years). We would thus only yield about 7 sensitised children from our cohort of 150 and this group would be too small to perform any meaningful statistical analysis on.

Inclusion Criteria for Control Group 2

- Age over 6 months but less than 4 years

AND

- Child attending general paediatric out patients for non allergic problem

Exclusion Criteria for Control Group 2

- Prior confirmed diagnosis of peanut or egg allergy at presentation

OR

- Parental reporting of an allergic reaction to peanut or egg

Initial Pilot Study

In order to establish whether there was likely to be a difference in household peanut consumption during infancy, an initial pilot study was conducted. Initial data was collected on a small sample of children allowing us to establish the practical feasibility of the project and basic features of the data. It is worth noting that all Cases and High Risk Controls recruited for this pilot study already had confirmed diagnoses due to prior clinic attendance. Data was obtained by postal questionnaire. Not excluding families where the diagnosis of PA was already established greatly facilitated rapid recruitment but allowed for the introduction of recall bias in to the data. The Normal Controls were obtained from attendees at the general paediatric outpatient clinic. Inclusion and exclusion criteria were otherwise the same as that proposed for the main study. After analysis of the data obtained, information from the Cases and High Risk Controls collected for this pilot study were discarded from the main study.

Approach to statistical analysis

The first step was to assess the normality of the data for each of the variables of interest in each of the groups. These proved to be not normally distributed, so non-parametric tests were appropriate. With three independent groups, a Kruskall Wallis test was the most appropriate test statistic to use. The descriptive statistics for this test were also necessary to obtain a better understanding of the data. These are provided below. A Bonferroni correction was used to account for the multiple tests

Results:

Descriptive statistics for overall average weekly household peanut consumption in grammes of peanut per household per week during infancy:

Group	Cases	High Risk Controls	Normal Controls
n	22	21	15
Median	69.5	3.45	24.2
Inter-quartile range	15.8, 140.11	0, 57.89	4.9, 54.53
Minimum,maximum	3.92, 575.89	0, 143.92	0, 255.63

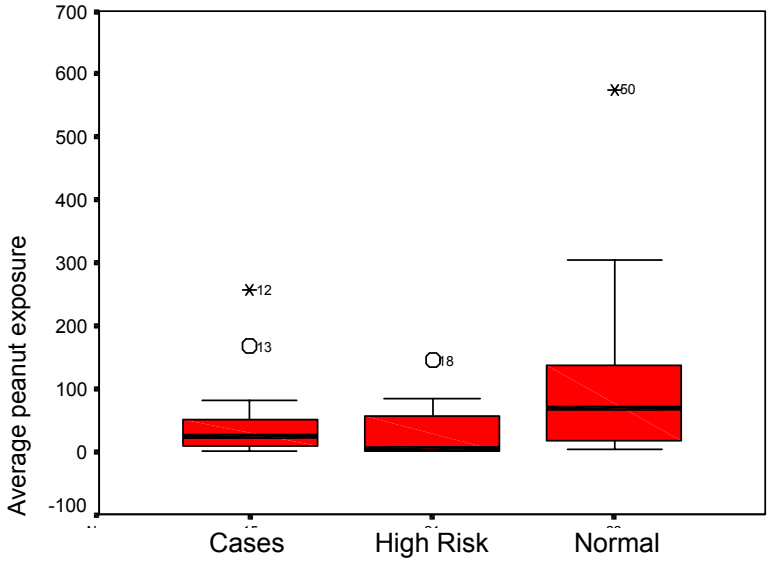


Figure 3: Average weekly household peanut consumption

The Kruskall Wallis test gave a p-value of 0.02. This provides sufficient evidence of a difference between the 3 groups. We observe from the descriptive statistics that the Cases score far higher than both of the two control groups.

Descriptive statistics for mother's peanut consumption during pregnancy in grammes per week:

Group	Cases	High Risk Controls	Normal Controls
Sample size	22	21	15
Median	13.5	0	14.7
Inter-quartile range	0.29, 68.8	0, 35.08	0, 47.19
Minimum,maximum	0, 261.2	0, 53.5	0, 106.4

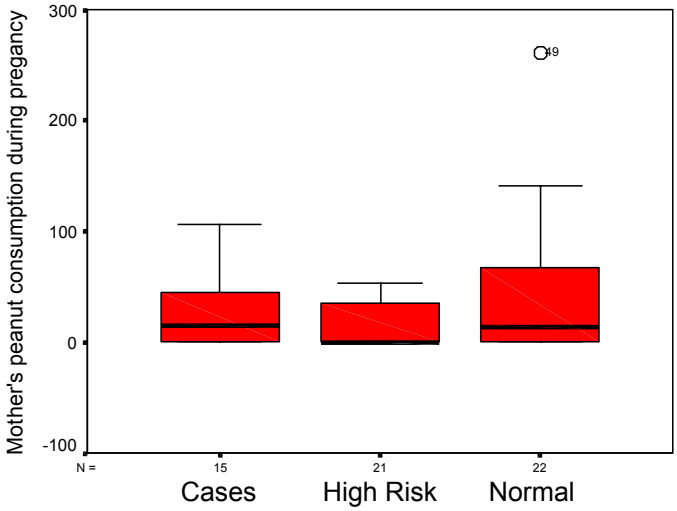


Figure 4: Average weekly maternal peanut consumption during pregnancy.

The Kruskall-Wallis test gave a p-value of 0.33. This provides insufficient evidence of a difference between the 3 groups.

Descriptive statistics for average mother's peanut consumption during breastfeeding in grammes per week:

Group	Cases	High Risk Controls	Normal Controls
Sample size	22	21	15
Median	3.88	3.71	12.1
Inter-quartile range	0, 19.06	0, 13.75	0, 43.94
Minimum,maximum	0, 59.15	0, 57.29	0, 84.15

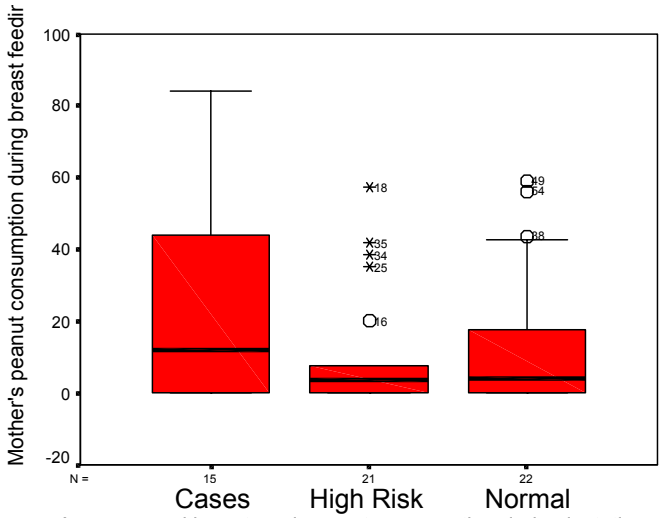


Figure 5: Average weekly maternal peanut consumption during lactation

The Kruskall-Wallis test gave a p-value of 1. This provides insufficient evidence of a difference between the 3 groups.

The preliminary data suggested that overall household peanut consumption is significantly higher in those infants who develop peanut allergy compared to the control groups. Furthermore, the peanut consumption in the High Risk Control group is substantially lower than that of the Normal Control group. Egg allergic children are at high risk of developing peanut allergy so this data suggests that the extremely low levels of environmental peanut may have exerted a protective effect over the children in the High Risk Control group.

We note that no significant difference is found between the different groups when comparing maternal peanut consumption during pregnancy or lactation.

These findings suggest that there is no systematic effect from recall bias amongst the parents of peanut allergic children, although this possibility will be avoided by the design of the main study. Feedback from parents reassured us that the study questionnaire was easy to understand and could be completed in a reasonable time frame. On the basis of this preliminary data, power calculations could be performed to establish sample size requirements.

Proposed Sample Size – The larger the study the more accurately we could estimate the effects of environmental peanut exposure on peanut allergy. Even in our pilot study with just 15 Normal Controls, some of the associations were statistically significant. We calculated the power that we will have to detect a variety of differences between cases and normal controls under assumptions derived from the preliminary data. All power calculations are based on a 2-sided test with 5% significance level without correction for multiple comparisons (see below for justification).

Our primary end point is the overall average weekly household peanut consumption. The difference between overall household peanut consumption during infancy in peanut allergic Cases versus High Risk Controls represents the largest difference between the groups. A logistic regression using overall household peanut consumption as a covariate based on preliminary data and selecting 15 cases at random results in a z-score of 2.05. In order to detect a significant difference of 5% with 90% power the sample size would need to be 37 in each group. However, if we power the study to detect a significant difference between the peanut allergic cases and the normal controls (where the difference is smaller) then this results in a z-score of 1.42. In order to detect a significant difference of 5% with 90% power the sample size would need to be 78 in each group.

We performed a further sample size calculation for the difference between maternal peanut consumption during breast feeding taking into account the effect of household peanut consumption (excluding maternal consumption) during breast feeding, in peanut and normal controls. A likelihood ratio test comparing a model based on maternal peanut consumption to the same model including household peanut consumption using preliminary data and selecting 15 cases at random, gives a chi-squared value of 1.67. A sample

size of 94 individuals per group would be required in order to detect a significant difference of 5% with 90% power.

It was also important that we try to confirm previous findings with regards to the effect of topical exposure to arachis oil containing creams during infancy. Observations in our pilot study regarding differences in proportion of infants with exposure to such skin creams revealed 80% of peanut allergic cases to have been exposed compared to 60% of the egg allergic controls. The power to detect a significant difference between the two groups, assuming the observed exposures are the underlying population exposures using a sample of 119 cases and controls is 90%. Analysis of exposure to arachis oil containing creams amongst the ALSPAC cohort[6], revealed a 90% exposure rate amongst peanut allergic children compared to 60% in controls.

Statistical note

Traditionally sample size calculations require a difference (d) that one wishes to detect and an assumption about its variance. Sample size estimates based on logistic regression carried out on the preliminary data use the square root of the observed chi-squared statistic (on 1 degree of freedom) as the difference d divided by it's standard error based on m cases and controls. The formula used is $n = \frac{(z_{1-a/2} + z_{1-B})^2}{x^2} \times m$ where $z_{1-a/2}$=1.96 (corresponding to a=0.05), z_{1-B}=1.28 (corresponding to 90% power), x^2 is the chi-squared value from the logistic regression and m is the number of cases and controls in the preliminary data (m=15).

Obtaining Information from Appropriate Cases and Controls

All children within the appropriate age range (6 months - 4 years) were approached upon their arrival at allergy/dermatology clinics. The parents of cases must not suspect peanut allergy, if unbiased data is to be obtained and thus prior to completion of the questionnaires, all parents were asked:

Has your child ever had an allergic reaction to a food? If so, which food?

If the patient responds with 'peanut' then the questionnaire was not administered and they did not take part in the study. Clearly the suspicion of a specific food allergy to peanut may well alter the parent's recollection of family peanut consumption. Nevertheless, this still does not remove the possibility that parents of children who are being referred to an allergy clinic, usually with bad eczema, may suspect food allergies. In our experience, parents of children with eczema nearly always suspect cow's milk as the incriminating allergen and sometimes wheat. They seldom consider egg or peanut as being a possibility.

However, even if the parents have a generalised suspicion that their child may be allergic, they do not know what the food allergen is when they complete our questionnaire (which is done before any formal allergy testing or consultation in the clinic). Therefore, if we find, as in our preliminary data, that familial peanut exposure is increased amongst the Peanut Allergic Cases and markedly decreased in the High Risk Controls, this difference simply could not be explained by recall bias or selective bias towards one particular food, as the parents do not know or suspect that their children have egg or peanut allergy. Recall bias would have to operate in the same direction for both the egg and peanut allergic children, not in two completely opposing directions given that parents do not suspect one specific food.

If the child fulfilled any other of the exclusion criteria, such as prior diagnosis of peanut allergy then the questionnaire was not administered. Remaining parents were asked to complete the questionnaire before they had either a consultation with a doctor or allergy testing. These could influence parental

perception of the child's allergic status and thus introduce recall bias. Questionnaires were collected and then the results of the child's allergy tests noted. Questionnaires from Cases and High Risk Controls were then drawn from those that fulfil the inclusion criteria for allergy diagnosis by either SPT, Specific IgE (see above) or later by DBPCFC. This method resulted in many parents filling in questionnaires that were not used in our analysis, as the child was not subsequently found to have peanut or egg allergy. However, this method minimised the risk of data being contaminated by recall bias, as it allows data collection before parents are aware of the child's allergic status.

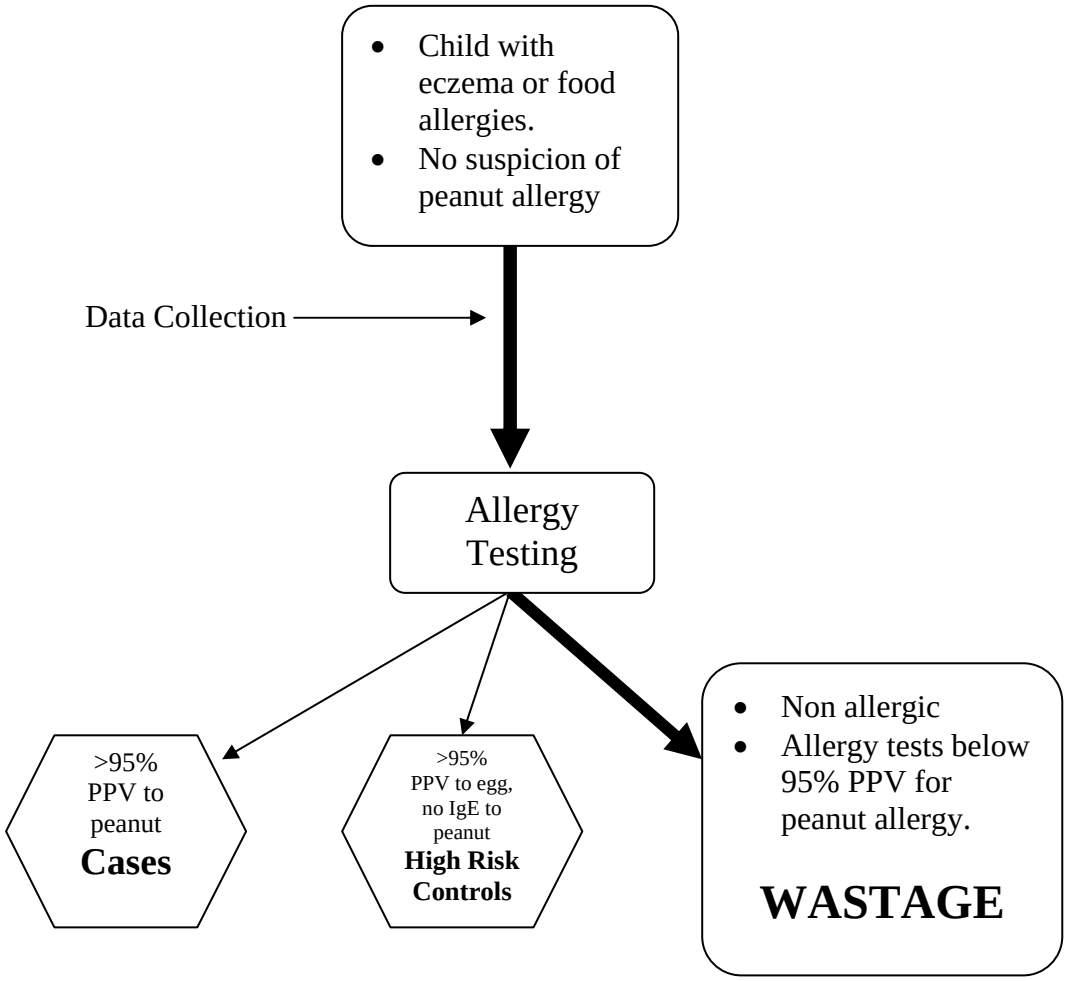


Figure 6: Study Recruitment algorithm

Questions relating to knowledge of DoH guidance were only asked after data on dietary peanut has been completed. The DoH guidance implies that maternal consumption during pregnancy and lactation has led to allergy and

exposing mothers to this information may influence recall of peanut consumption, even prior to knowledge of a diagnosis.

Further to the recruitment of cases as they attended routine clinics, we also drew on the lengthy allergy service waiting list of new patients (approximately 700 children). One third of these had been referred for assessment of the role of allergy in their eczema as well as being in the 0 to 3 year age group. These families were contacted and information regarding history of reactions to peanut obtained. Those children who remained suitable for inclusion were invited to attend special extra clinics aimed at identifying new cases of peanut or egg allergy.

Many patients for the High Risk Control group were obtained by the same method as the peanut allergic controls. Many children with eczema were given firm diagnosis of egg allergy based on allergy tests. However, given that previous reactions to egg, was not an exclusion criteria for entry, this group were much easier to identify and recruit. Care was taken to repeat SPT to peanut to ensure the child remained non sensitized to peanut rather than relying on older tests that may have changed.

Patients for our Normal Control group were drawn from the general paediatric clinic attendees at St Mary's Hospital. Parents were approached as they arrived at clinic and asked about the age of their child and the reason for their attendance at the clinic. Those attending clinic regarding an allergic problem (asthma, eczema, rhinitis, food allergy) were excluded. Parents were then asked if the child has a known allergy to egg or peanut or if they think the child has ever had an allergic reaction to peanut. Those who did were excluded. The remaining children did not have formal allergy testing and thus all questionnaires that were administered could be used.

All parents who received the questionnaire were given an 'Information for Parents' leaflet (Appendix B) describing the study. No formal consent forms were signed as consent could be implied by the completion of the questionnaire. Parents were given assistance in completing the questionnaire

by a researcher and if required, an interpreter. St Mary's hospital serves a multiethnic community and in the clinic setting, interpreters are arranged in advance for out patient appointments. In order to ensure maximum inclusivity in the study sample, it is essential not to exclude those with limited English language skills.

Entry of raw data into computer

All the data obtained from each of the patients was collected in paper form. This data was entered into a computer database. Microsoft Excel 2003 spreadsheet software was used for this purpose. This involved approximately 60 data points per patient excluding the Food Frequency Questionnaires (FFQ) for all family members.

Data from FFQs of each family was entered into a separate Excel datasheet. This datasheet incorporated a computer model designed to integrate information on the exact peanut content of each food in the FFQ food list (fig 2). Thus raw data on food consumption was converted into a quantification of the amount of peanut that it represents. This model provides a number of outcome measures relating to peanut consumption:

- average household peanut consumption expressed as grams per week per household during pregnancy, lactation and the period of infant's first year of life when mother is not breast feeding

- average number of peanut eating episodes per week per household during pregnancy, lactation and the period of infant's first year of life when mother is not breast feeding

- average household peanut butter consumption expressed as grams per week per household during pregnancy, lactation and the period of infant's first year of life when mother is not breast feeding

- average number of peanut butter eating episodes per week per household during pregnancy, lactation and the period of infant's first year of life when mother is not breast feeding

- maternal average weekly peanut consumption during pregnancy and lactation.

- index child's average weekly peanut consumption during 1st year of life

These composite values were then entered into the main database. Outcomes relating to average household consumption during infant's first year of life were then calculated using data on the average weekly peanut consumption of each family member as well as a weighted value derived from maternal consumption during breastfeeding and after breastfeeding (if lactation was discontinued before the index case was one year of age).

All data was double-checked once entered into our main database, before any statistical analysis was carried out. Data was initially entered by one of the researchers and then checked by another individual independent of our group. All our original questionnaires and databases are available for the FSA to view, if required.

Statistical analysis of results and their implications

Statistical analysis was untaken under the supervision of Professor Peter Sasieni PhD, Professor of Biostatistics, Department of Epidemiology, Mathematics and Statistics, Wolfson Institute of Preventive Medicine, London.

Details of statistical methods used will be outlined with the presentation of results in the section below.

We wish to note that confounding variables that are thought to affect atopy per se, were included in our analysis (number of siblings, socio-economic group, prematurity, contact with pets and smokers, history of bronchiolitis). Nevertheless, there may be other factors that effect atopy, which we are unaware of. This is one of the reasons why we have included a High Risk group as controls. We would thus expect that the peanut allergic and egg allergic group would be balanced for genetic and general environmental factors that predispose to atopy. If, indeed, differential routes and amount of exposure do play a causal role in sensitization, our data comparing these two groups is less likely to be confounded by these genetic and environmental factors.

Results

Assessment of Recall Accuracy of FFQ

A total of 30 of the 40 mothers completed both the initial and follow up questionnaire (Table 2). The remaining 10 mothers were either not contactable on follow up or were not willing to complete the follow up questionnaire.

Case	Initial recall (g/week)	Follow up recall (g/week)
1	25	1.61
2	0	0
3	0	0
4	25.19	13.34
5	10	10
6	6.44	6.44
7	1.35	2.7
8	32.74	29.4
9	44.44	41.44
10	47.71	59.22
11	0	0
12	0	0
13	0	0
14	94.5	96.5
15	37.5	36.9
16	0	0
17	0	0
18	6.3	6.3
19	0	0
20	111.14	83.84
21	13.5	13.5
22	29.1	54
23	14.7	14.7
24	0	0
25	0	0
26	0	4.95
27	0	2.1
28	32.75	26
29	0	0
30	10.5	13.93

Table 2 Reported peanut consumption (grams of peanut per week) on initial and follow up recall.

Figure 6 shows a plot of peanut consumption (grams/week) on initial recall against the follow up recall as a linear- and a smoothed-fit line. Due to the initial consumption value given by case 20 as 111.14g/week being considerably underestimated on follow up recall, the smooth-fit dips lower at

higher values, otherwise it stays close to the linear-fitted line, suggesting a linear relationship between the initial and follow up questionnaire of peanut consumption. The lack of a significant difference between the best fit line and y=x plot indicates that there is no apparent bias in predicting the recall values of one questionnaire from the other.

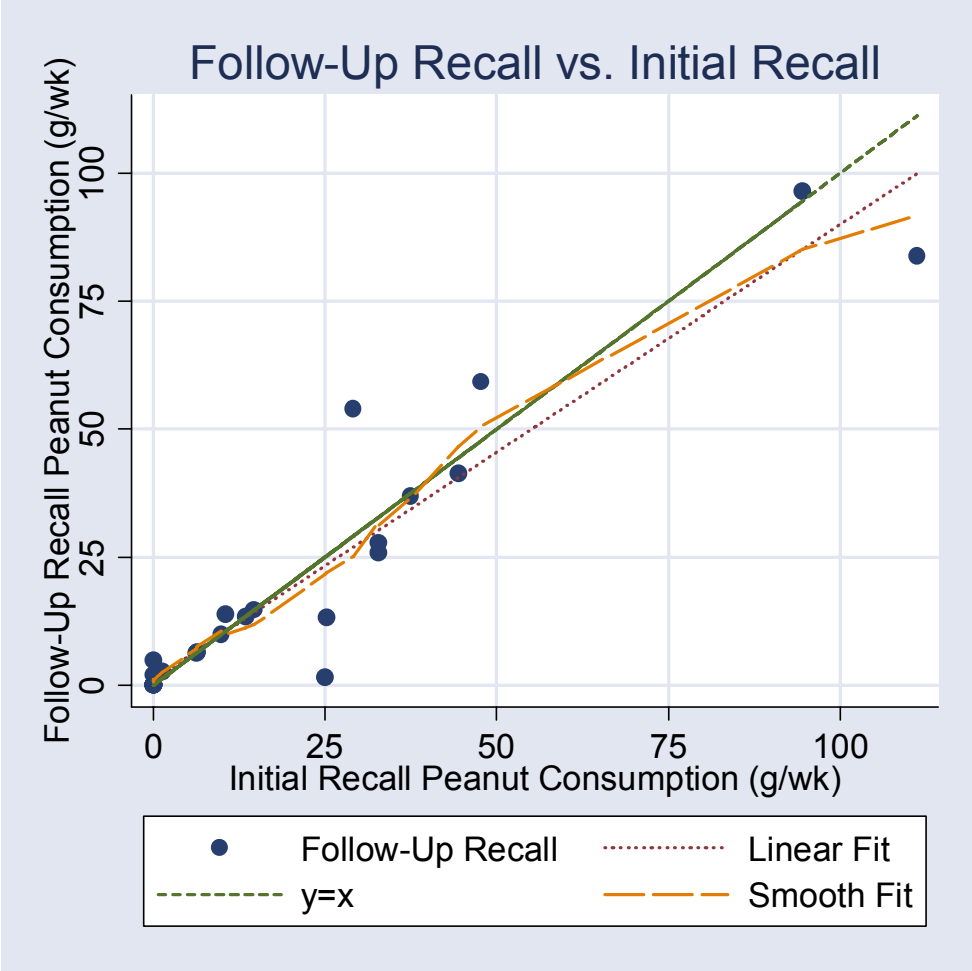


Figure 6 Graph of initial recall against follow up recall

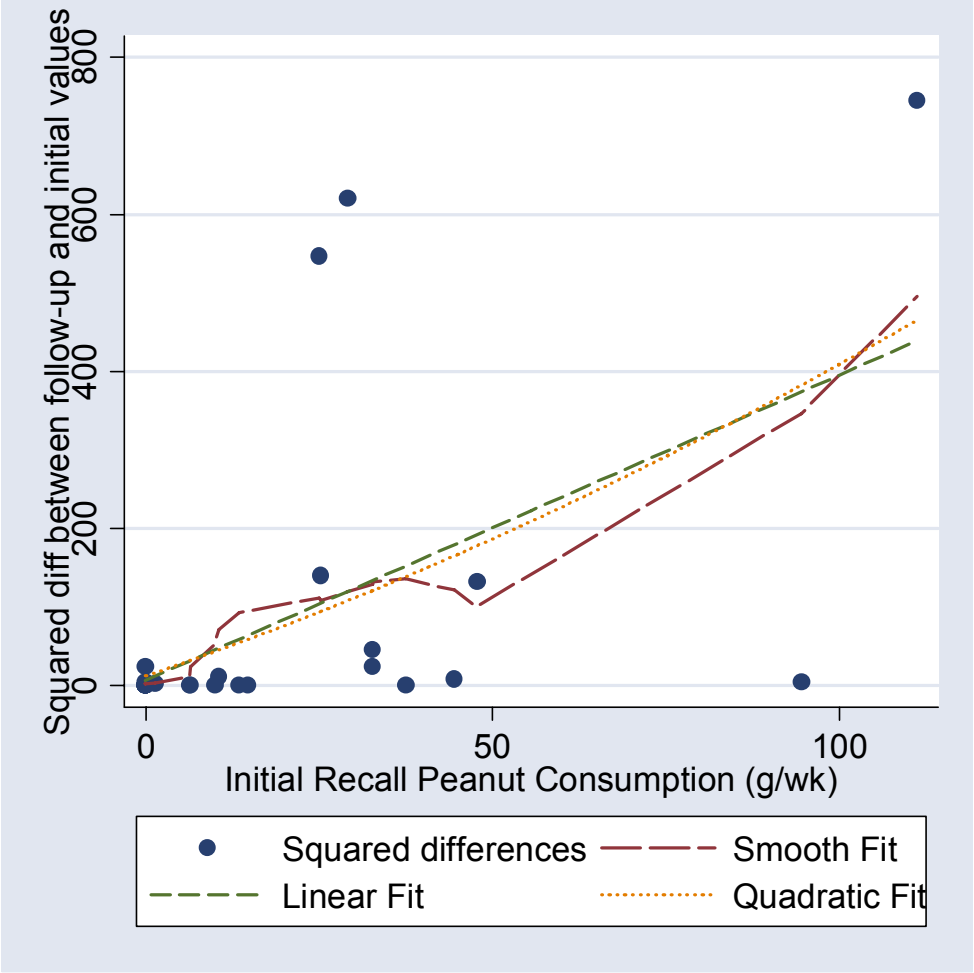


Figure 7 Graph of squared difference between the follow-up and initial values plotted against the initial recall values

The squared difference between the follow-up and initial values are plotted against the initial recall values (figure 7). This informs us that the variability in the follow up recall from the initial recall increases with higher initial recall values. As more peanut consumption is initially recalled, the greater variability there is in recalling it at a later date. This means that a simple range of possible initial recall values cannot be provided for any given follow up recall value, as this range will vary depending on the size of the follow up recall value. Using the predicted values from a regression line we can generate confidence intervals for a likely value of the initial recall given a particular follow up recall value, as seen in the figure 8 below. This simulates the anticipated practical usage of the SFFQ, where only retrospective data will be available. Figure 8 also illustrates the variance increasing as the follow up recall value increases.

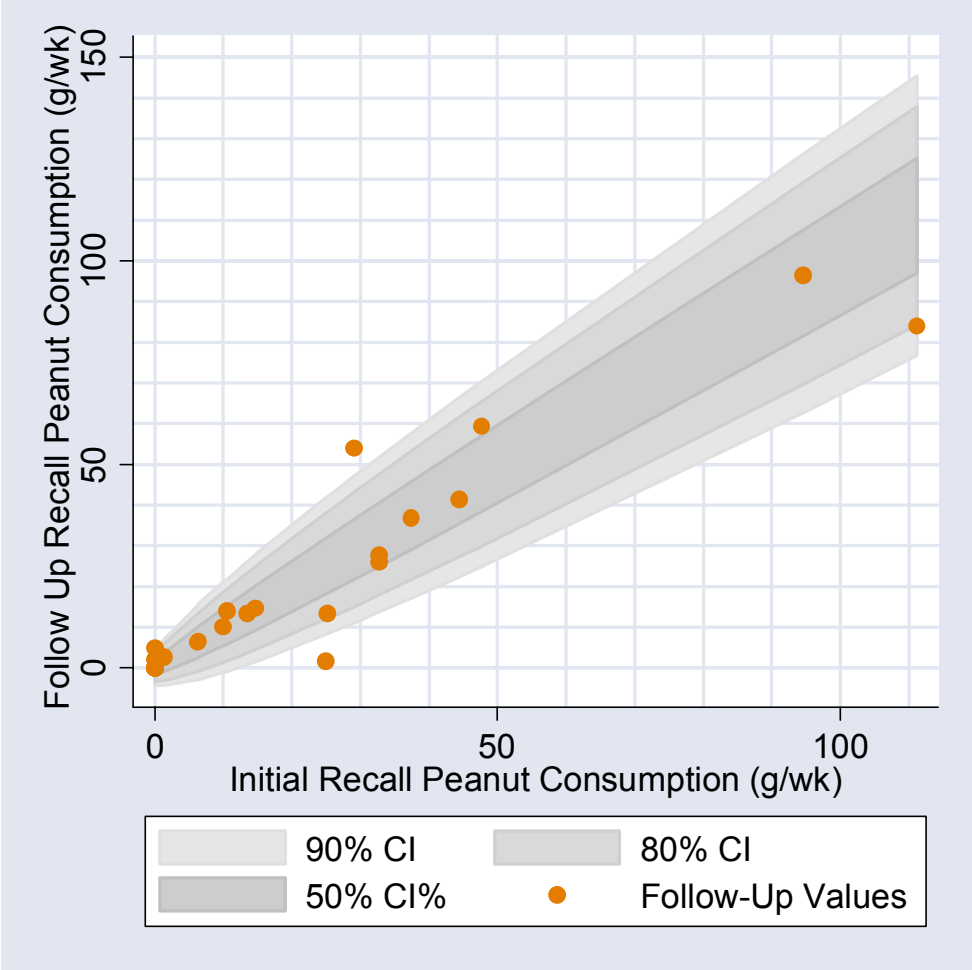


Figure 8 Confidence intervals for prediction of initial recall value from follow up recall value

Table 3 gives the ranges for which there are 50%, 80% and 90% probability of containing the initial recall values for a given follow up recall value.

Follow up value	50%	80%	90%
0	0 – 2	0 – 3	0 – 4
15	10 - 20	5 -25	2 -28
50	40 – 60	32 - 68	27 - 73
100	87 – 113	75 – 126	67 - 133

Table 3 Ranges of initial recall value at different levels of confidence for a given follow up recall (g/peanut)

Results of Main Study

We successfully recruited

- 133 Peanut allergic Cases
- 160 High Risk (Egg allergic) Controls
- 150 Normal Controls

The results of the study will be presented as they relate to the objectives stated above. All analyses were carried out using Stata 8.0 for Windows (StataCorp LP, College Station, TX, USA) statistical software package.

Basic Demographics

Gender

28 records in the Normal Controls group did not have data on gender.

Group	Female	Male	Total
Cases	39 (29.32%)	94 (70.68%)	133 (100%)
High Risk Controls	51 (31.88%)	109 (68.12%)	160 (100%)
Normal Controls	50 (40.98%)	72 (59.02%)	122 (100%)
Total	140 (33.73%)	275 (66.27%)	415 (100%)

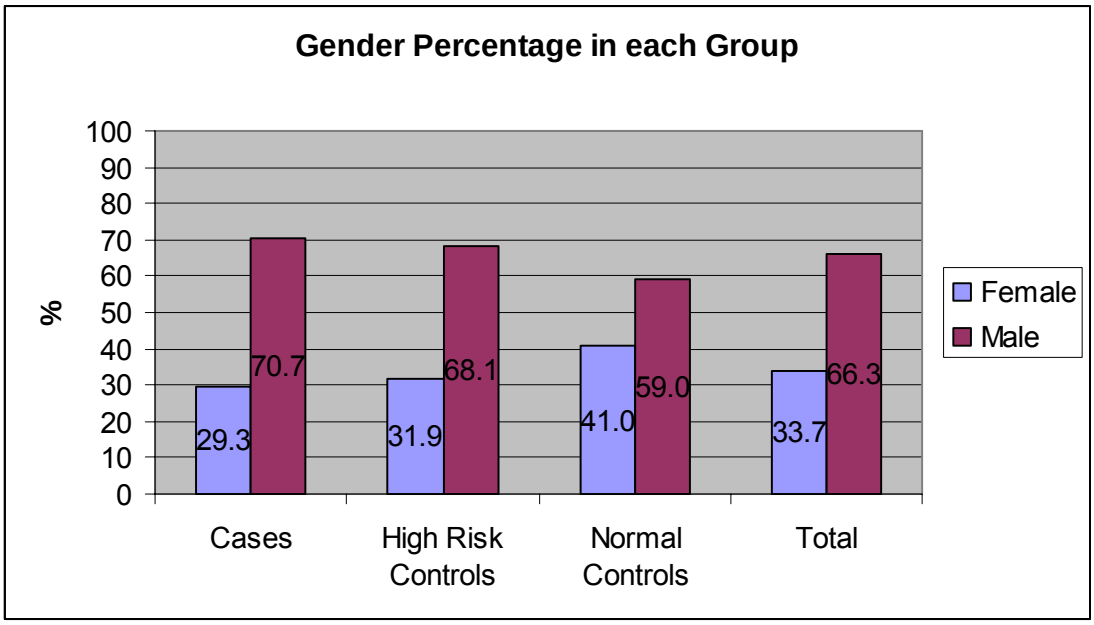


Figure 9: Proportion of males and females in each group

Proportions were compared using the χ^2 test. No significant difference was found between the three groups by gender.

Age (Months) when questionnaire was completed

Group	Median	Min,Max
Cases	28	5,50
High Risk Controls	23	6,49
Normal Controls	26	6,51
Total	26	5,51

The percentiles for age at completing the questionnaire for the whole sample were -

Percentile	10^{th}	25^{th}	50^{th}	75^{th}	90^{th}
Age (months)	10	14	26	36	45

The numbers of children falling into each of these percentile categories were compared using χ^2 test. No significant differences were found between the groups.

Group	5–10mth	10.5–14mth	14.5-26mth	26.5-36mth	36.5–51mth	Total
Cases	12 (9.02%)	21 (15.79%)	28 (21.05%)	33 (24.81%)	39 (29.32%)	133 (100%)
High Risk Controls	23(14.37%)	20 (12.50%)	49 (30.63%)	41 (25.62%)	27 (16.88%)	160 (100%)
Normal Controls	19(12.67%)	17 (11.33%)	40 (26.67%)	33 (22.00%)	41 (27.33%)	150 (100%)
Total	54(12.19%)	58 (13.09%)	117(26.41%)	107 (24.15%)	107 (24.15%)	443 (100%)

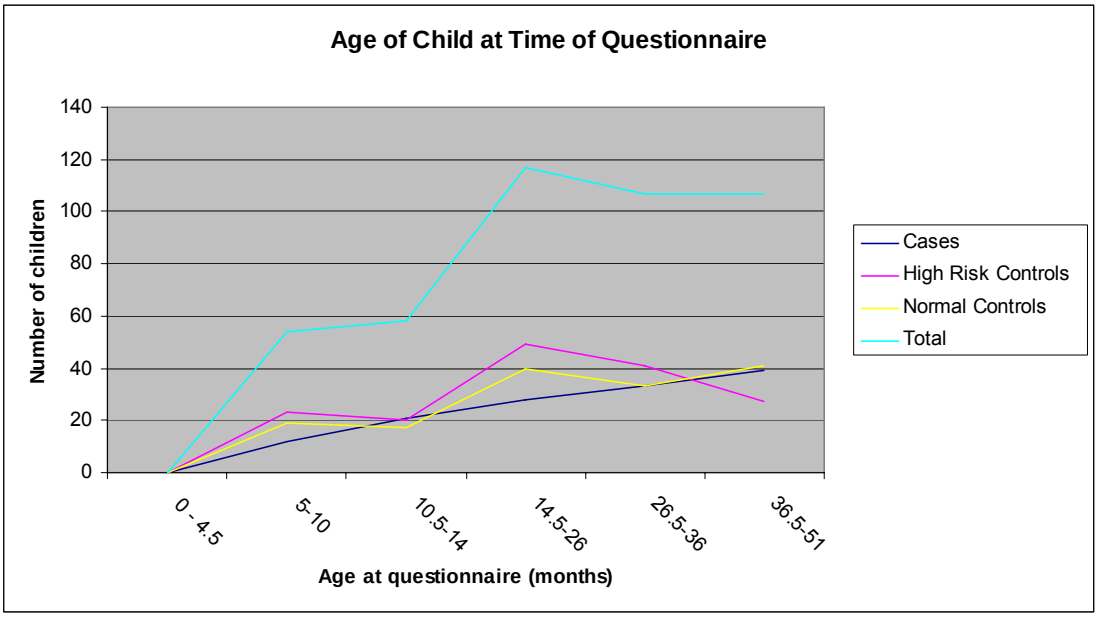


Figure 10: Age of child at time of questionnaire

Objective 1

The aim of this part of the study was to demonstrate that a high level of exposure to environmental peanut during infancy is a risk factor for later PA. This can be done in a number of ways using our data:

1) Comparison of **total weekly household consumption of peanut during infant's first year of life**. This includes the consumption of all family members including the infant (if they were eating peanut) and the mother expressed as grammes of peanut per week. In order to average this value out over the whole one year period, the infant and maternal values have been weighted. For example, infants may have consumed 10g peanut/week but only from 6 months of age and thus the contribution to annual consumption is only 5g/week. Similarly, mothers provided different data for their peanut consumption during lactation and also for any period during the infant's first year when they were not lactating. The length of lactation in each individual case is taken into account to produce an average value for the entire year.

Group	Number	25th centile	Median	75th centile
Cases	133	33.33	78.87	157.00
High Risk Control	160	0.00	7.83	38.14
Normal Control	150	4.20	29.14	82.10

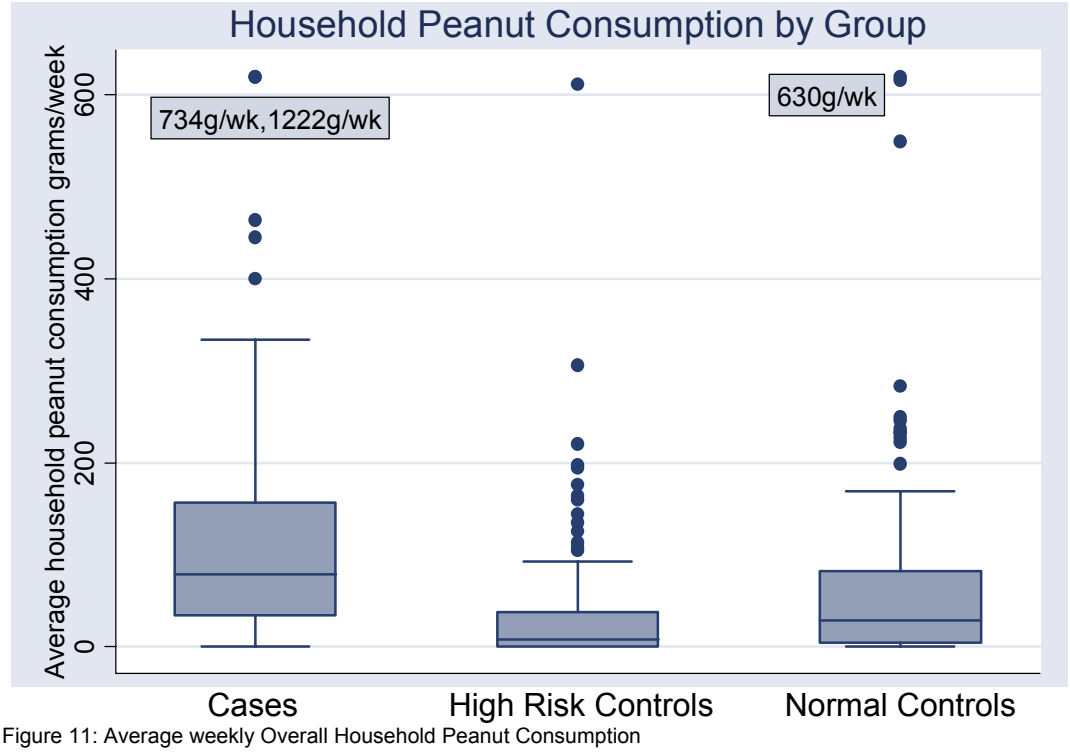


Figure 11: Average weekly Overall Household Peanut Consumption

A Kruskal-Wallis test rejects the hypothesis that the populations are the same with a p-value of 0.0001, which indicates a highly significant difference in the total weekly household consumption of peanut during infant's first year of life between the three groups.

Pair wise comparisons between the three groups each gives highly significant differences-

Cases vs High Risk Controls	p value < 0.0001
Cases vs Normal Controls	p value < 0.0001
High Risk Controls vs Normal Controls	p value < 0.0001

2) Comparison of **total weekly household episodes of consumption of peanut during infant's first year of life**.

Group	Number	25th centile	Median	75th centile
Cases	133	2.25	5.25	9
High Risk Control	160	0	1	4
Normal Control	150	0.50	2.33	6

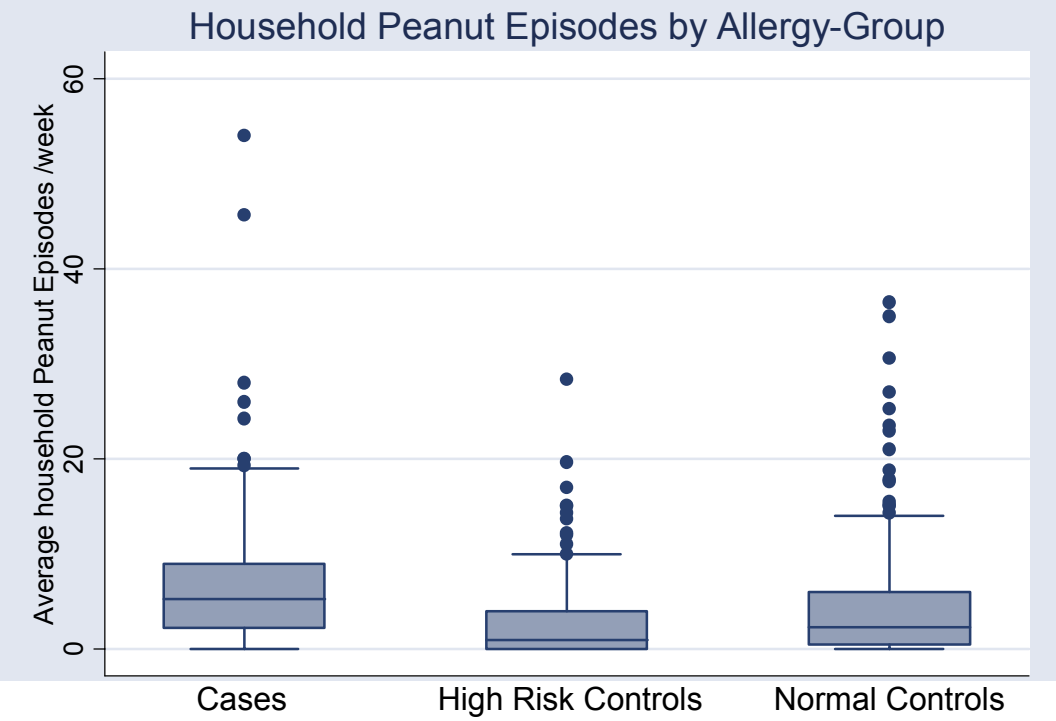


Figure 12: Average weekly Overall Household Episodes of Peanut Consumption

Kruskal-Wallis rejects the hypothesis that the populations are the same with a p-value of 0.0001, which indicates a highly significant difference in the Average Total Household Peanut Episodes per week between the three groups.

Pair wise comparisons between the three groups each gives highly significant differences –

Cases vs High Risk Controls	p value < 0.0001
Cases vs Normal Controls	p value = 0.0001
High Risk Controls vs Normal Controls	p value = 0.0002

3) Comparison of **total weekly household consumption of peanut butter during infant's first year of life**. This was calculated in the same manner as total household consumption of peanut but only taking peanut butter consumption into account.

Group	Number	25th centile	Median	75th centile
Cases	133	0	22.48	67.5
High Risk Control	160	0	0	0
Normal Control	150	0	0	19.69

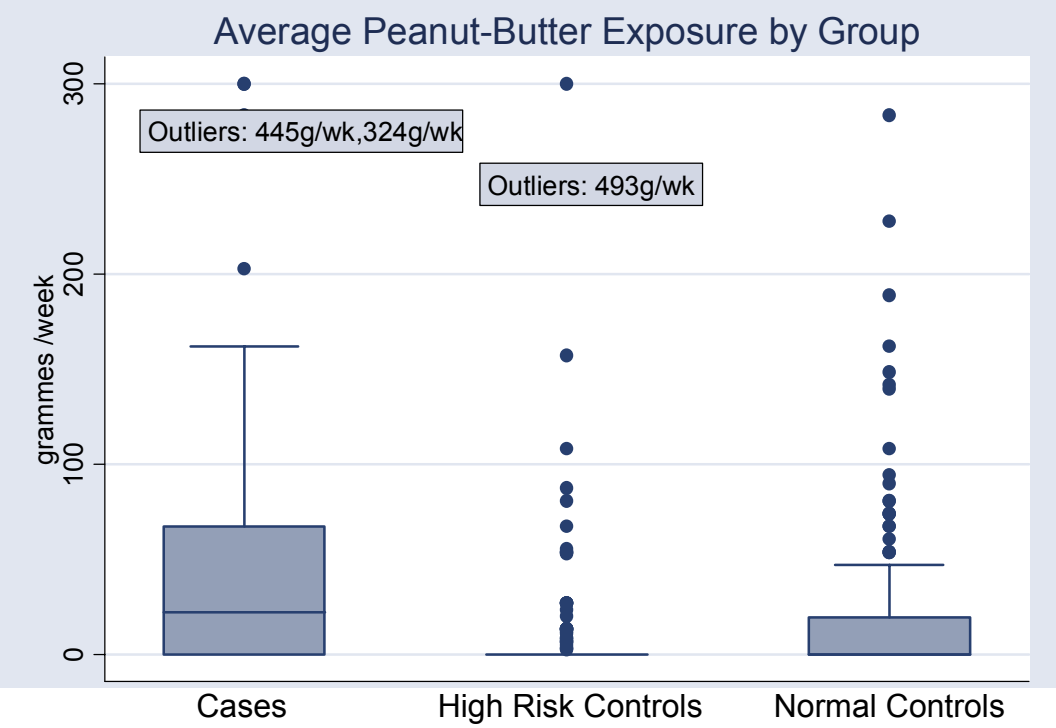


Figure 13: Average weekly Overall Household Peanut Butter Consumption

Kruskal-Wallis rejects the hypothesis that the populations are the same with a p-value of 0.0001, which indicates a highly significant difference in the total weekly household consumption of peanut butter between the three groups.

Pair wise comparisons between the three groups each gave significant differences –

Cases vs High Risk Controls	p value < 0.0001
Cases vs Normal Controls	p value < 0.0001
High Risk Controls vs Normal Controls	p value = 0.0023

4) Comparison of **total weekly household episodes of peanut butter consumption of during infant's first year of life**. This was calculated in the same manner as total household episodes of peanut consumption but only taking peanut butter consumption into account.

Group	Number	25th centile	Median	75th centile
Cases	133	0	1	3
High Risk Control	160	0	0	0
Normal Control	150	0	0	1

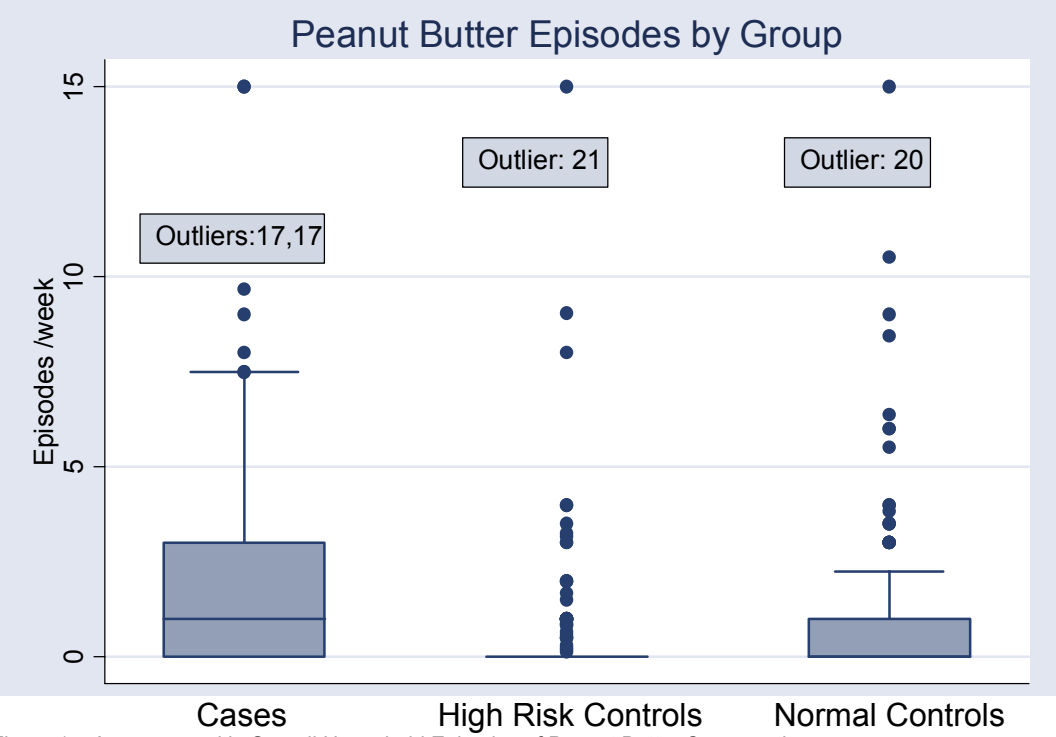


Figure 14: Average weekly Overall Household Episodes of Peanut Butter Consumption

Kruskal-Wallis rejects the hypothesis that the populations are the same with a p-value of 0.0001, which indicates a highly significant difference in Total Weekly Peanut Butter Episodes between the three groups.

Cases vs High Risk Controls	p value < 0.0001
Cases vs Normal Controls	p value < 0.0001
High Risk Controls vs Normal Controls	p value = 0.0028

An analysis of the proportion of household peanut that was consumed in the form of peanut butter was carried out on all families where any peanut was consumed in the household during the first year of life.

Group	Number*	25th centile	Median	75th centile
Cases	124	0	0.26	0.61
High Risk Control	109	0	0	0
Normal Control	120	0	0	0.46

*Households with no peanut consumption were not included in the analysis.

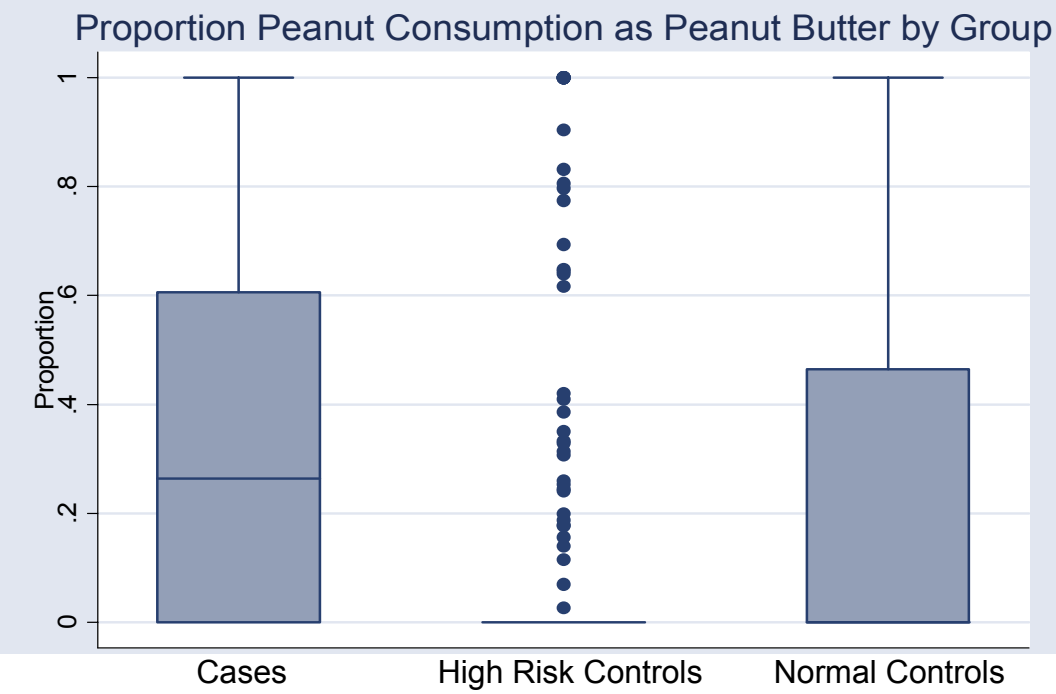


Figure 15: Proportion of Household Peanut Consumption in form of Peanut Butter

Kruskal-Wallis rejects the hypothesis that the populations are the same with a p-value of 0.0001, which indicates a highly significant difference in Proportion between the three groups.

Pair wise comparisons between the three groups each gave significant differences –

Cases vs High Risk Controls	p value < 0.0001
Cases vs Normal Controls	p value = 0.0004
High Risk Controls vs Normal Controls	p value = 0.0020

5) A further consideration when analysing outcomes based on different dietary components, was the availability of the peanut contained in different foods to act as a potential environmental allergen. We thus also considered differences between our 3 groups based not only on peanut butter consumption alone, but also on peanut consumed in forms other than peanut butter as well as in terms of Snickers alone. We also analysed snickers consumption in terms of total episodes per week of consumption and the proportion of total peanut consumption that was accounted for by snickers.

i) Average Weekly Household Consumption excluding Peanut-Butter

Group	Number	25th centile	Median	75th centile
Cases	133	13.89	31.82	85
High Risk Control	160	0	5.98	27.94
Normal Control	150	0	15.2	51.6

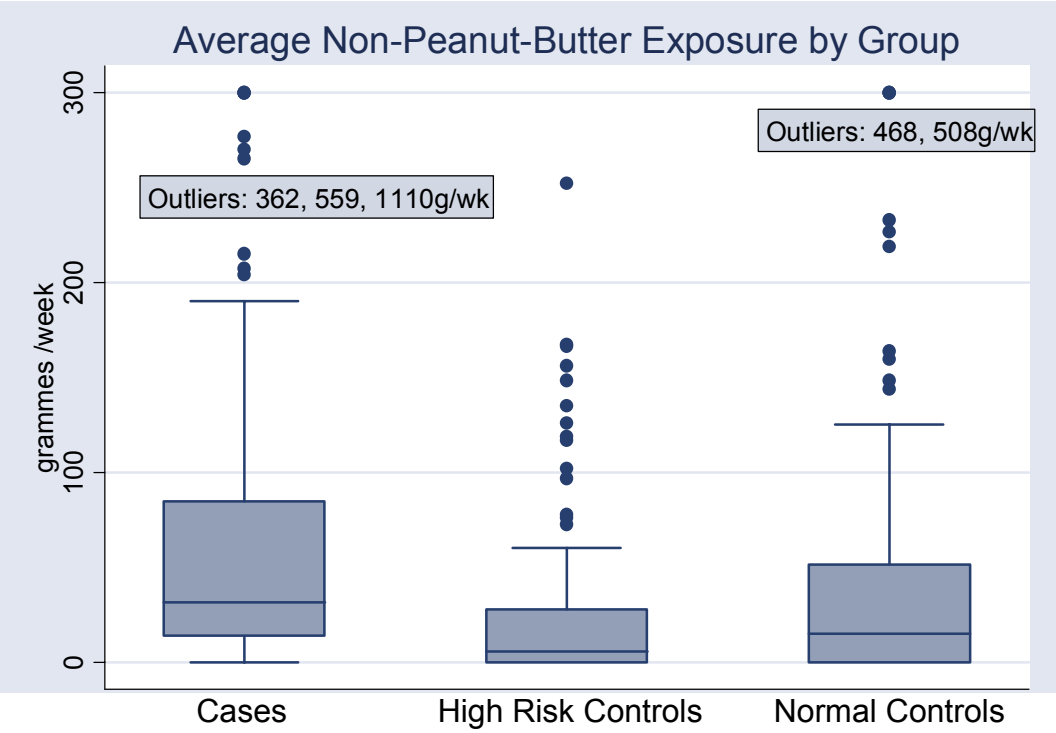


Figure 16: Average weekly Overall Household Consumption of Peanut excluding Peanut Butter

Kruskal-Wallis rejects the hypothesis that the populations are the same with a p-value of 0.0001, which indicates a highly significant difference in the AverageHousehold Peanut Exposure (not through Peanut Butter) between the three groups.

Pair wise comparisons between the three groups each gave significant differences -

Cases vs High Risk Controls	p value < 0.0001
Cases vs Normal Controls	p value = 0.0001
High Risk Controls vs Normal Controls	p value = 0.0040

ii) Average weekly household Snickers Consumption

Using data for the Cases and High Risk groups only:

Group	Number	Median	75th centile	90th centile
Cases	133	0	15.40	30.8
High Risk Controls	160	0	1.46	15.4

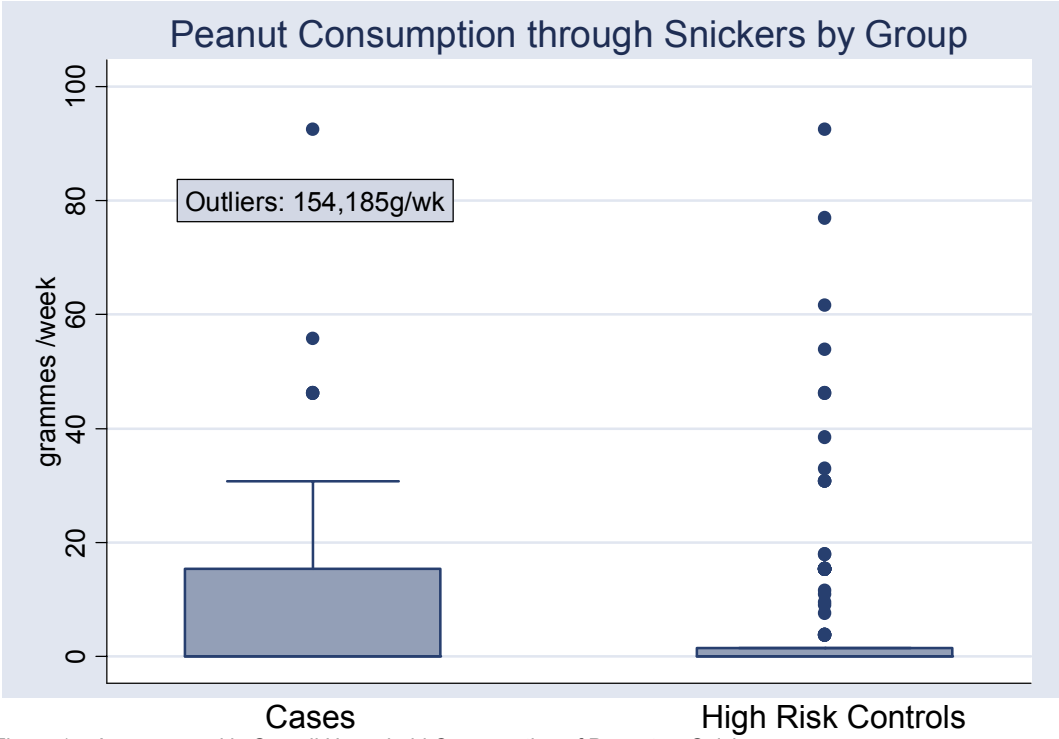


Figure 17: Average weekly Overall Household Consumption of Peanut as Snickers

These groups are significantly different (p=0.0028).

iii) Average weekly household episodes of Snickers Consumption

Using data for the Cases and High Risk groups only:

Group	n	Median	75th centile	90thcentile	Max
Cases	133	0	1	2	12
High Risk Controls	160	0	0.095	1	5

Figure 18: Average weekly Overall Household Episodes of Snickers Consumption

The difference between the two groups was found to be highly significant (p-value = 0.0024).

iv) Proportion of Average Total Household Peanut Exposure through Snickers

Using data for the Cases and High Risk Control groups only where there was some household peanut consumption.

Group	n	25th centile	Median	75th centile
Cases	124	0	0	0.18
High Risk Controls	109	0	0	0.28

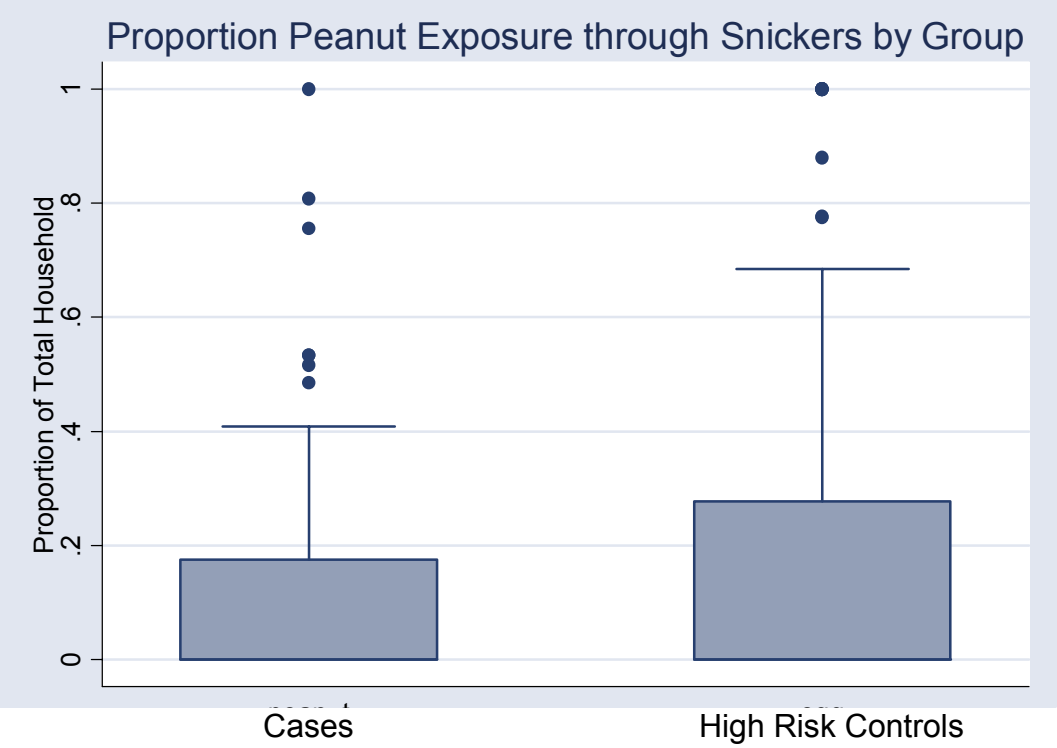


Figure 19: Proportion of Average weekly Overall Household Peanut Consumption as Snickers

The difference between the two groups was found to be non- significant (p-value = 0.2655).

4) A further approach to considering the differences in environmental exposure in these 3 groups is by comparing the number of people in each household who consumed peanut containing products during the first year of the child's life.

Group	Number	25th centile	Median	75th centile
Cases	133	1	2	3
High Risk Control	160	0	1	2
Normal Control	150	1	2	2

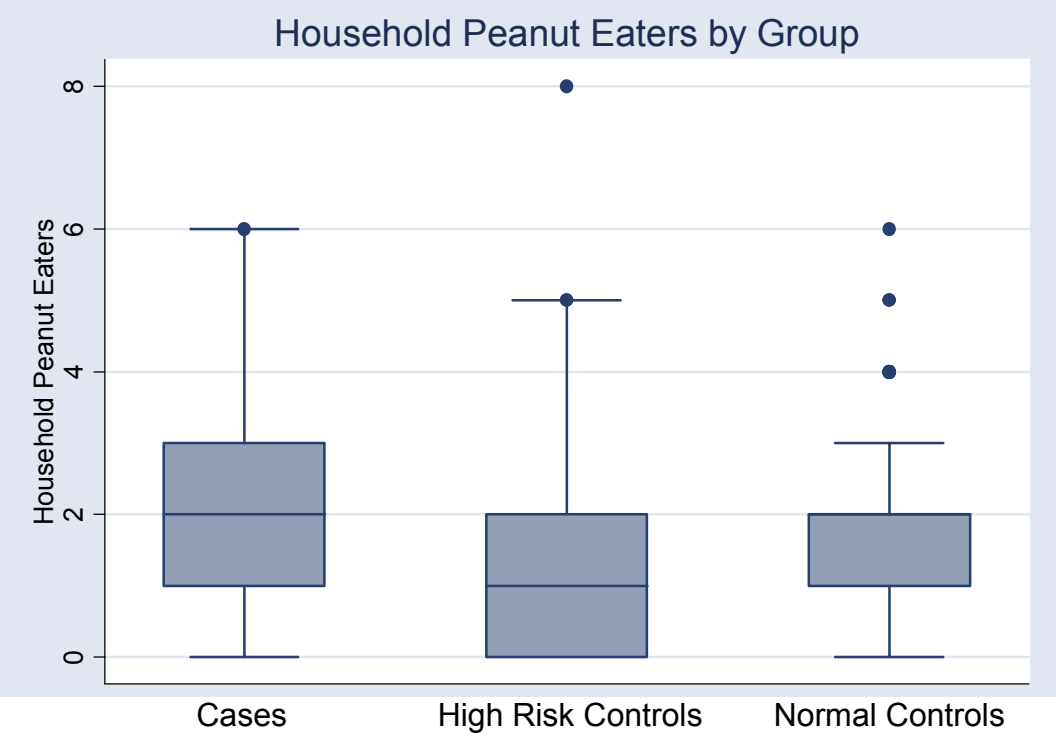


Figure 20: Number of Peanut Eaters in Household

Group	No. of Peanut-Eaters in Household (%-age of Group)				
	0	1	2	3+	Total
Cases	9 (6.77%)	33 (24.81%)	52 (39.10%)	39 (29.32%)	133 (100%)
High Risk Controls	51 (31.87%)	51 (31.87%)	41 (25.62%)	17 (10.63%)	160 (100%)
Normal Controls	30 (20.00%)	40 (26.67%)	49 (32.67%)	31 (20.67%)	150 (100%)
Total	90 (20.32%)	124 (27.99%)	142 (32.05%)	87 (19.64%)	443 (100%)

Kruskal-Wallis rejects the hypothesis that the populations are the same with a p-value of 0.0001, which indicates a highly significant difference in the Total Number of Peanut Eaters in Household between the three groups.

Pair wise comparisons between the three groups each gave significant differences –

Cases vs High Risk Controls	p value < 0.0001
Cases vs Normal Controls	p value = 0.0023
High Risk Controls vs Normal Controls	p value = 0.0009

This is specific to the number of household members who eat peanut and contrasts with the lack of difference between total numbers of people in each household overall:

Group	Number	25th centile	Median	75th centile
Cases	133	3	4	4
High Risk Controls	160	3	3	4
Normal Controls	150	3	3	4

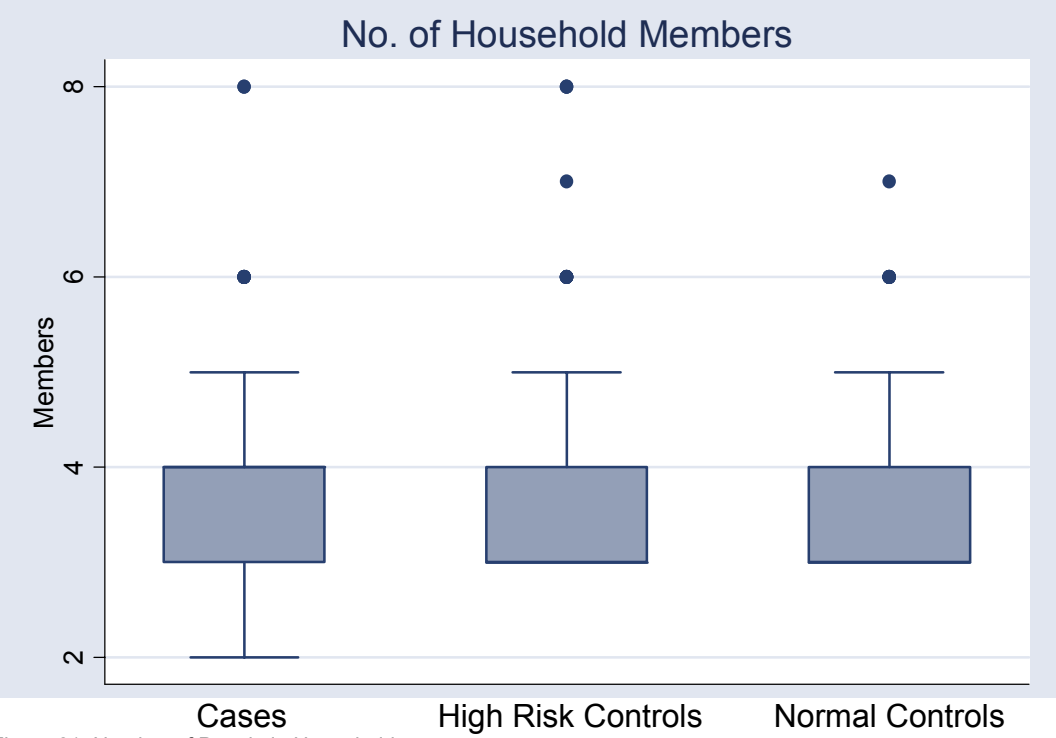


Figure 21: Number of People in Household

The Kruskal-Wallis test does not reject the hypothesis that the number of household members are the same.

6) Finally, a number of comparisons were made between subpopulations of the Cases and High Risk Controls to ensure that the differences in the complete groups were not due to systematic bias within these subpopulations. There are 3 analyses, the relevance of which will be discussed in greater detail in the discussion:

i) Cases who had egg allergy as well as PA with High Risk Controls.

ii) Cases with High Risk Controls who had not eaten peanut before.

iii) Cases with SPT >10mm to peanut with High Risk Controls.

i) Cases who had egg allergy as well as peanut allergy with High Risk Controls:

Group	Number	25th centile	Median	75th centile
High Risk Controls	160	0	7.825	38.14008
Cases with concurrent egg allergy	75	29.83	71.75	141.1

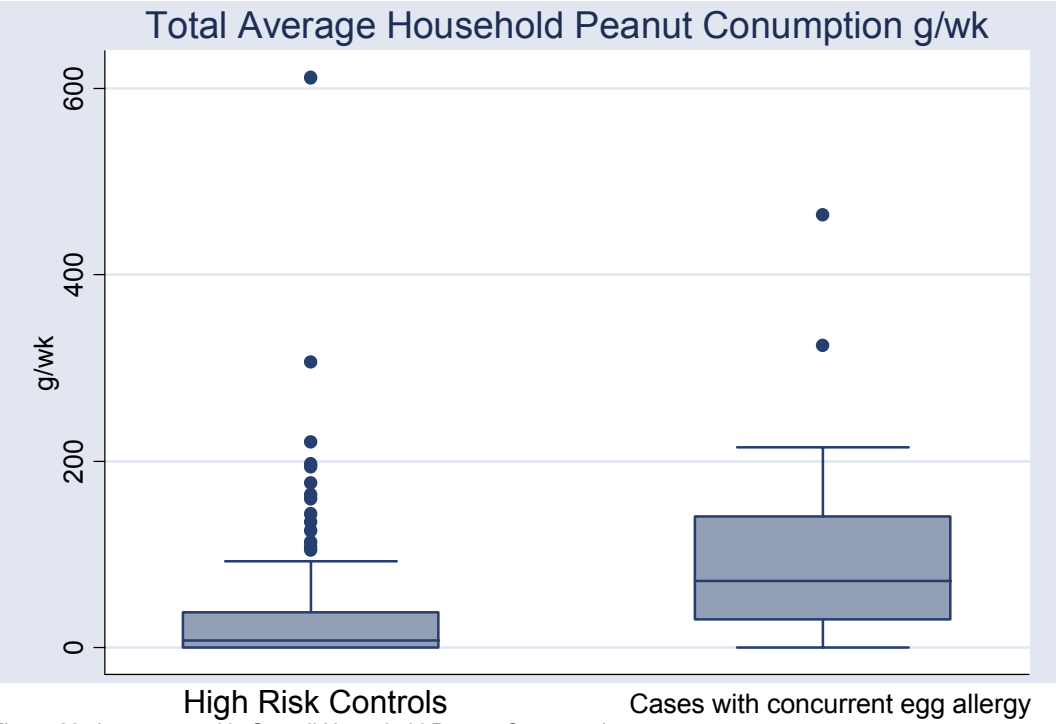


Figure 22: Average weekly Overall Household Peanut Consumption

The Kruskal-Wallis test rejects the hypothesis that the groups are the same (p=0.0001).

ii) Cases with High Risk Controls who had not eaten peanut before:

Group	Number	25th centile	Median	75th centile
High Risk Controls, not eaten peanut	122	0	3.04	26.0
Cases	133	33.33	78.87	157.00

The Kruskal-Wallis test rejects the hypothesis that the groups are the same (p=0.0001).

iii) Cases with SPT =/>10mm to peanut with High Risk Controls.

Group	Number	25th centile	Median	75th centile
High Risk Controls	160	0	7.83	38.14
Cases with SPT>10mm	54	26.25	58.82	125.81

The Kruskal-Wallis test rejects the hypothesis that the groups are the same (p=0.0001).

Maternal consumption of peanut during pregnancy and breastfeeding will be considered in Objective 3 below.

Department of Health Guidance

The second part of this objective was to compare Cases (n=133) and High Risk Controls (n=160) with regards to their prior knowledge of the DoH guidance[28] and, where appropriate, if they adhered to the advice regarding maternal and infant peanut avoidance.

We considered the efficacy of the guidelines in 4 ways:

I. Awareness of the guidance amongst the target population
II. Maternal decision to follow advice
III. Maternal decision to follow advice coupled with evidence of successful avoidance based on FFQ study data
IV. Reduction in likelihood of PA

Consideration of our entire sample for outcomes 1-3 would provide useful information about how the guidelines have been received by mothers and how it has influenced their behaviour. In order to obtain a better understanding of whether the advice may actually reduce the likelihood of peanut allergy, we considered the relative adherence to the guidelines amongst the Cases and High Risk Controls.

χ^2 test has been used to compare proportions.

Using our entire sample (n=443) we compared those 'high risk' mothers to whom the advice applies (women who are atopic or have an atopic first degree relative) to those to whom it does not (low risk), with regards to awareness of and adherence to the guidelines.

DoH Guidance	n	Awareness of Guidance	Reported Adherence to Guidance	True intentional adherence based on FFQ
High Risk Mothers	322	174(54.0%)	112(34.8%)	54(16.8%)
Low Risk Mothers	121	54(44.6%)	23(19.0%)	12(9.9%)
p value		NS	<0.01	NS

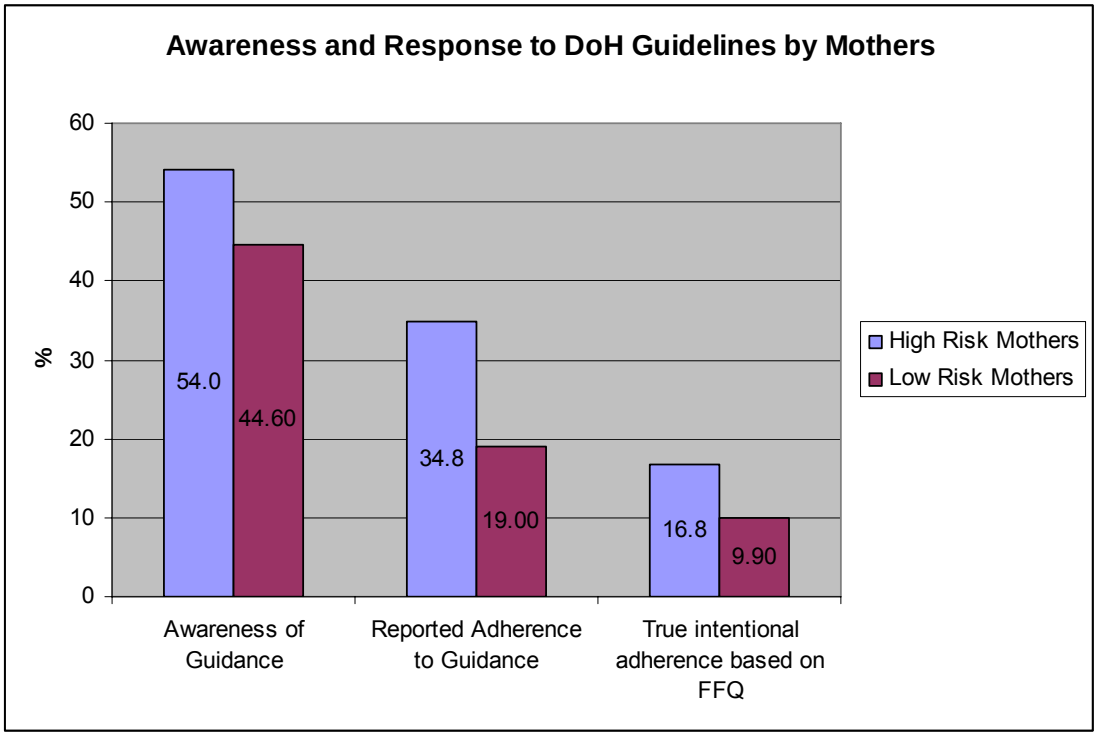


Figure 23: Awareness and response to DoH guidelines

The chart below maps the response to the advice amongst high risk mothers.

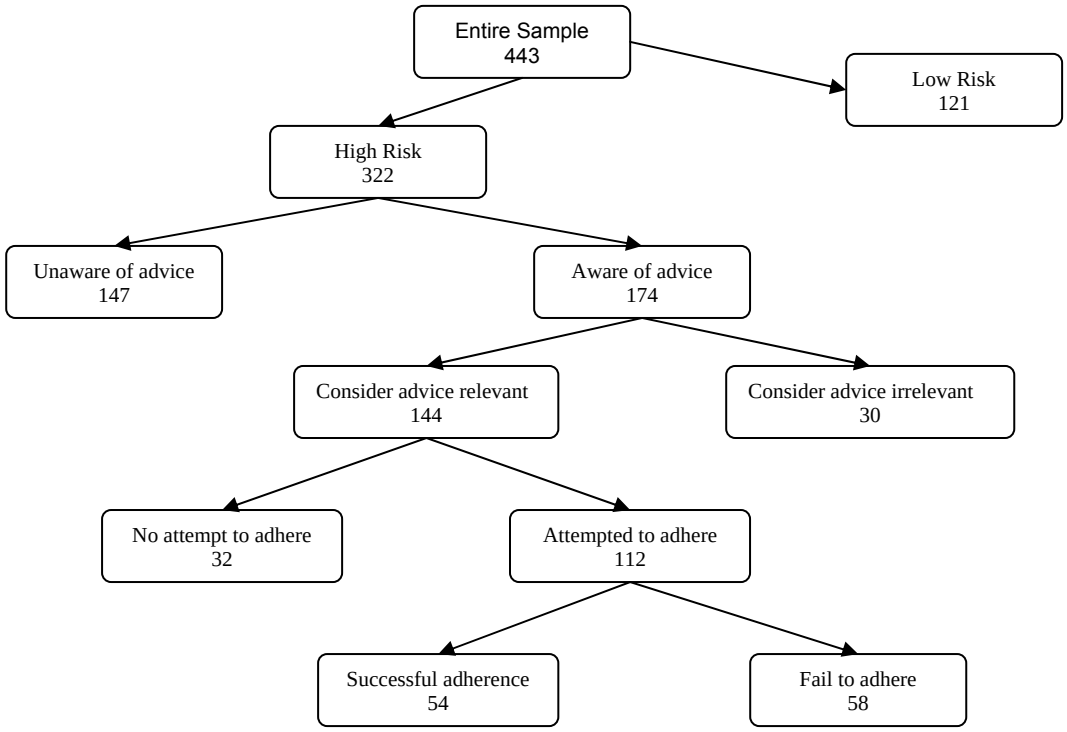


Figure 24: Awareness and response to DoH guidelines by 'High Risk' mothers

Efficacy of Guidelines in Reduction of Peanut Allergy

The DoH advice is targeted at mothers' with a personal or family history of atopy. Only this population, to whom the advice was targeted, was considered further. There was no significant difference between the Cases and High Risk Controls in terms of number of mothers to whom the advice would be relevant for.

DoH Guidance	n	Awareness of guidance	Aware and considered relevant	Maternal Report of Adherence	True adherence based on FFQ
Cases	112	51(45.5%)	38(33.9%)	28(25%)	28(25%)
High Risk Control	124	72(58.1%)	63(50.8%)	55(44.4%)	53(42.7%)
p value	NS	NS	<0.01	<0.01	<0.01

Whilst awareness of the advice is similar in the 2 groups, there are significantly greater proportions of mothers amongst the High Risk Controls who correctly considered the advice to be relevant to them, attempted to follow it and successfully did so.

Those mothers who claimed to adhere to the advice and those who successfully followed it, were not the same mothers. The results were thus also considered only amongst those mothers to whom the advice applied, were aware of it and chose to follow it.

DoH Guidance	n	Avoidance during preg/lactation	Infant & Maternal avoidance
Cases	28	15 (53.6%)	13 (46.4%)
High Risk Controls	55	36 (65.5%)	30 (54.5%)
p value		NS	NS

Thus amongst mothers to whom the advice was relevant, in the Cases 13/112 (11.6%) mothers were aware of the advice and successfully followed it. When compared to 30/124 in the High Risk Controls (24.2%), there is a significantly greater proportion amongst the High Risk Controls (p=0.025).

Objective 2

The aim of this part of the study was to investigate the role of direct application of preparations containing peanut or soya oil to inflamed skin in sensitization to peanut allergen. Our previous published data[6] identified the application of peanut containing creams as an independent risk factor for PA, as was the presence of an oozing/crusting rash.

Information was gathered for all children on the use of topically applied creams during the first year of life. Parents were able to select which creams they had used from a large list of options and also add any other preparations used. Our knowledge of which commercially available creams contain peanut or soya oil allowed comparison of the proportion of children in each group who were using these products. Further analysis was performed on the number of different peanut/soya-oil–containing preparations used by the children in the three groups. This included an analysis confining the comparisons only to the children who were exposed to these products within each group.

Information was also gathered on the presence of either eczema or other oozing/crusting rash (nappy rash, cradle cap etc) during first year of life. Age of eczema onset and the maximal strength of steroid treatment required (see table 4) were used as crude markers of severity.

0	No steroid used
1	Mild steroid used
2	Moderately potent steroid used
3	Potent steroid used

Table 4: Scoring system for eczema severity based on highest strength of steroid used

Preparations containing Peanut Oil	Preparations containing Soya Oil
Oily Calamine Lotion	Balneum
Polytar Emollient Bath Additive	Balneum Plus
Polytar AF Shampoo	
Polytar Plus Liquid	
Polytar Liquid plus	
Zinc & Castor Oil Ointment	
Zinc Cream	
Siopel	
Calendula Baby cream / Nappy cream	

Table 5: Commercially available preparations containing peanut or soya oil

Eczema & Rash in First Year of Life

Rash in first year of life	Presence of eczema	Presence of oozing/crusting rash	Presence of eczema or oozing/crusting rash
Cases	122(91.7%)	104(78.2%)	130(97.7%)
High Risk Controls	141(88.1%)	126(78.8%)	152(95%)
Normal Controls	63(42%)	90(60%)	114(76%)
p value	<0.0001	<0.0001	<0.0001

The highly significant differences found on χ^2 analysis are due to the differences between Cases and Normal Controls and between High Risk and Normal Controls. There are no significant differences between the Cases and High Risk Controls for any of these outcomes.

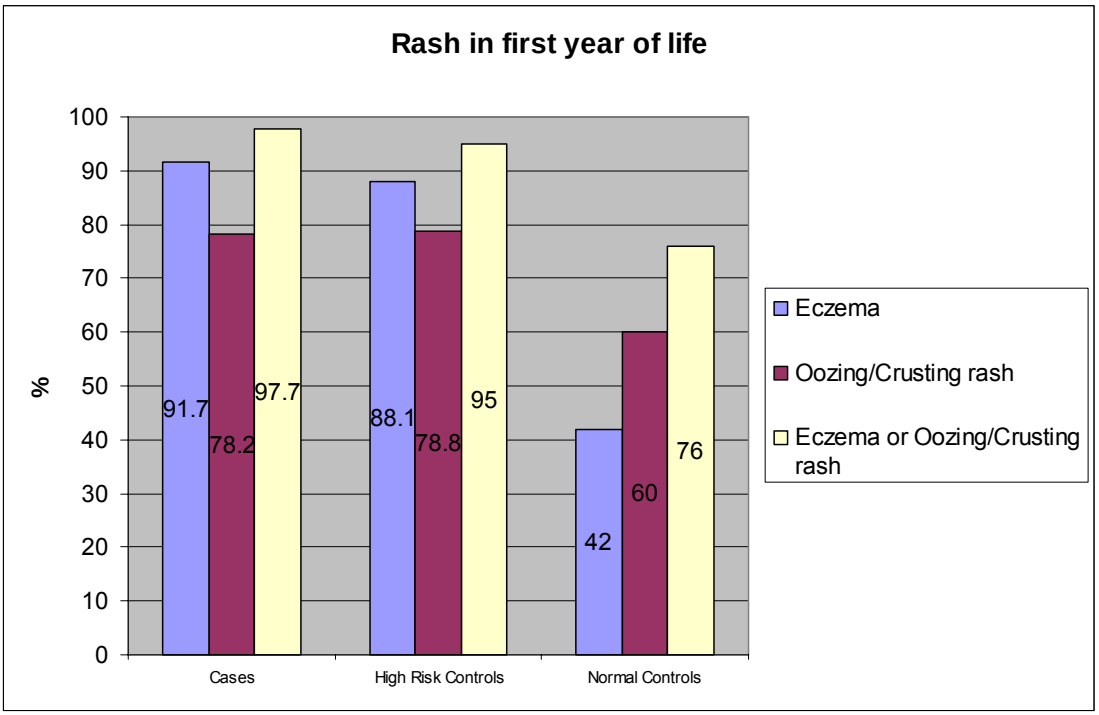


Figure 25: Presence of rashes in first year of life

Age of onset of eczema was compared:

	n	25th centile	Median	75th centile
Cases	116	1	2.5	4.5
High Risk Controls	135	1	2	4
Normal Controls	60	1	3.75	6

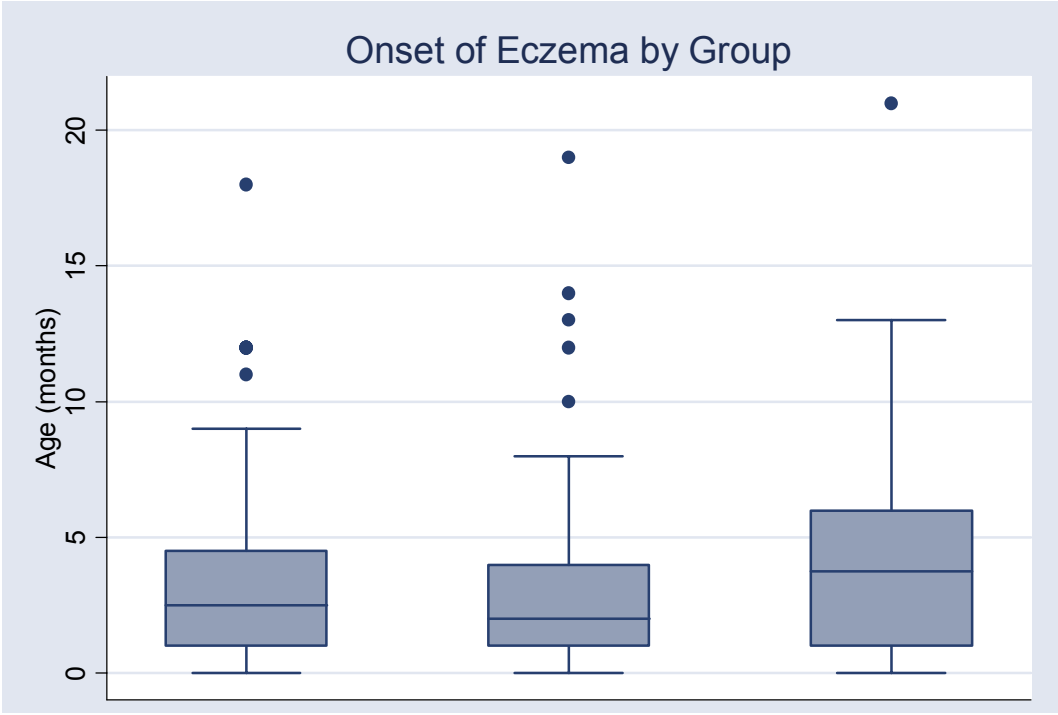


Figure 26: Age of onset of eczema

Pair-wise comparison again reveals significant differences between Cases and Normal Controls and between High Risk and Normal Controls.

Cases vs High Risk Controls	NS
Cases vs Normal Controls	p value < 0.0001
High Risk Controls vs Normal Controls	p value < 0.0001

Severity of eczema was compared between the Cases and High Risk Controls using the crude severity score based on maximal steroid strength (Table 4).

	n	25th centile	Median	75th centile
Cases	117	1	1	2
High Risk Controls	140	1	1	2

No significant differences were detected between the 2 groups for eczema severity.

Use of Creams

Groups were compared for their total use of any topical creams applied to the infant over the first year of life.

	n	Proportion applying creams	25th centile	Median	75th centile	Mean no. creams/child
Cases	133	131(98.5%)	3	4	6	4.6
High Risk Controls	160	155(96.9%)	2	4	6	4.3
Normal Controls	150	132(88%)	1	2	4	2.6

Kruskal-Wallis rejects the hypothesis that the populations are the same with a p-value of 0.0001. Pair-wise analysis reveals significant difference between Cases and Normal Controls and High Risk and Normal Controls. The Cases and High Risk Controls do not differ significantly.

Cases vs High Risk Controls	NS
Cases vs Normal Controls	p value < 0.0001
High Risk Controls vs Normal Controls	p value < 0.0001

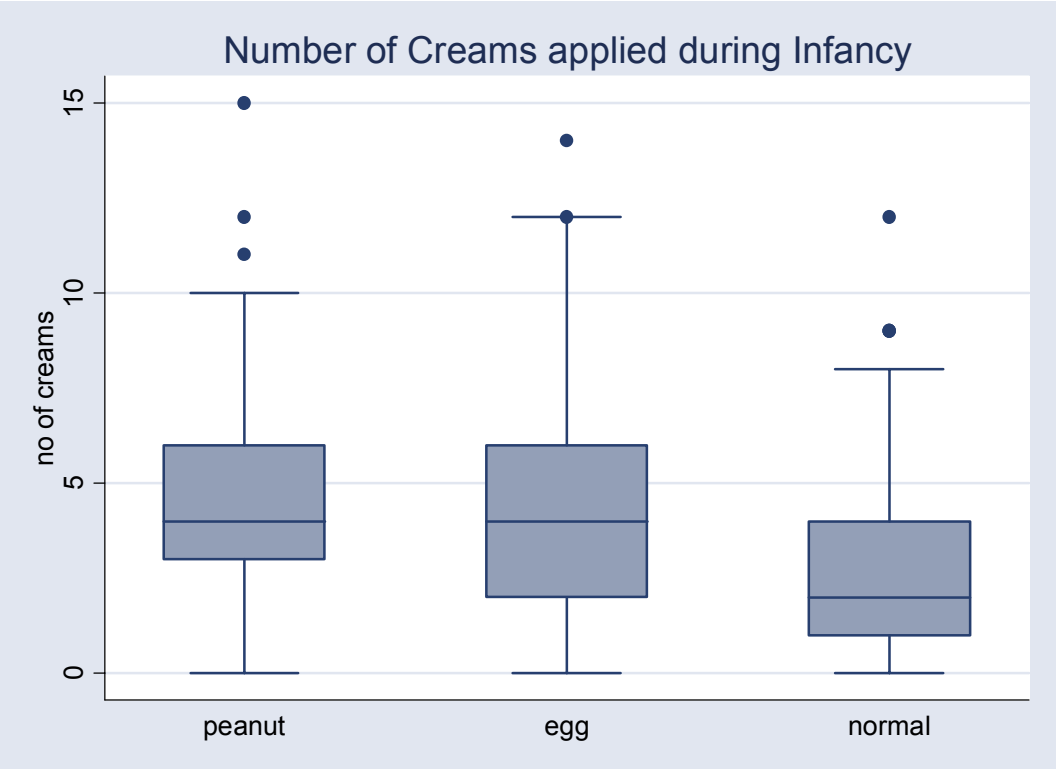


Figure 27: Number of creams applied to infant during first year of life

Application of creams containing peanut or soy

The groups were compared with regards to total number of peanut/soy containing creams applied to the skin during first year of life. The average number of peanut/soy containing creams per child was also compared, over the entire group and also just amongst those children who used the creams.

Application of peanut containing creams	Number of children applying peanut containing creams	Mean number of peanut containing creams per child	Mean Number of peanut containing creams per child using them
Cases	47 (35.3%)	0.51	1.45
High Risk Controls	54 (33.7%)	0.45	1.33
Normal Controls	46 (30.7%)	0.39	1.28

There were no significant differences between the groups in terms of the proportion of children applying creams containing peanut , the mean numbers of peanut containing creams per child over the whole group or just those using them.

Application of soy containing creams	Number of children applying soya containing creams	Mean Number of soya creams per child	Mean Number of soya containing creams per child using them
Cases	32 (24.1%)	0.26	1.09
High Risk Controls	24 (15%)	0.19	1.25
Normal Controls	5 (0.3%)	0.04	1.20
p value (K-W)	<0.0001	<0.0001	NS

Kruskal-Wallis rejects the hypothesis that these populations are the same and thus pair wise comparisons were carried out. Again, significant differences are only detected between Cases and Normal Controls and High Risk and Normal Controls. The Cases and High Risk Controls do not differ significantly.

	Number of children applying soya containing creams	Mean Number of soya creams per child
Cases vs High Risk Controls	NS	NS
Cases vs Normal Controls	p value < 0.0001	p value < 0.0001
High Risk vs Normal Controls	p value < 0.0001	p value < 0.0004

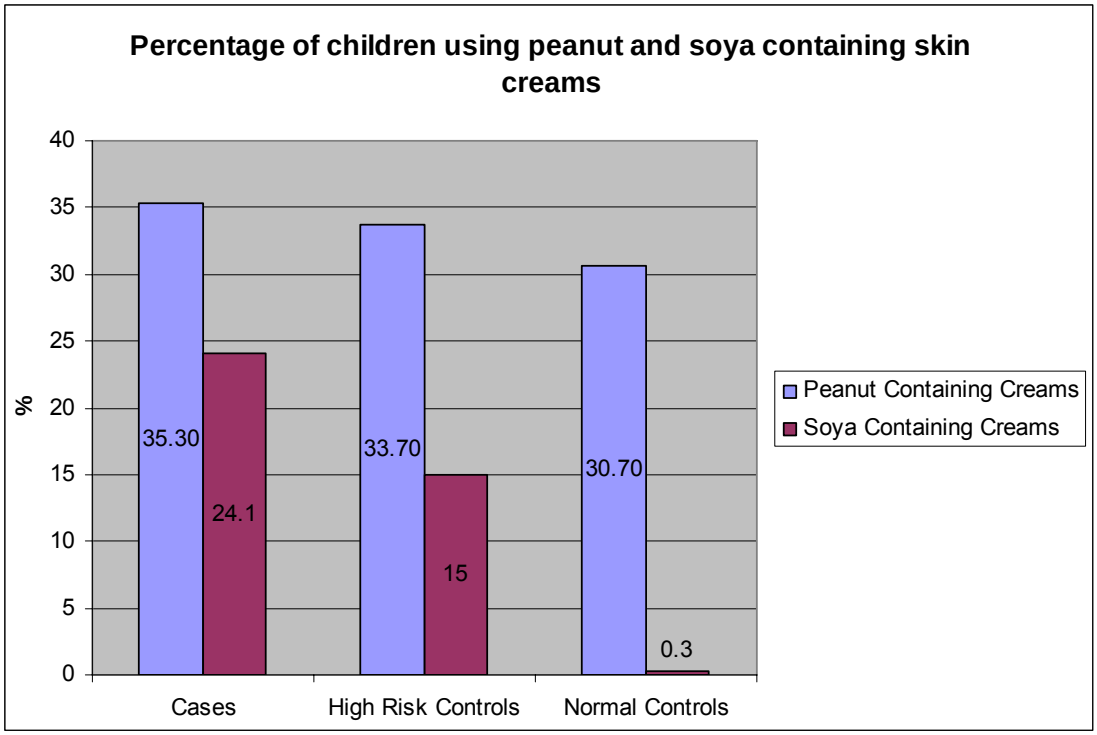


Figure 28: Proportion of children applying creams containing peanut or soy to infant during first year of life

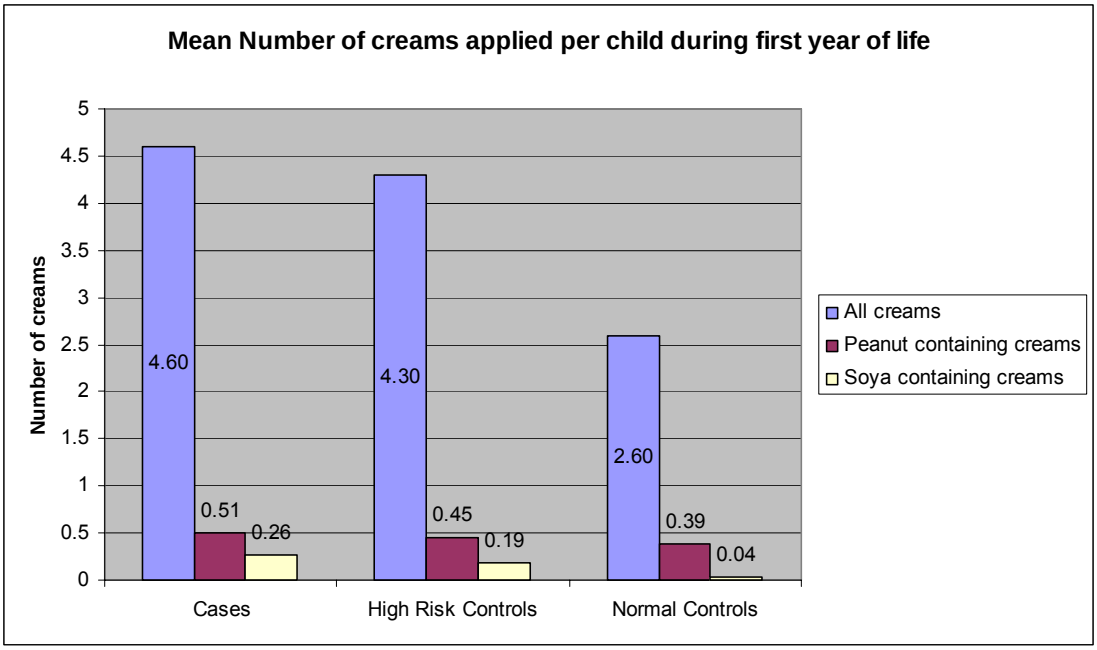


Figure 29: Proportion of children applying creams containing peanut or soy to infant during first year of life

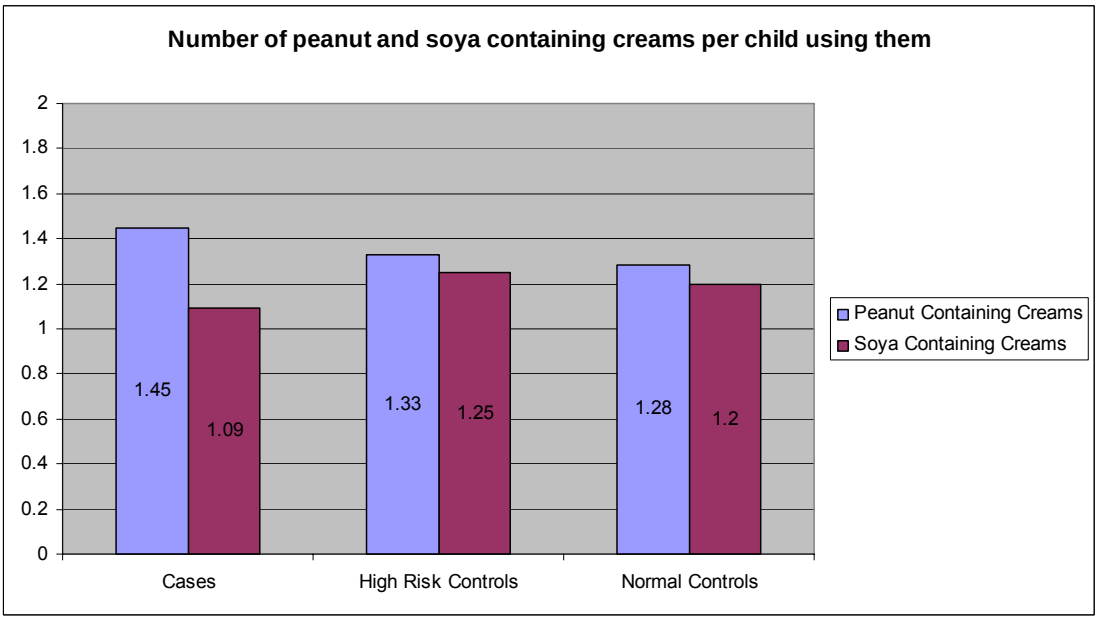


Figure 30: Number of creams containing peanut or soy applied to each infant using them during first year of life

Objective 3

As discussed earlier, possible routes of sensitisation to peanut include in utero (due to maternal consumption during pregnancy), breastfeeding or environmental exposure. Although there is a clear difference between our 3 groups regarding environmental peanut consumption, this difference may not itself be causal.

Maternal Peanut Consumption during Pregnancy

Each mother completed an FFQ specific to their peanut consumption during pregnancy:

grammes peanut/week	n	25th centile	median	75th centile
Cases	133	0	10	37.74
High Risk Controls	160	0	0	10
Normal Controls	150	0	4.8	32.06

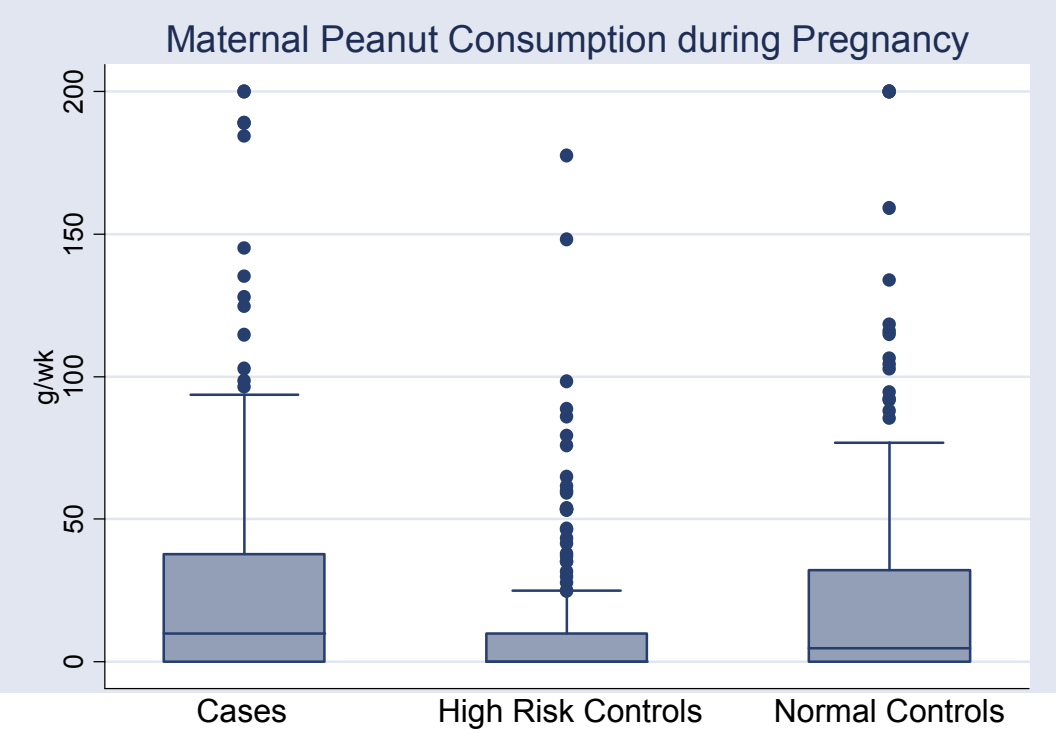


Figure 31: Average Weekly Maternal Peanut Consumption During Pregnancy. Note there are an additional two outliers with peanut-consumption values >200 in the Cases, and three such outliers in the Normal Controls.

Maternal Peanut Consumption during Lactation

Firstly, the number of mothers who breast fed was considered.

	Breast-fed	Breast-Fed > 6/12
Cases	121 (91.0%)	83(62.4%)
High Risk Controls	149 (93.1%)	94(58.8%)
Normal Controls	128 (84.3%)	74(49.3%)

χ^2 test reveals no significant differences between the groups.

If we consider only those mothers who breastfed their child then we can consider peanut consumption as a) an average over a 1 year period (thus taking into account how long the mother breastfed for) or b) the stated weekly consumption for the lactation period, unadjusted for how long breast feeding was continued

a) Average weekly maternal peanut consumption during lactation over first year of life.

Reported weekly peanut consumption during lactation for each mother was multiplied by the proportion of the first year of life that the baby was breastfed for. Therefore a mother who ate 50g of peanut a week during lactation and breast fed for 6 months of the child's first year of life will have the same average value as a mother who ate 25g of peanut but breastfed for the whole first year.

	n	25th centile	Median	75th centile
Cases	121	0	1.68	13.5
High Risk Controls	148	0	0	2.85
Normal Controls	127	0	1.76	11.64

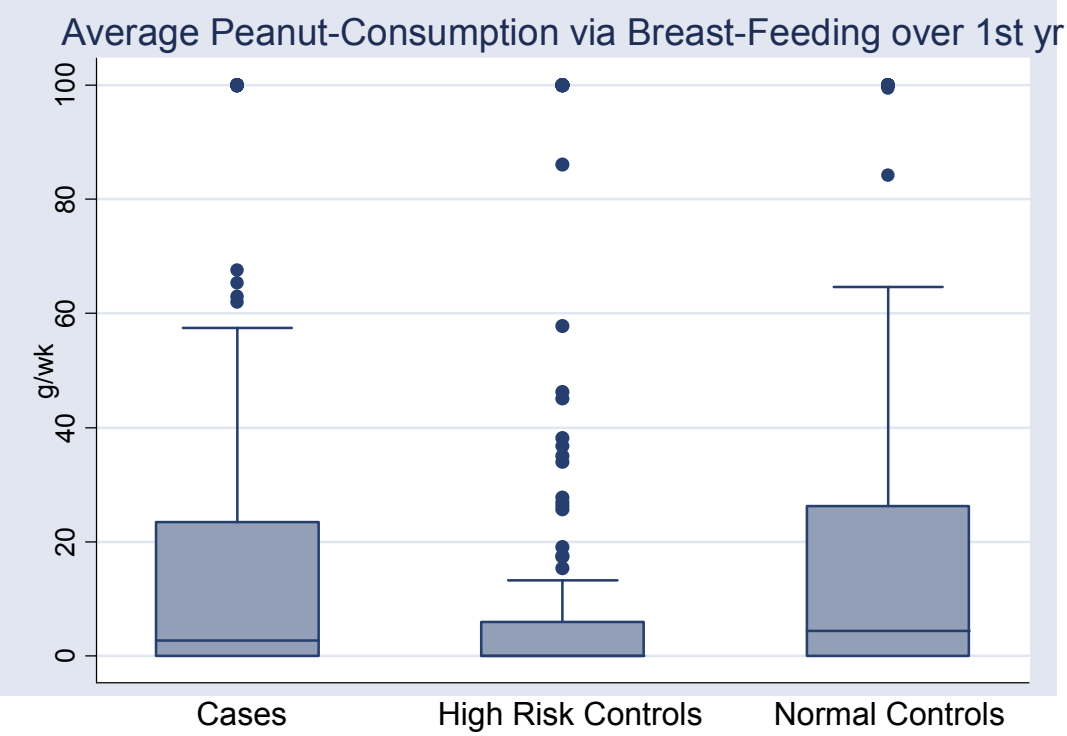


Figure 32: Average Weekly Maternal Peanut Consumption During Lactation. The 100g/wk data-point above identify the outliers - 3 in Cases, 2 in Normal Controls.

b) Weekly maternal peanut consumption during lactation, unadjusted for length of breast feeding

Reported weekly peanut consumption during lactation for each mother was not adjusted for this analysis and therefore a mother who ate 50g of peanut a week during lactation and breast fed for 6 months of the child's first year of life will have the same value as a mother who ate 50g of peanut but breastfed for the whole first year.

	n	25th centile	Median	75th centile
Cases	121	0	2.5	27
High Risk Controls	149	0	0	5
Normal Controls	129	0	3.53	30

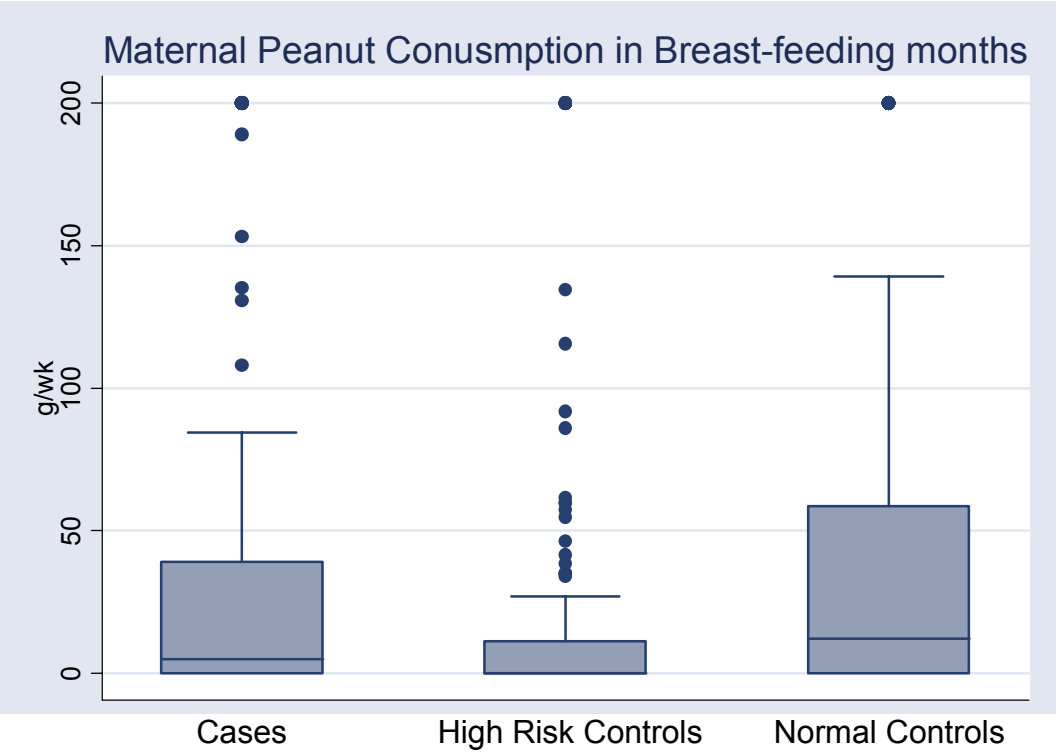


Figure 33: Peak Weekly Maternal Peanut Consumption During Lactation. Outliers shown at 200g/wk - 2 in Cases, 2 in Normal Controls.

	Maternal Peanut Consumption during Pregnancy	Maternal Peanut Consumption during Lactation (averaged)	Maternal Peanut Consumption during Lactation (peak)
Cases vs High Risk Controls	p value < 0.0001	p value < 0.0008	p value < 0.0009
Cases vs Normal Controls	NS	NS	NS
High Risk vs Normal Controls	p value < 0.001	p value < 0.0009	p value < 0.0002

Correlation Coefficients

Total weekly household consumption of peanut during infant's first year of life vs maternal peanut consumption during pregnancy = 0.45

Total weekly household consumption of peanut during infant's first year of life vs maternal peanut consumption during lactation is 0.51.

Logistic Regression Analyses:

Total weekly household consumption of peanut during infant's first year of life versus maternal peanut during pregnancy

This analysis considers the peanut-allergic Cases (n=133), High Risk Controls (n=160) and Normal Controls (n=150), comparing:

1. the Cases and High Risk Controls and gives the Relative Risk of being peanut allergic based on the amount of peanut consumption
2. the Odds Ratio of being peanut allergic based on the amount of peanut consumption using the Cases and Normal Controls.

Peanut Consumption was grouped in the following way-

Peanut-Consumption grammes/week	Group
0	1
0.1 - 15	2
15.1 – 50	3
50+	4

Table 6: Groups according to weekly peanut consumption

If we fit a generalised linear model with Maternal Peanut Consumption During Pregnancy (MP-PREG, truncated at 150g/wk) to whether or not the child is peanut-allergic amongst the Cases & High Risk Controls, this comes out as being highly significant ($p<0.001$). The relative risk of being peanut allergic is shown in the table below.

MP-PREG Group *	Risk Relative to Group 1 (no peanut consumption) (with 95% CI)	Odds Ratio with Group 1 (no peanut consumption) (with 95% CI)
2	1.40 (0.98 - 1.99)	1.23 (0.66- 2.31)
3	1.86 (1.36 – 2.54)	1.76 (0.93- 3.31)
4	1.76 (1.23 – 2.50)	1.06 (0.55- 2.10)

*as described in table above

Table 7: Relative risk & Odds ratio of peanut allergy, grouped by maternal peanut consumption

The relative-risks were found to have a significant trend ($p<0.001$).

However, when the same variable is fit via a logistic regression to whether or not a child is peanut allergic then this was not found to be significant.

Similarly, when data from Normal Controls is used, there is no significant trend.

If we do the same again but this time fit Overall weekly household peanut consumption (PX-MEAN, truncated at 150g/wk) instead of MP-PREG, this is found to be highly significant ($p<0.001$) for both analyses.

PX-MEAN Group	Risk Relative to Group 1 (no peanut consumption)		Odds Ratio with Group 1(no peanut consumption)
	Un-adjusted	Adjusted for MP-PREG	
2	1.3 (0.55– 3.19)	1.3 (0.56 - 3.19)	1.4(0.47- 4.19)
3	3.4 (1.78– 6.66)	3.4 (1.76 – 6.57)	2.9 (1.21 – 6.99)
4	5.3 (2.87– 9.88)	5.1 (2.74 – 9.58)	5.5 (2.43 -12.29)

Table 8: Relative risk & Odds ratio of peanut allergy, grouped by overall weekly household peanut consumption.

The relative-risks were found to have a significant trend for each of the three columns ($p<0.001$).

On including both MATP-PREG and PX-MEAN with a backward stepwise logistic regression, the MATP-PREG is dropped ($p=0.55$). This indicates that the MP-PREG is not adding any further information once allowing for PX-MEAN values (as is apparent from comparing risk and adjusted risk values in the above table).

Total weekly household consumption of peanut during infant's first year of life versus maternal peanut consumption during lactation

This analysis considers the peanut-allergic Cases (n=133), High Risk Controls (n=160) and Normal Controls (n=150), comparing:

Peanut Consumption was grouped in the following way-

Peanut-Consumption grammes/week	Group
0	1
0.1 - 15	2
15.1 – 50	3
50+	4

Table 9: Groups according to weekly peanut consumption

If we fit a generalised linear model with Maternal Peanut Consumption during lactation (MP-BFED, truncated at 150g/wk) to whether or not the child is

peanut allergic, this comes out as being highly significant (p=0.007). The relative risk of being peanut allergic is shown in table 10.

MP-BFED Group	Risk Relative to Group 1 (no peanut consumption) (with 95% CI)
2	1.18 (0.82- 1.70)
3	1.63 (1.19 – 2.23)
4	1.62 (1.09 – 2.39)

Table 10: Relative risk of peanut allergy grouped by maternal peanut consumption during lactation

As we know from the previous analysis, if we fit PX-MEAN instead of MP-BFED, this is found also to be highly significant (p<0.001) for both analyses.

PX-MEAN Group	Risk Relative to Group 1 (no peanut consumption)	
		Adjusted for MP-BFED
2	1.3 (0.55– 3.19)	1.3 (0.56 - 3.19)
3	3.4 (1.78– 6.66)	3.4 (1.77 – 6.63)
4	5.3 (2.87– 9.88)	5.3 (2.83 – 9.81)

Table 11: Relative risk of peanut allergy grouped by overall household peanut consumption

The relative-risks were found to have a significant trend (p<0.001).

On including both terms with a backward stepwise logistic regression the MP-BFED is dropped (p=0.67). This indicates that the MP-BFED is not adding any further information once allowing for PX-MEAN values (as is apparent from comparing risk and adjusted risk values in table 11).

Another mode of analysis is to consider the running means, which look at the proportion of children that are peanut allergic at different consumption values. There is a positive association between PX_MEAN and the likelihood of being peanut allergic when the Cases and High Risk Controls are considered. Figure 34 shows how the likelihood of PA increases steeply as the household consumption increases from 0, but levels off after about 85g/week.

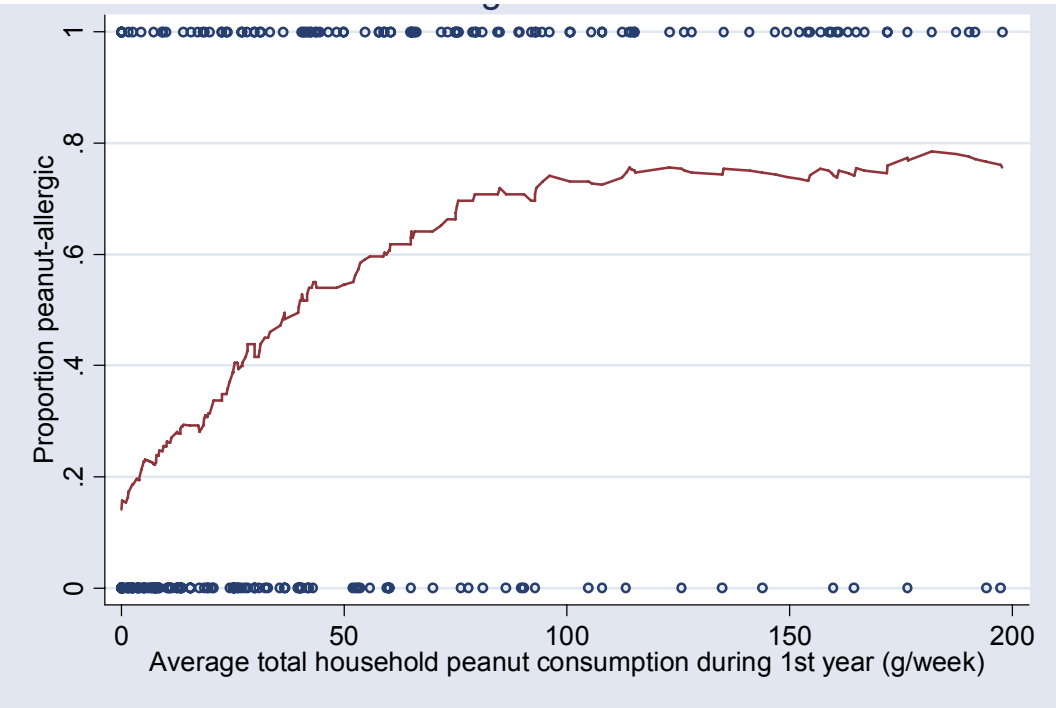


Figure 34: Proportion of children with peanut allergy by level of average weekly overall household peanut consumption.

This analysis still tells us little about the relative importance of different routes of exposure as the increasing likelihood of peanut allergy may simply be due to the association between high household consumption and high maternal consumption during pregnancy or lactation. However, by examining only the cases where these relationships between maternal peanut consumption during pregnancy and total household consumption have broken down does provide further insight. 4 situations are thus examined :

1) Families where the mother did not eat any peanut during pregnancy (but the household may have eaten peanut during the child's first year of life)
2) Families where there was no household consumption in the child's first year of life (but the mother may have eaten peanut during pregnancy)
3) Families where the mother breastfed but did not eat any peanut during lactation (but the household may have eaten peanut during the child's first year of life)
4) Families where there was no household consumption in the child's first year of life except for peanut eaten by the lactating mother

1) There were 134 cases in the Cases and High Risk Controls where the mother had no peanut consumption whilst pregnant. PX-MEAN was found to be highly significant in the model (p<0.001).

PX-MEAN Group	Risk Relative to Group 1 (no peanut consumption)
2	0.5 (0.07 – 3.70)
3	2.8 (1.20 – 6.58)
4	6.1 (2.96 – 12.39)

Table 12: Relative risk of peanut allergy, grouped by overall weekly household peanut consumption

The relative-risks were found to have a significant trend (p<0.001).

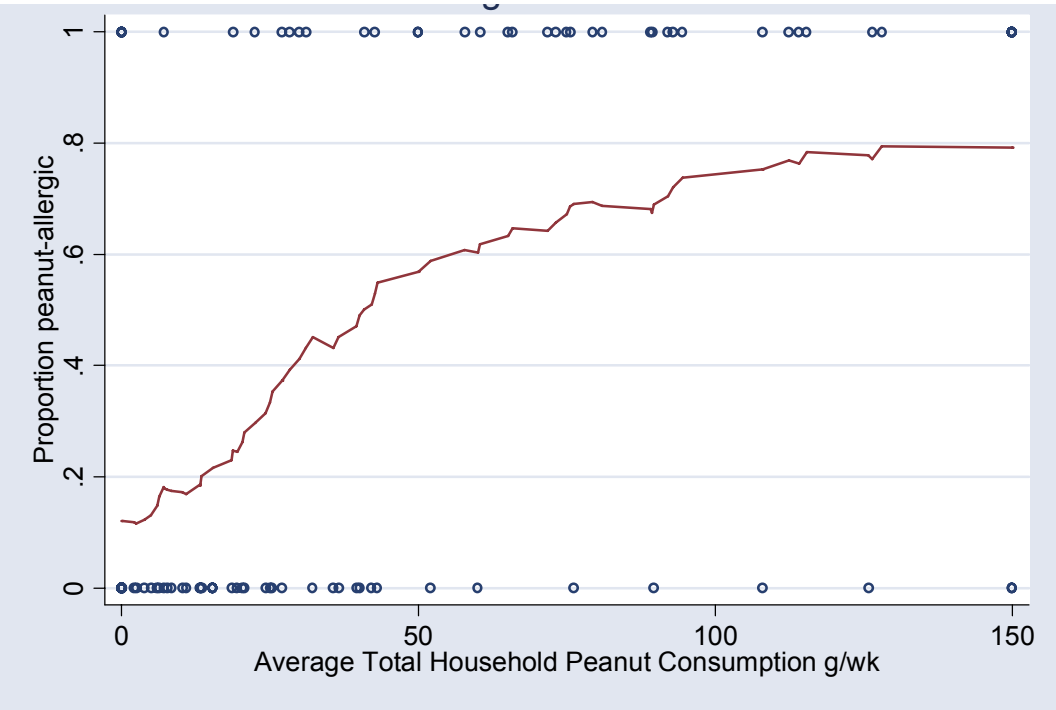


Figure 35: Proportion of children with peanut allergy by level of average weekly overall household peanut consumption.

2) Considering the 100 Cases and High Risk Controls where there was no household consumption in the child's first year of life, the maternal peanut consumption during pregnancy was not found to be significant.

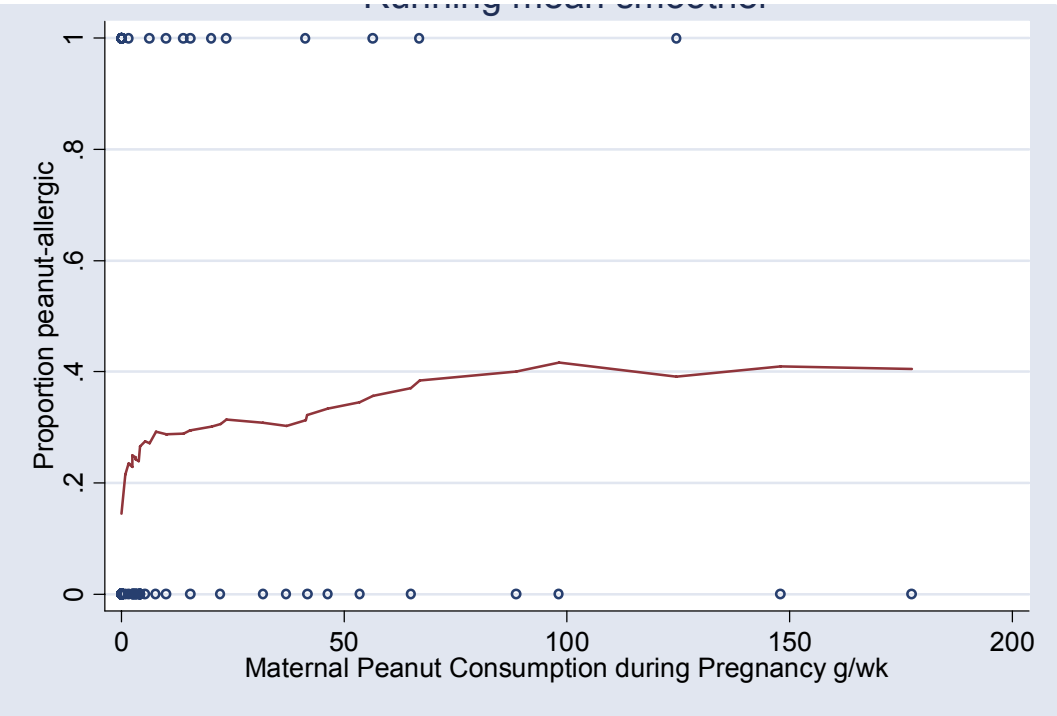


Figure 36: Proportion of children with peanut allergy by level of maternal peanut consumption during pregnancy.

3) Considering the Cases and High Risk Controls whose mothers breastfed but did not consume any peanuts whilst breastfeeding (n=149), the average total household consumption was still found to be highly significant in explaining peanut allergy (p-value <0.0001).

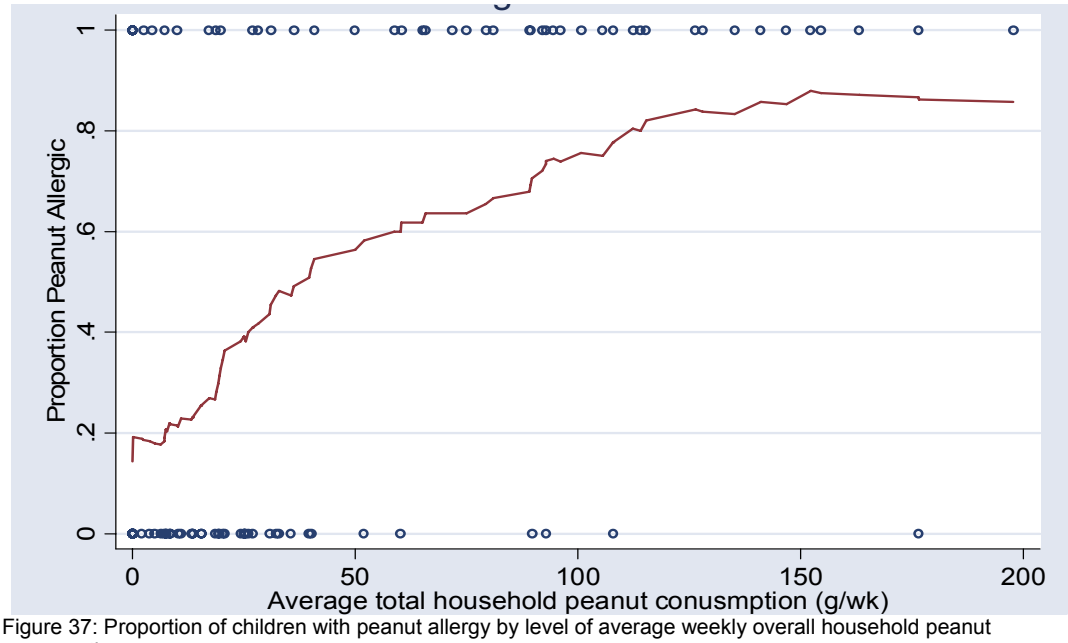


Figure 37: Proportion of children with peanut allergy by level of average weekly overall household peanut consumption.

4) Of the 94 Cases and High Risk Controls where there was no household consumption in the child's first year of life except for peanut eaten by the lactating mother the maternal peanut consumption during breast feeding was not found to be significant.

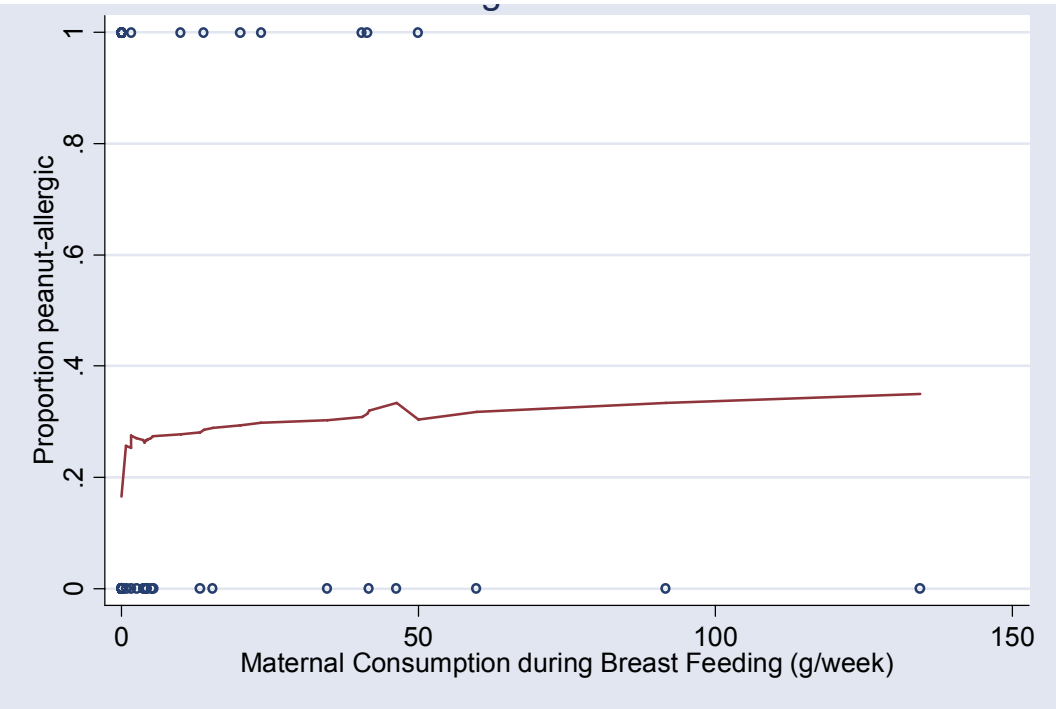


Figure 38: Proportion of children with peanut allergy by level of maternal peanut consumption during lactation.

Objective 4

The aim of this part of the study was to consider whether other factors that have been linked to increased risk in allergic diseases are important in peanut allergy. An additional factor, the number of older siblings, has been added to this section.

Our main focus in this project was risk factors specific to peanut allergy. The presence of these will lead to significant differences between Cases and High Risk Controls. However, for most outcomes Cases and High Risk Controls have been combined and then compared to Normal Controls. When combined, the Cases and High Risk Controls form a single larger group of food allergics. If significant differences are limited to comparison between this composite group and the Normal controls then this suggests a risk factor for food allergy. However, we have yet to perform a logistical regression analysis for factors between these 2 groups. Unless specified otherwise, χ^2 test has been used to compare proportions.

1) Socio-economic status

Socio-economic status was defined according to **Standard Occupational Classification 2000 (SOC2000).** The Standard Occupational Classification was first published in 1990 to replace both the Classification of Occupations 1980 (CO80) and the Classification of Occupations and Dictionary of Occupational Titles (CODOT). SOC 1990 was revised and updated to produce SOC2000.

The two main concepts of the classification remain unchanged:

- kind of work performed - job, and
- the competent performance of the tasks and duties - skill.

The Standard Occupational Classification consists of the following major groups:

1 Managers and Senior Officials

2 Professional Occupations

3 Associate Professional and Technical Occupations

4 Administrative and Secretarial Occupations

5 Skilled Trades Occupations

6 Personal Service Occupations

7 Sales and Customer Service Occupations

8 Process, Plant and Machine Operatives

9 Elementary Occupations

For each case and control, data was obtained on the occupation of both parents. For analysis of Socio-Economic status, only the higher ranking of the two parents was considered. If only one parent lived with the child, then the socio-economic status of that parent is considered. There is no code for housewives, the unemployed or students and thus a further group (0) has been added.

Group	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total**
Cases	10 (7.52%)	21 (15.79%)	55 (41.35%)	15 (11.28%)	10 (7.52%)	11 (8.27%)	3 (2.26%)	1 (0.75%)	2 (1.50%)	5 (3.76%)	133(100%)
High Risk	8 (5.00%)	18 (11.25%)	73 (45.63%)	27 (16.88%)	9 (5.63%)	11 (6.88%)	3 (1.88%)	5 (3.13%)	1 (0.63%)	5 (3.13%)	160 (100%)
Normal	7 (4.67%)	19 (12.67%)	81 (54.00%)	18 (12.00%)	2 (1.33%)	6 (4.00%)	2 (1.33%)	6 (4.00%)	5 (3.33%)	4 (2.67%)	150 (100%)
Total	25 (5.64%)	58 (13.09%)	209 (47.18%)	60 (13.54%)	21 (4.74%)	28 (6.32%)	8 (1.81%)	12 (2.71%)	8 (1.81%)	14 (3.16%)	443 (100%)

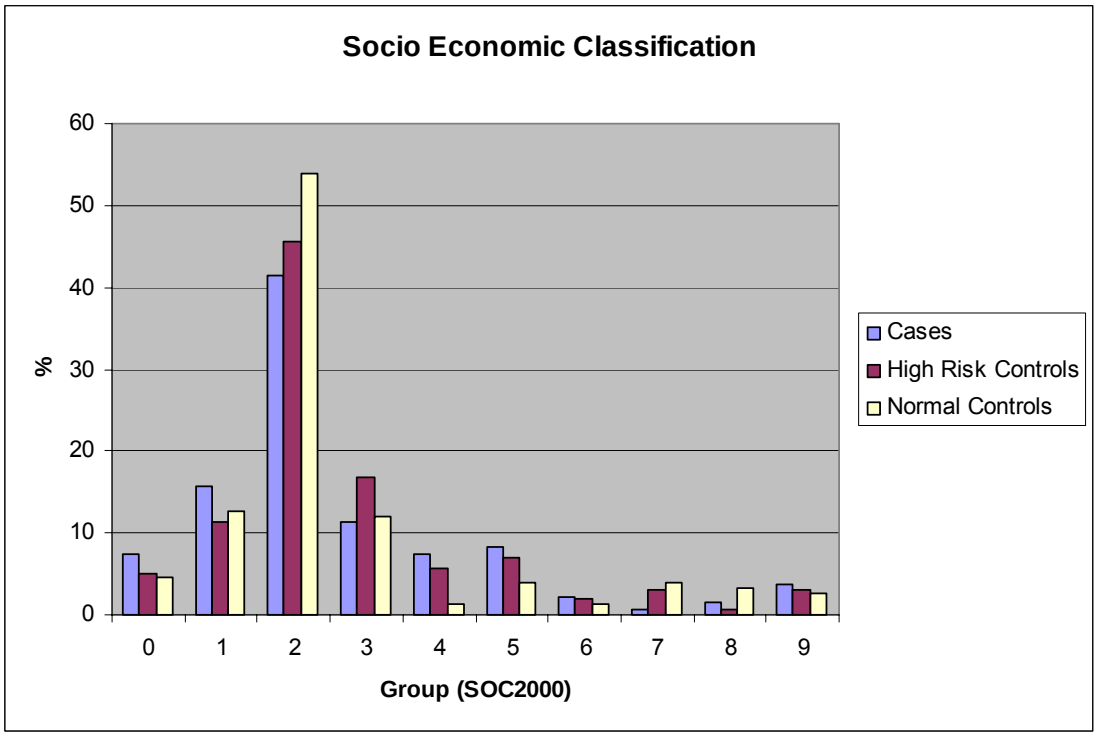


Figure 39: Socio-Economic Group of families (SOC2000 classification)

No significant difference was found between the three groups by SEG.

2) Ethnicity of child based on parental ethnicity

Details of ethnic group were obtained from both parents. Ethnicity was considered in 6 groups, with children of mixed parentage coded separately.

1 Caucasian
2 Asian Indian
3 Arabic/Middle Eastern
4 Afro-Caribbean/African
5 Asian Chinese
6 Other
7 Mixed

Group	1	2	3	4
Cases	68(51.1%)	13 (9.8%)	5 (3.7%)	13 (9.8%)
High Risk Controls	96(60%)	17 (10.6%)	8 (5%)	7 (4.4%)
Normal Controls	103(68.7%)	14 (9.3%)	13 (8.7%)	4 (2.7%)
Total	264(60.6%)	43(9.9%)	27 (6.2%)	22 (5.0%)

5	6	7	Total
6 (4.5%)	6 (4.5%)	22 (16.5%)	133 (100%)
5 (3.1%)	6 (3.8%)	21 (13.1%)	160 (100%)
1 (0.7%)	4 (2.7%)	11 (7.3%)	150 (100%)
12 (2.8%)	14 (3.2%)	54 (12.4%)	436 (100%)

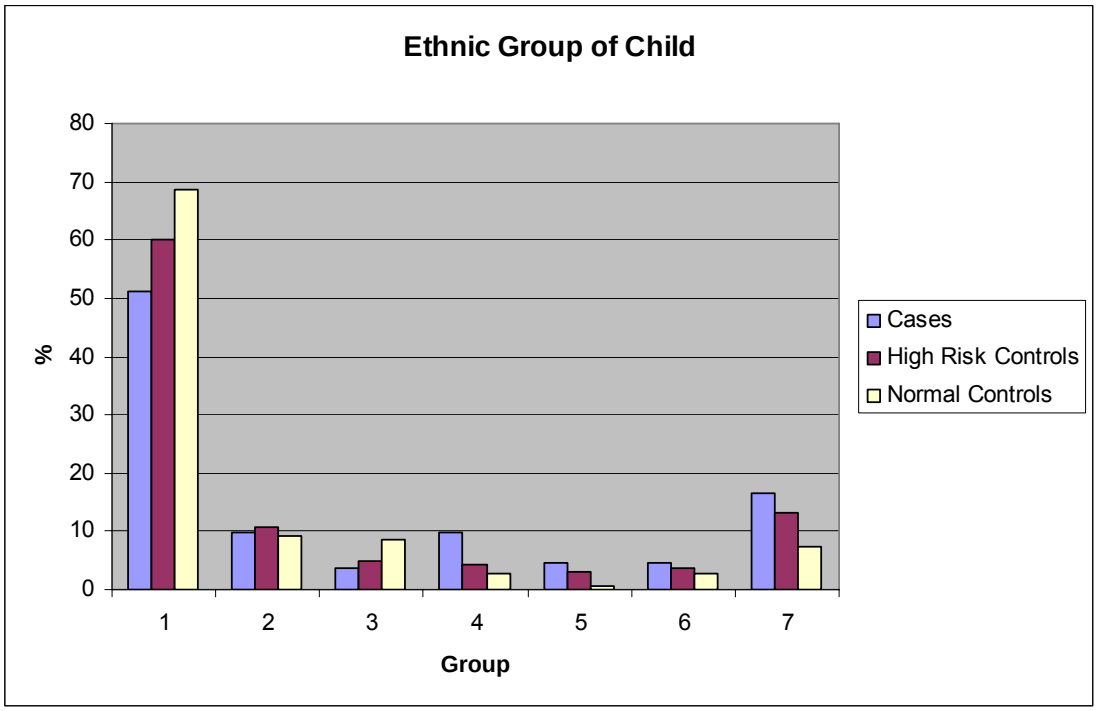


Figure 40: Ethnic Group of Child

Whilst there is no significant difference between Cases and High Risk Controls, there are differences between the combined food allergic group (Cases and High Risk Controls) compared to the Normal Controls. This is due to the greater proportion of Caucasians (p=0.01) and lower proportion of mixed ethnicity (p=0.05) amongst the Normal Controls.

3) Nationality of infant based on parental nationality

Details of country of birth were obtained from both parents. Mixed nationality was considered only in children of Caucasian ethnicity. The nationality of all those born in England, Scotland, Wales and Northern Ireland was considered

British. Unfortunately, this question was omitted from the first print run of our questionnaire and thus data was missing on a number of Caucasian families. No significant differences were found between the groups.

	n=	Caucasians	Mixed nationality	Missing Data	% mixed nationality
Cases	133	67	20	14	37.7
High Risk Controls	160	96	22	20	28.9
Normal Controls	150	103	21	14	23.6

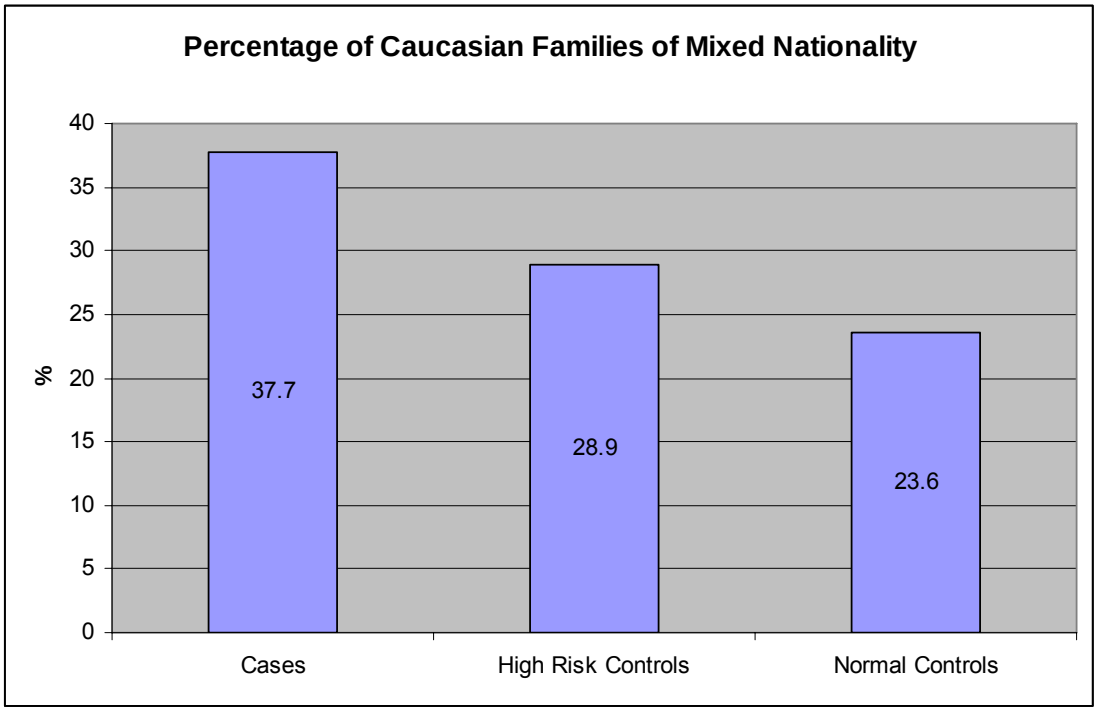


Figure 41: Mixed parental nationality amongst Caucasian families

The differences between the groups are not statistically significant.

4) Age of initial peanut consumption by infant

There is a significant difference amongst the groups with regards to the proportion of children who had eaten peanut in the past (p<0.0001).

	Number who have eaten peanut	Median age of consumption	Median weekly consumption (g/peanut)
Cases	14(11%)	23.5	0*
High Risk Controls	37(23%)	17.5	0
Normal Controls	76(50.6%)	16.5	0

*implies that exposures to peanut were single episodes, with no ongoing regular peanut consumption

Pair wise comparison reveals a difference when comparing Cases to both High Risk and Normal controls as well as between the two control groups.

Cases vs High Risk Controls	p value = 0.009
Cases vs Normal Controls	p value < 0.0001
High Risk Controls vs Normal Controls	p value = 0.001

Mothers of infants who had eaten peanut were also asked to recall when they first had peanut in their diet and to estimate their regular intake. It was clear from responses that recall tended to be discretised eg as 18 months or 1 year. For this reason we have considered the data in categories.

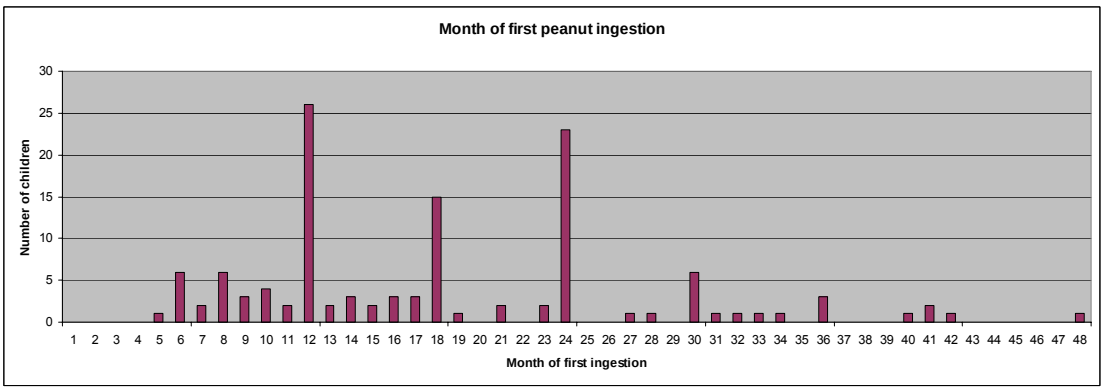


Figure 42: Age of first peanut ingestion in months

	Cases	High Risk Controls	Normal Controls
<13 months	4(28.6%)	15(40.5%)	33(43.4%)
<25 months	6(42.8%)	20(54.1%)	33(43.4%)
<37 months	3(21.4%)	0(0%)	8(10.5%)
>36 months	1(7.1%)	2(5.4%)	2(2.6%)
Total	14(100%)	37(100%)	76(100%)

5) Soy consumption in infancy

Data was obtained for each child regarding whether they had ever ingested soya milk, when they first had it and how long it was taken for.

	Number who had taken soya milk	Number who had taken soya milk in first year of life
Cases	52(39.1%)	30(22.6%)
High Risk Controls	68(42.5%)	51(31.9%)
Normal Controls	25(16.7%)	14(9.3%)

There are significant differences in the proportion of children who consumed soya milk amongst the groups ($p<0.0001$). This difference is due to the low proportion in the Normal Controls relative to the food allergic children ($p<0.0001$). Similar differences exist for soya milk consumption during the first year of life, with no differences between Cases and High Risk Controls but significantly lower proportion of Normal Controls consuming soya milk ($p<0.001$).

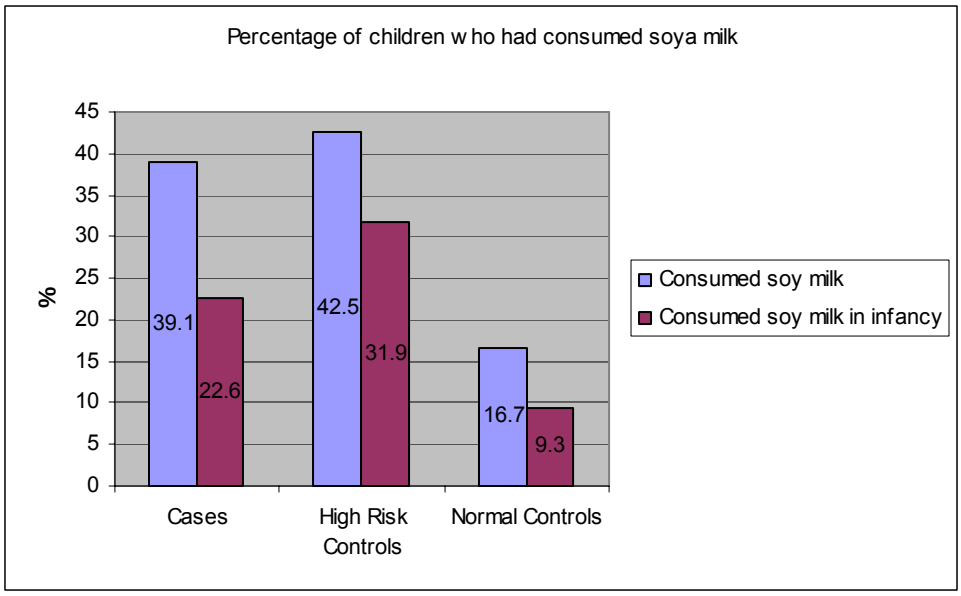


Figure 43: Percentage of children consuming soya milk

The age at which soya milk was first taken did not show any significant differences between the groups.

	n	25th centile	Median	75th centile
Cases	52	5.5	8	13.5
High Risk Controls	68	4	6	9.5
Normal Controls	25	5	6	15

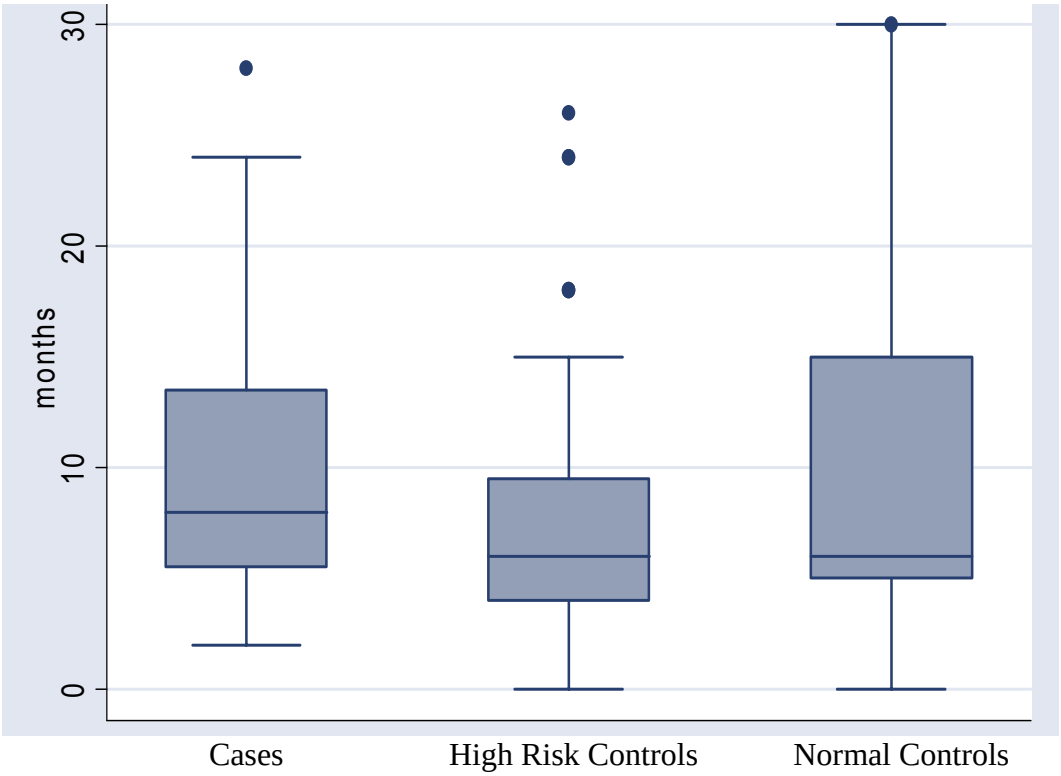


Figure 44: Age at which soya milk was first consumed

The length of soya milk consumption during the first year of life did not show any significant differences between the groups.

	n	25th centile	Median	75th centile
Cases	52	1	5	19.5
High Risk Controls	68	1	7	22.5
Normal Controls	25	1	6	12

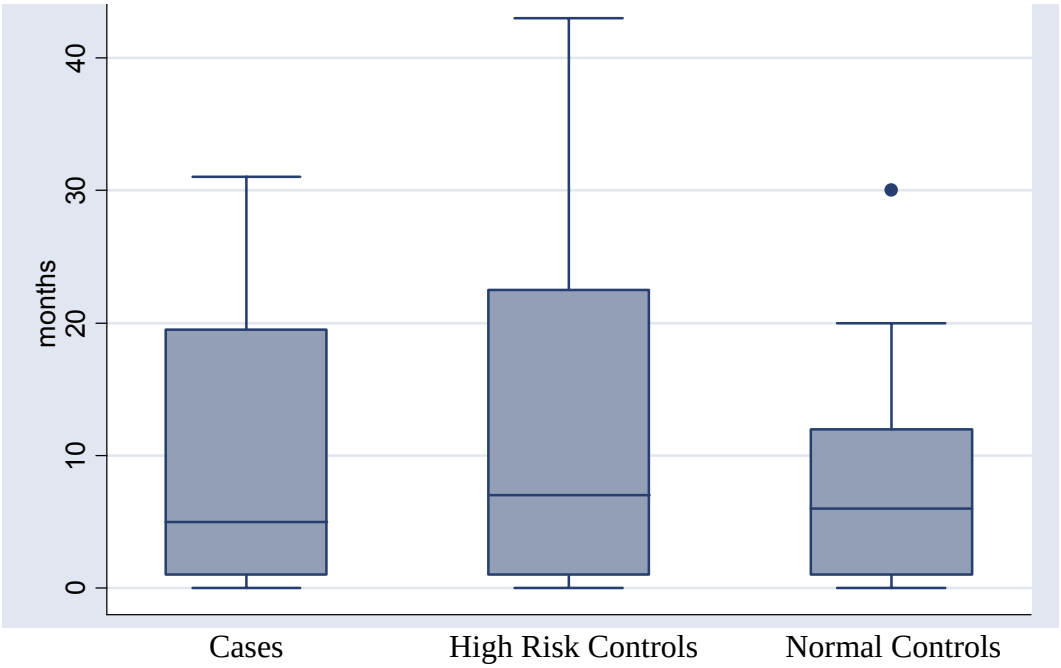


Figure 45: Length of soya milk consumption in first year of life

5) Prematurity

Prematurity was defined as less than 37 weeks gestation.

	Born prematurely
Cases	3(2.3%)
High Risk Controls	2(1.3%)
Normal Controls	9(6%)

There is a significant difference in the proportion of children with prematurity amongst the 3 groups (p=0.05). Pair-wise comparison reveals :

Cases vs High Risk Controls	NS
Cases & High Risk Controls vs Normal Controls	p value =0.025

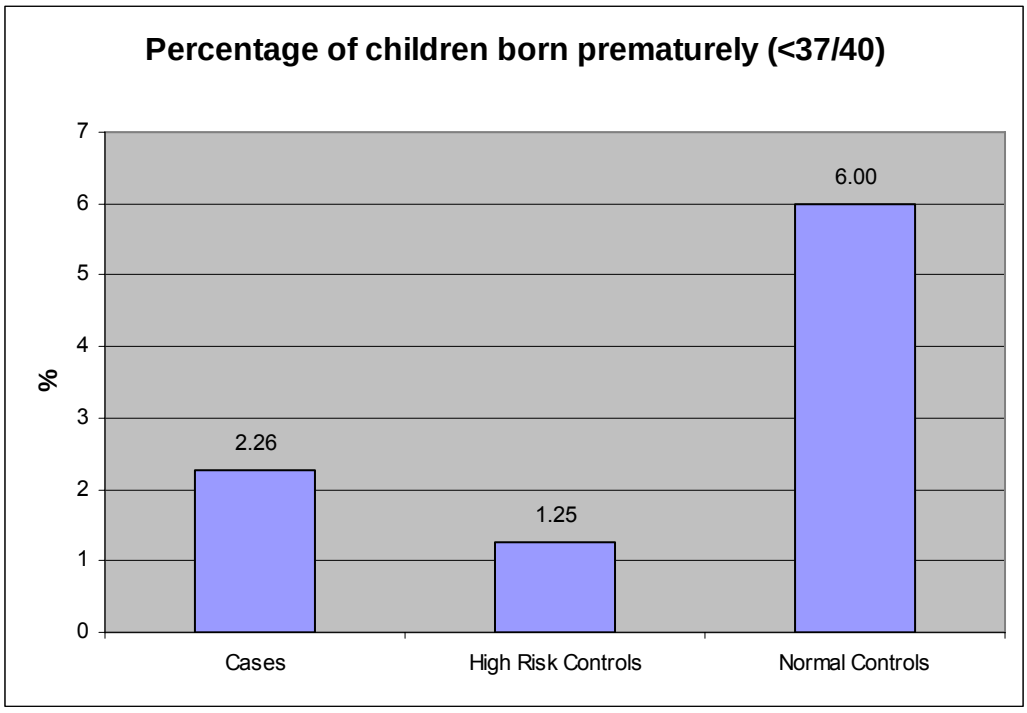


Figure 46: Percentage of children born at <37weeks gestation

6) Presence of cat and dog in household during infancy

Details of cat and dog ownership during first year of child's life were obtained.

	Cat ownership	Dog Ownership	Either
Cases	9(6.8%)	3(2.3%)	12(9.0%)
High Risk Controls	10(6.3%)	3(1.9%)	14(8.8%)
Normal Controls	15(10.0%)	12(8.0%)	25(16.7%)
Combined Food Allergics	19(6.5%)	6(2.0%)	26(9.1%)

2 owners (both in the Normal Control group) had both cat & dog.

There is no significant difference in cat ownership or pet ownership overall but there is in dog ownership (0.025). This is due to the higher rates in the Normal Controls whilst the two food allergic groups do not differ.

Cases vs High Risk Controls	NS
Cases vs Normal Controls	p value =0.05
High Risk Controls vs Normal Controls	p value = 0.025

When Cases and High Risk Controls are considered as a single group of food allergic children, this group differs significantly from the Normal Controls in respect to dog ownership (p=0.007), either pet ownership (p=0.019) but not cat ownership (p=0.21).

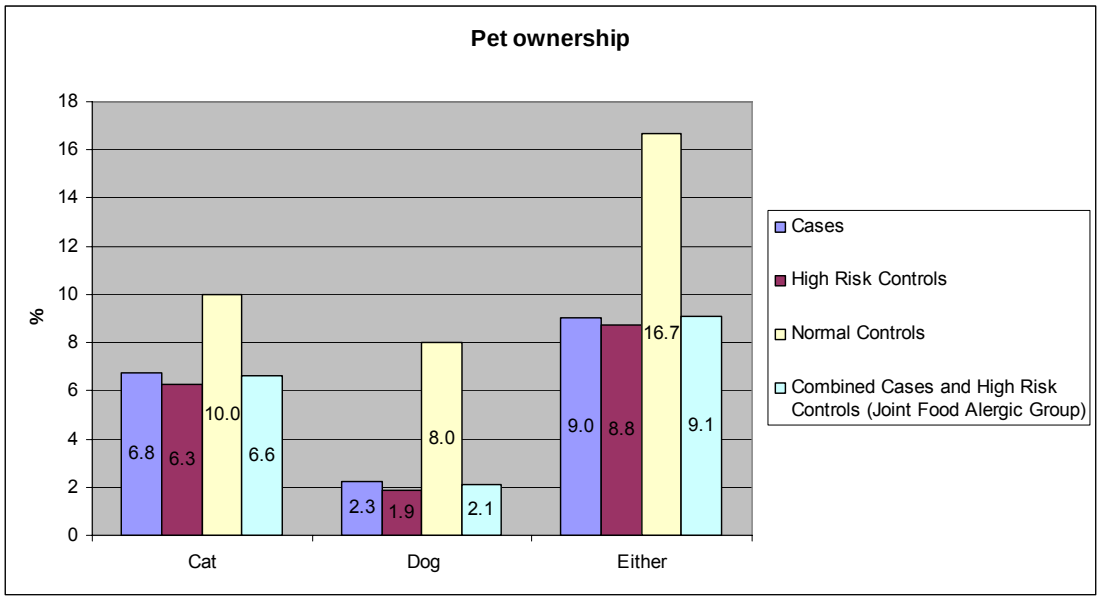


Figure 47: Pet Ownership during first year of life

7) Bronchiolitis, wheeze and asthma

All respondents were questioned with regards to occurrence of wheeze and when it first occurred. Further questioning related to episodes of bronchiolitis, when it occurred and whether it was known to be due to Respiratory Syncytial Virus (RSV). Parental report of asthma and use of medications was also recorded.

	Wheeze	Wheeze during first six months of life	Wheeze during first year of life
Cases	66(49.6%)	15(11.3%)	44(33.1%)
High Risk Controls	64(40.0%)	21(13.1%)	46(28.8%)
Normal Controls	38(25.3%)	16(10.7%)	30(20.0%)

The proportion of children who wheezed, wheezed in first 6 months or first year of life did not differ significantly between the Cases and the High Risk controls. However, the combined group of food allergic children (Cases and the High Risk controls) were significantly more likely to have wheezed ($p<0.001$) and wheezed by 1 year ($p=0.025$) than the Normal Controls. There were no significant differences between the groups in wheeze by 6 months.

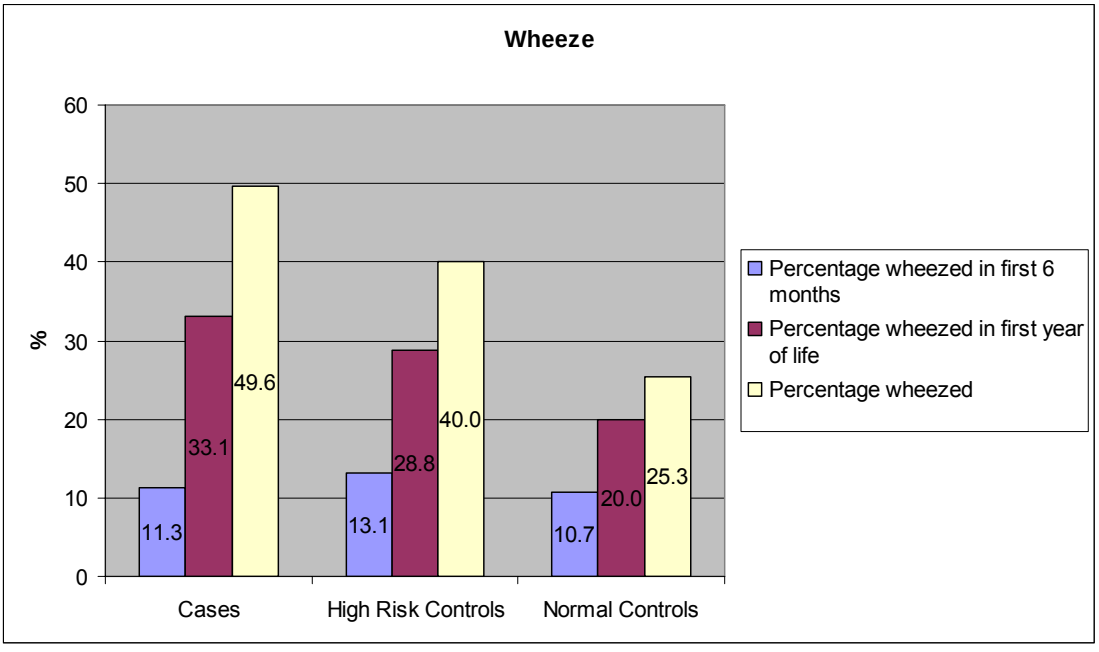


Figure 48: Wheeze amongst children

The age of first wheezing did not differ between the groups.

	n	25th centile	Median	75th centile
Cases	63	7	11	16
High Risk Controls	63	6	9	14
Normal Controls	38	5	8	12

There was no significant difference between the groups in terms of previous report of bronchiolitis or known RSV+ve bronchiolitis.

	Suffered from bronchiolitis	Suffered from known RSV +ve bronchiolitis
Cases	16(12.0%)	5 (3.8%)
High Risk Controls	15(9.4%)	3 (1.9%)
Non food allergic Controls	14(9.3%)	1 (0.7%)

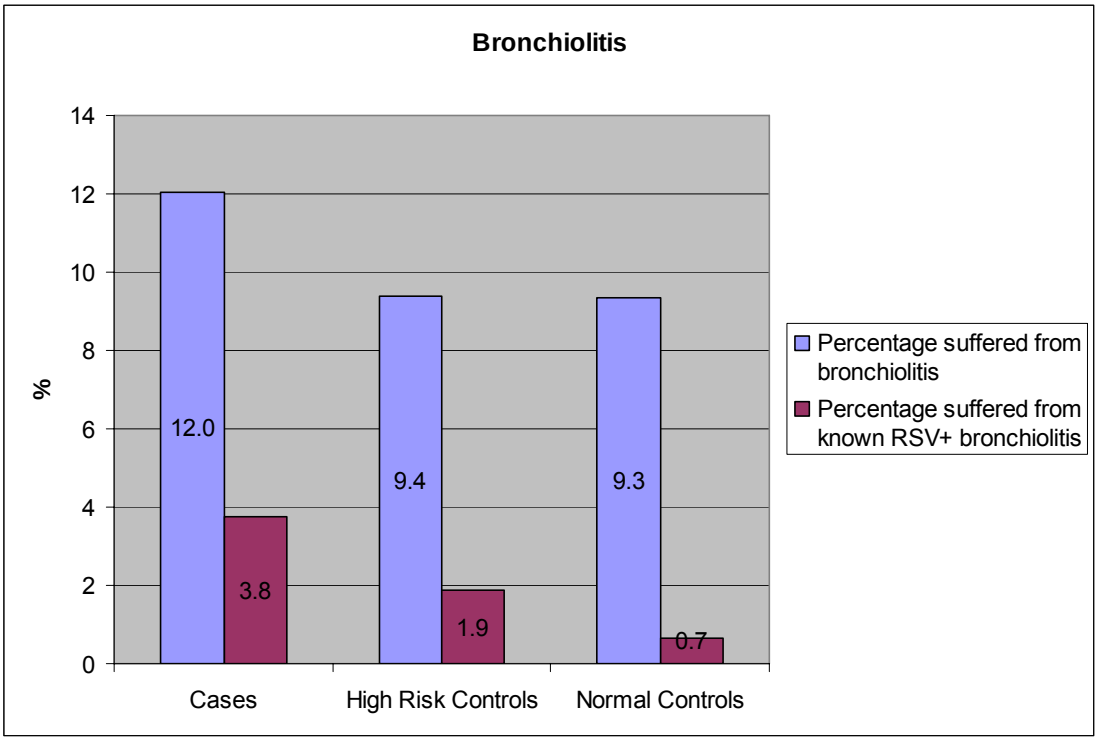


Figure 49: History of bronchiolitis

There was no significant difference between the groups in terms of age of diagnosis with bronchiolitis:

	n	25th centile	Median	75th centile
Cases	16	3	6	11
High Risk Controls	15	5	10	17
Normal Controls	14	5	6	6

There are significant differences in the prevalence of asthma reported in the 3 groups ($p<0.0001$) but no significant differences between the Cases and High Risk Controls. When these 2 groups are considered together, they differ significantly from the Normal Controls ($p<0.0001$). Similarly, the prevalence of asthma requiring inhaled steroids did not differ significantly between the Cases and High Risk Controls. When these 2 groups are considered together, they differ significantly from the Normal Controls ($p<0.01$).

	Parental report of asthma	Parental report of asthma requiring ICS
Cases	33 (24.8%)	16 (12.0%)
High Risk Controls	26 (16.3%)	14 (8.8%)
Normal Controls	9 (6.0%)	4 (2.7%)

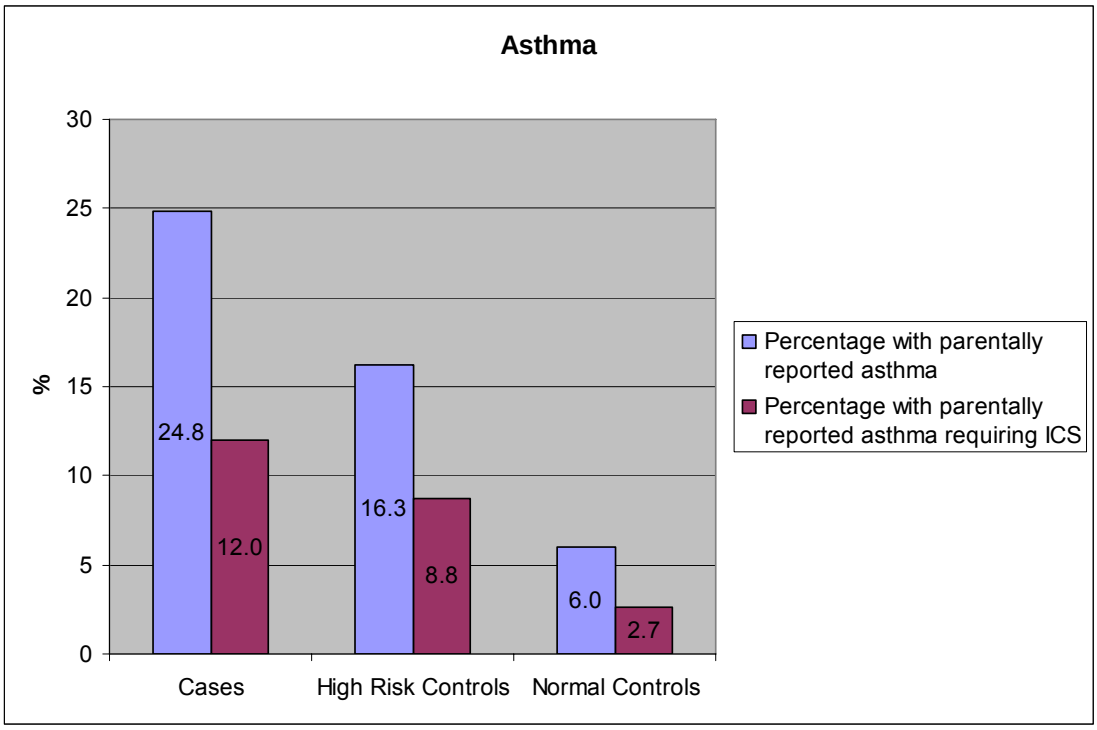


Figure 50: History of asthma

8) Passive smoking

Details were obtained of the number of smokers in each household during child's first year of life. Information was also obtained of average number of cigarettes smoked per day by each resident smoker.

	Number of households with a smoker
Cases	19(14.3%)
High Risk Controls	26(16.3%)
Normal Controls	22(14.7%)

There is no significant difference between the number of households with a smoker in.

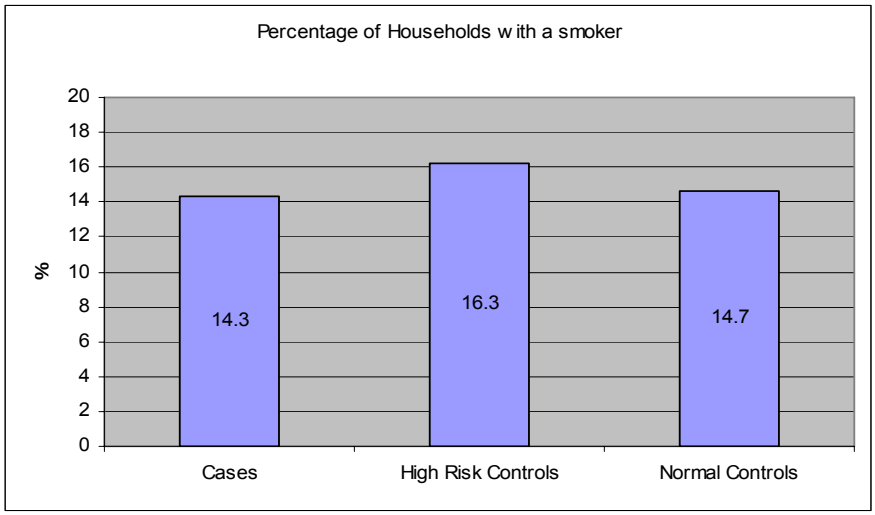


Figure 51: Percentage of Households with a smoker

There is no significant difference between the groups in terms of number of smokers per household or average number of cigarettes per smoker.

Number of smokers	0	1	2	3	Total
Cases	114	16	3	0	133
High Risk Controls	134	23	2	1	160
Normal Controls	128	17	5	0	150
Total	376	56	10	1	443

Number of cigarettes	0	10	20	100	Total
Cases	114	12	4	3	133
High Risk Controls	135	17	6	2	160
Normal Controls	128	15	4	3	150
Total	377	44	14	8	443

9) Family history of allergy

Details were obtained of self reported diagnosis of asthma, eczema or hayfever by mother, father and where present, older siblings.

Maternal Atopy	Asthma	Eczema	Hayfever	Any atopy
Cases	26(19.5%)	34(25.6%)	44(33.1%)	75(56.4%)
High Risk Controls	28(17.5%)	41(25.6%)	54(33.8%)	76(547.%)
Normal Controls	20(13.3%)	33(22.0%)	31(20.7%)	59(39.3%)

No significant differences are found between the groups for maternal asthma (p=0.25) or eczema (p=0.76). Significant differences were found for maternal hayfever (p=0.025) and any atopy (p=0.025). In both cases, the differences between Cases and High Risk Controls were not significant. Using the combined food allergic group (Cases and High Risk Controls), differences to the Normal Controls were significant for maternal hayfever (p=0.01) and any maternal atopy (p=0.025).

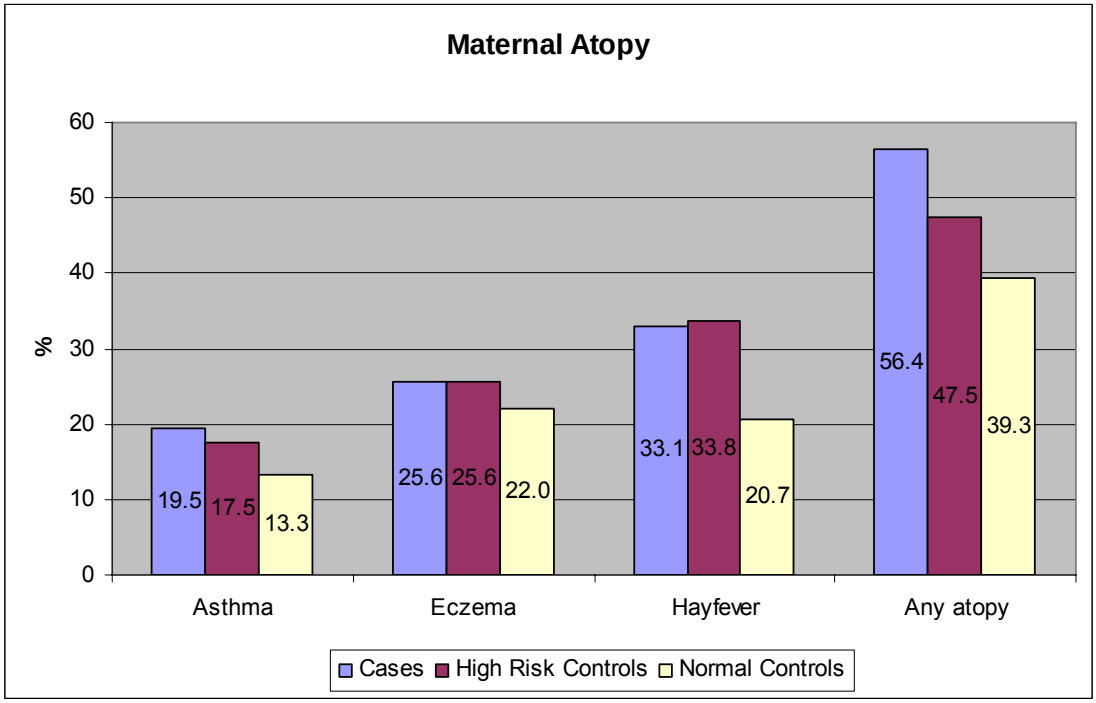


Figure 52: Percentage of children with maternal history of atopy

Paternal Atopy	Asthma	Eczema	Hayfever	Any atopy
Cases	28(21.1%)	25(18.8%)	41(30.8%)	65(48.9%)
High Risk Controls	21(13.1%)	24(15.0%)	57(35.6%)	76(47.5%)
Normal Controls	12(8.0%)	14(9.3%)	32(21.3%)	48(32.0%)

Significant differences were found between the 3 groups in terms of Paternal asthma (p=0.01), hayfever (p=0.025) and any atopy (p=0.01). Cases and High Risk Controls were not significantly different in relation to Paternal asthma, eczema, hayfever or any atopy but the combined group of food allergic children was significantly different to the Normal Controls (p=0.025, p=0.05, p=0.01, p=0.01).

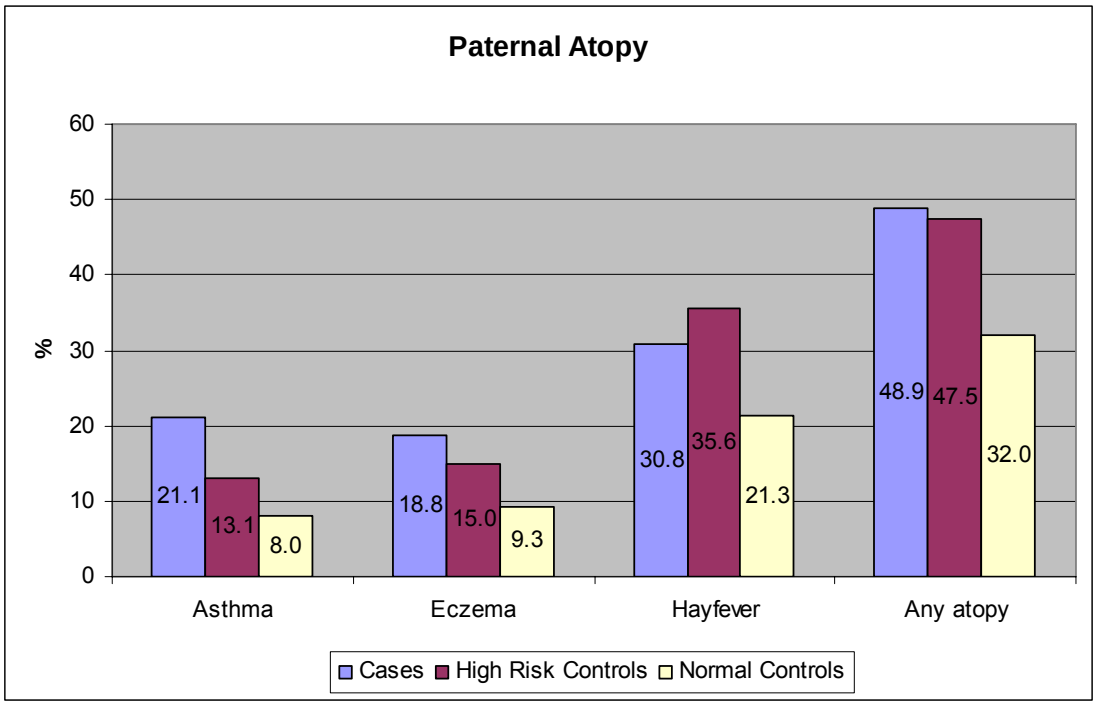


Figure 53: Percentage of children with paternal history of atopy

Older Sibling Atopy	No. with older sibs	Asthma*	Eczema*	Hayfever*	Any atopy*
Cases	52(39.1%)	10(19.2%)	25(48.1%)	7(13.5%)	28(53.8%)
High Risk Controls	76(47.5%)	23(30.3%)	36(47.4%)	13(17.1%)	40(52.6%)
Normal Controls	59(39.3%)	9(15.3%)	19(32.2%)	9(15.3%)	25(42.4%)

* as a percentage of those children with older siblings

Atopy amongst older siblings could only be considered amongst those children who had older siblings. The 3 groups did not differ in the proportion having an older sibling (see also 10 below). There were no significant differences between the 3 groups in terms of sibling asthma, eczema, hayfever or combined atopy.

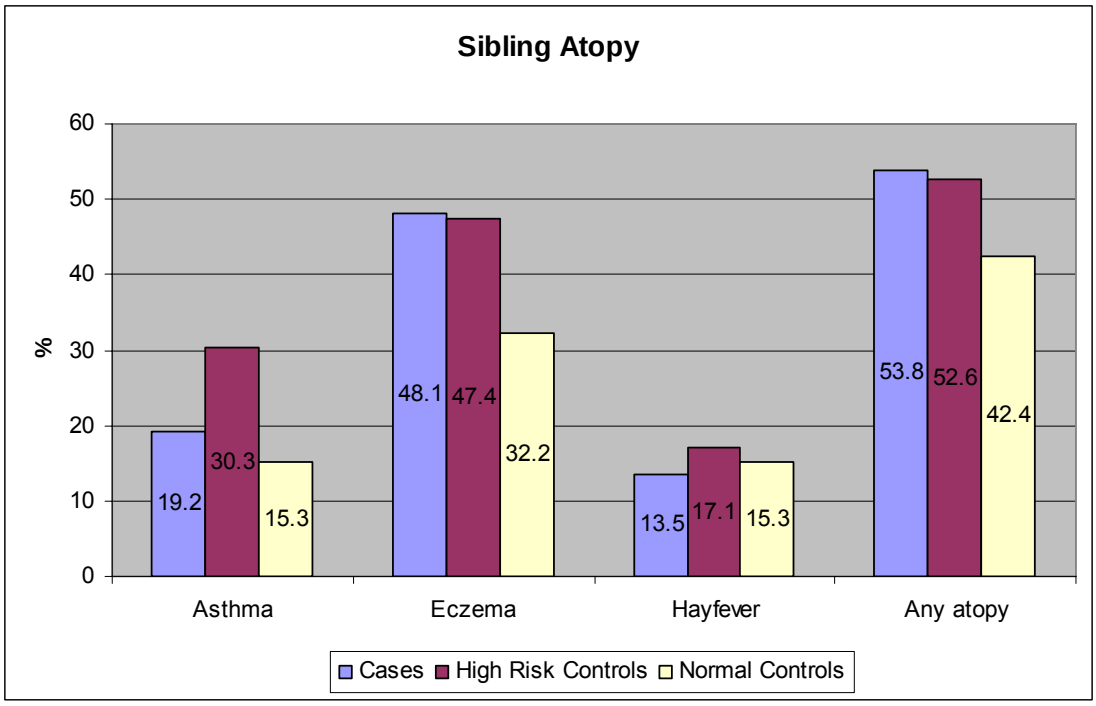


Figure 54: Percentage of children with sibling history of atopy

Composite Family Atopy	Asthma	Eczema	Hayfever	Any atopy
Cases	55(41.4%)	65(48.9%)	76(57.1%)	113(85.0%)
High Risk Controls	58(36.3%)	77(48.1%)	90(56.3%)	123(76.9%)
Normal Controls	38(25.3%)	52(34.7%)	56(37.3%)	85(56.7%)

When the presence of atopic disorders in either parent or older sibling were considered together, there were no significant differences between cases and high risk controls. However, combining these 2 groups showed significant differences to the non food allergic controls for family history of asthma (p=0.007), eczema (p=0.007), hayfever (p<0.0001) or any atopy (p<0.0001).

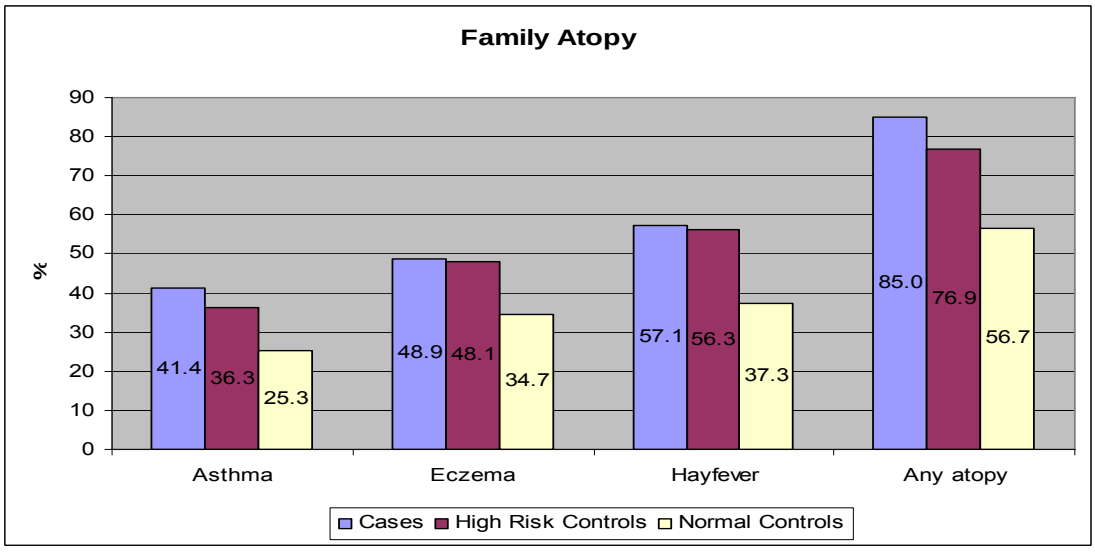


Figure 55: Percentage of children with family history of atopy

Details of family history of food allergy were also requested for each family member.

Food Allergy	Maternal	Paternal	Sibling*	Any family
Cases	21(16.7%)	10(7.9%)	4(8.2%)	32(25.4%)
High Risk Controls	30(18.8%)	15(9.4%)	29(38.2%)	62(38.8%)
Normal Controls	19(12.7%)	9(6.0%)	13(22.0%)	41(23.3%)

* as a percentage of those children with older siblings

No significant difference in reporting of food allergy amongst parents was found between the 3 groups (maternal p=0.28, paternal p=0.54). Differences were found between the groups with regard to sibling food allergy:

Cases vs High Risk Controls	p value =0.0001
Cases vs Normal Controls	p value =0.05
High Risk Controls vs Normal Controls	p value = 0.05

Differences were also found in the composite score reflecting any food allergy in the family.

Cases vs High Risk Controls	p value =0.001
Cases vs Normal Controls	NS
High Risk Controls vs Normal Controls	p value = 0.05

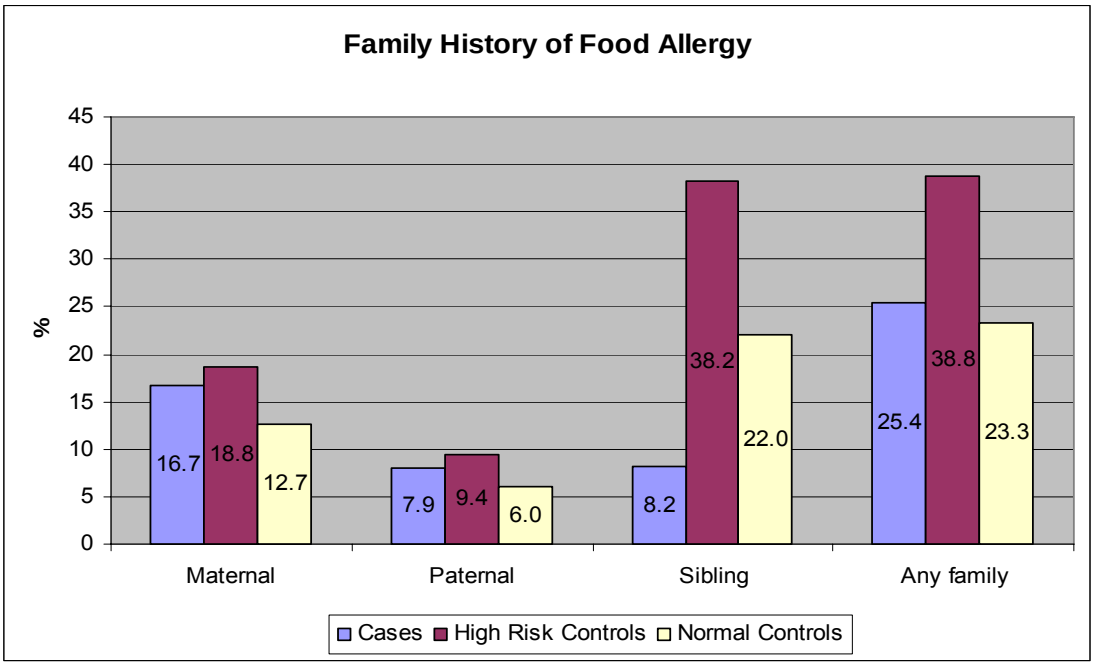


Figure 56: Percentage of children with family history of reported food allergy.

9) Breastfeeding - This outcome has been considered in Objective 3.

10) Number of Older Siblings

Kruskal-Wallis test rejects the hypothesis that the groups differ in terms of the number of older siblings.

	n	Median	75th percentile	90th percentile	Max
Cases	133	0	1	2	6
High Risk Controls	160	0	1	2	5
Normal Controls	150	0	1	2	3

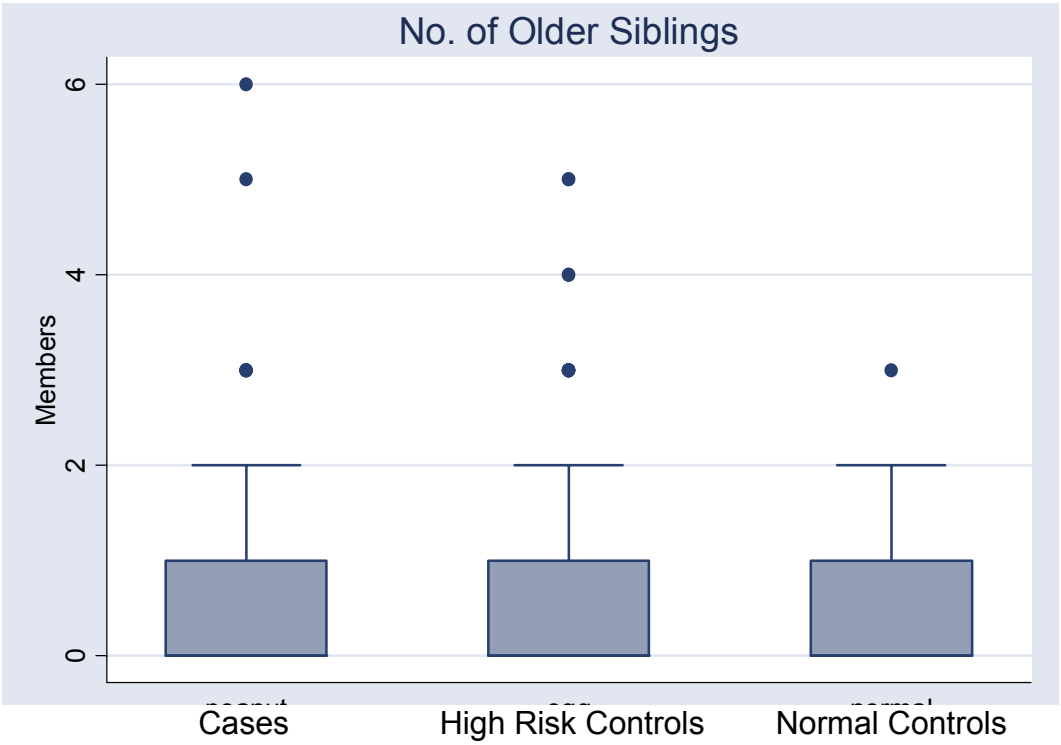


Figure 57: Number of older siblings

Additional Results

According to our hypotheses, exposure to high levels of environmental peanut in early life would lead to peanut allergy, whilst low levels would protect against it. Although this hypothesis has been supported by the analyses outlined in Objective 1 & 3, this remains a simplistic model. Many factors would be expected to influence the exposure of the infant's immune system to environmental peanut beyond just the amount of peanut being consumed by household members. This concept was considered above when the type of peanut consumption (peanut butter or snickers) or the application of creams containing peanut oil or soy were compared. It is clear from the data that

1) some of the Cases have PA despite low levels of exposure to peanut through household consumption of others
2) some of the High Risk Controls do not have PA despite high levels of exposure.

The possible explanations for this are explored in the discussion of these results. This section includes analyses that compare these anomalous cases/controls to the other children in their own groups to see whether they differ.

1) Cases with total weekly household consumption below 33.33g/week (Lower quartile) (Low HPC) were compared with the remainder of the Cases (High HPC).

a) Eczema or eczema/rash in first year of life

Cases	n	Presence of Eczema	Presence of Eczema/Rash in first year of life
Low HPC	33	31(93.9%)	32(97.0%)
High HPC	100	91(91%)	98(98.0%)
p value		NS	NS

Severity of eczema (based on steroid score)

Steroid Score	Low HPC	High HPC	Total
0	19	6	25
1	34	14	48
2	16	4	20
3	20	4	24
Total	89	28	117

Onset of eczema (months)

Onset (months)	Low HPC	High HPC	Total
0	8	4	12
.5	2	1	3
1	14	3	17
1.5	2	0	2
2	16	8	24
3	16	4	20
4	8	1	9
5	4	2	6
6	7	3	10
7	1	0	1
8	1	2	3
9	1	0	1
11	1	0	1
12	6	0	6
18	1	0	1
Total	88	28	116

There are no statistically significant differences between low and high HPC Cases with regard to presence of eczema, eczema or rash in infancy, onset or severity of eczema.

b) Creams including Peanut & Soy containing creams applied during first year of life

Mean number of creams per child	n	All creams	Peanut containing creams	Soy containing creams
Low HPC	33	4.55	0.57	0.24
High HPC	100	4.78	0.33	0.33

Creams containing Peanut	Low HPC	High HPC	Total
0	61	25	86
1	26	5	31
2	9	3	12
3	3	0	3
4	1	0	1
Total	100	33	133

Creams containing Soy	Low HPC	High HPC	Total
0	77	24	101
1	22	7	29
2	1	2	3
Total	100	33	133

There are no statistically significant differences between low and high HPC Cases with regard to application of creams including those containing peanut or soya.

c) Use of soya milk

	n	Used of soya milk
Low HPC	33	11(30%)
High HPC	100	41(41%)

Age of fist soya milk consumption (months)	n	25th percentile	Median	75th percentile
Low HPC	33	5.5	8	12
High HPC	100	6	8	16

Length of soya milk consumption in first year of life (months)	n	25th percentile	Median	75th percentile
Low HPC	11	0	0	3
High HPC	41	0	1	4

There are no statistically significant differences between low and high HPC Cases with regard to the use of soya milk, age of first consumption or length of use in first year of life.

d) Oral exposure of child to peanut – 10.5% of Cases had an oral exposure to peanut which did not lead to an allergic reaction, prior to diagnosis of PA.

	n	Exposure to peanut
Low HPC	33	2(6.1%)
High HPC	100	12(12%)

There was no statistically significant difference between low and high HPC Cases with regard to exposure of the child to peanut.

e) Proportion of peanut consumed as peanut butter

	n	25th centile	Median	75th centile
Low HPC	24	0	0	0.44
High HPC	100	0.11	0.35	0.72

Cases with no HPC were excluded. Kruskal-Wallis test returns a p value of 0.0001.

2) High Risk Controls with total weekly household consumption above 38.14g/week (upper quartile) (High HPC) were compared with the remainder of the High Risk Controls (Low HPC).

a) Eczema or eczema/rash in first year of life

Cases	n	Presence of Eczema	Presence of Eczema/Rash in first year of life
Low HPC	120	106(88.3%)	114(95.0%)
High HPC	40	35(97.5%)	38(95.0%)
p value		NS	NS

Severity of eczema (based on steroid score)

Steroid Score	Low HPC	High HPC	Total
0	15	7	22
1	63	19	82
2	10	5	15
3	17	4	21
Total	105	35	140

Onset of eczema (months)

Onset (months)	Low HPC	High HPC	Total
0	7	4	11
.25	3	0	3
.5	2	0	2
.75	2	0	2
1	19	7	26
1.5	1	2	3
2	24	5	29
2.5	2	3	5
3	13	4	17
4	10	6	16
5	7	1	8
6	5	1	6
7	1	0	1

8	1	0	1
10	1	0	1
12	0	1	1
13	1	0	1
14	1	0	1
19	0	1	1
Total	100	35	135

There are no statistically significant differences between low and high HPC High Risk Controls with regard to presence of eczema, eczema or rash in infancy, onset or severity of eczema.

b) Age of child when questionnaire completed

Age (months)	n	25th percentile	Median	75th percentile
Low HPC	120	14	22	34
High HPC	40	15	26	32.5

There is no statistically significant difference between low and high HPC High Risk Controls with regard to age at which the child was when the questionnaire (and allergy testing) was completed.

c) Exposure of child to peanut – 23.8% of Cases had oral exposure to peanut.

	n	Eaten peanut	Eaten peanut by 3 years	Eaten peanut by 2 years	Eaten peanut by 18 months
Low HPC	120	21	18	16	3
High HPC	40	17	17	16	2
p value		<0.001	<0.001	<0.001	NS

There is a difference between low and high HPC Cases. A significantly higher proportion of High Risk Controls with high levels of exposure to household peanut have eaten peanut themselves than those High Risk Controls exposed to lower levels of environmental peanut. This difference remains when only peanut consumption prior to age 3 or 2 is considered.

Although we have shown that the groups do not differ in terms of age when questioned, if we exclude all children younger than 3, then the difference

between the groups regarding peanut consumption by the age of 3 (as recommended by DoH guidelines) remains significant.

	n	Eaten peanut by 3 years
Low HPC	24	3
High HPC	6	3
p value		0.05

Furthermore, the average weekly amount of peanut eaten by the children is compared:

Peanut Consumption (g/week)	n*	25th percentile	Median	75th percentile
Low HPC	19	0	0	0
High HPC	15	6.75	13.9	81

*data was not complete for 4 peanut consuming children.

Krushkal-Wallis returns a value of 0.0001, rejecting the hypothesis that these groups are the same.

d) Proportion of peanut consumed as peanut butter

	n	25th centile	Median	75th centile
Low HPC	63	0	0	0
High HPC	40	0.11	0.35	0.72

Cases with no HPC were excluded. Kruskal-Wallis test returns a p value of 0.0001.

DISCUSSION

Assessment of Recall Accuracy of FFQ

Our results confirm that a retrospective FFQ can effectively predict prior peanut consumption at a point in the past. As mother's retrospective recall of peanut consumption increases we can reliably predict that their initial, contemporaneous assessment of their peanut consumption would also have been increasingly large. There is no systematic bias between the initial and retrospective reporting of peanut consumption. Validation of this SFFQ provides us with a powerful tool in the assessment of the role of maternal peanut consumption during pregnancy and early infancy in the development of later peanut allergy.

An obvious limitation to the use of this FFQ is the inability to obtain absolute, accurate measures of peanut protein intake. This is a problem encountered with many dietary intake studies[47], due to the lack of external 'gold standard' measures available for research[42]. A gold standard measure, such as a biomarker, would allow us to compare FFQ data contemporaneously and thus provide validation of it's accuracy. Weighed records and 24 hour recalls would be a possible reference measure but are not without their own errors[46]. It would also be difficult to validate the FFQ in our actual study population as we will be referring to consumption in the past and thus contemporaneous measurement is simply not possible for retrospective data collection. However, in our proposed studies, accurate assessment of peanut consumption is less important than relative values for comparison between those mothers whose children develop peanut allergy and those who do not. Furthermore, although the confidence intervals are broad, we are still able to clearly differentiate mothers with high peanut consumption from those with low peanut consumption. It is worth noting, in consideration of the validation confidence intervals, that they are based on the assumption of normal errors. In reality, the difference between the initial and follow-up values will be discrete. In many women there may be no difference between the values but for others, erroneous recall of one peanut dense item, could result in a large discrepancy from the initial value.

A further criticism of our methodology is the use of the same FFQ to obtain both initial and follow up data. This in itself may have influenced follow up recall, when mothers simply remembered how they had completed the initial FFQ and used this memory to complete the follow up questionnaire, rather than reflected on their prior peanut consumption. This may have been avoided by obtaining initial consumption data by a different method, such as diet diaries. This different approach is being adopted in our other, ongoing validation studies of the FFQ.

It is important to appreciate the context of this validation study and the limitations of it's value in different settings. The population studied, as well as the foods included in the FFQ list, restricts it's generalisability to populations within the UK. Peanut consumption, and the forms in which that peanut is consumed, vary widely in different countries[32] making our SFFQ unsuitable for use outside of the UK without further validation. Additionally, the recall of consumption during pregnancy may well not be comparable to recall of consumption during a different period in the past. Women may well be paying particular attention to their diet during pregnancy especially with regards to food such as peanut, where there is specific advice in the public domain to recommend avoidance[28]. As a result, recall of this period may well be significantly better than that of periods outside of pregnancy. Further validation would be required for recall of consumption during periods outside of pregnancy.

Our study was validated on the basis of a 2 year delay between initial recall of peanut consumption and the later follow up. It is known that recall accuracy decreases as the time between the period of interest and retrospective recall increases. This is due to the increasing influence of current intake on recall of past intake[42]. The 2 year time interval in this study was utilized because of our knowledge of the average age a child reaches before their allergy to peanut is discovered[1]. Asking a mother to recall earlier peanut consumption after she is aware of her child's peanut allergy will introduce considerable possibility of recall bias, for two reasons. Firstly, current Department of Health advice[28] to avoid peanut consumption during pregnancy and breast feeding strongly implies that there is an established mechanistic link between such exposure and later allergy. Although this link, in truth, remains elusive, it is likely to

focus the mothers mind on times that she may have deviated from this advice and thus lead to exaggerated recall. Secondly, once a child has received a diagnosis of peanut allergy, the families will receive a considerable amount of education regarding which foods do and do not contain peanut protein from both formal (eg Internet) and informal sources (eg dietetic advice). This knowledge will influence a mother's response on specific questioning about consumption of peanut containing foods. Thus information on maternal peanut consumption must be obtained prior to her child's diagnosis and will need collection, in the majority of cases, before the child reaches the age of three. In our study, by administering the FFQ to mothers of selected children at high risk of peanut allergy eg atopic children with troublesome eczema, before they have had any perceived reactions to peanut, would allow a relatively high yield of peanut allergic cases, apparent when these children have subsequent allergy tests or food challenges. This approach avoids the need to obtain a very large amount of data prospectively, without introducing the recall bias that would occur as soon as a diagnosis of peanut allergy is made. Assessment of recall accuracy of the questionnaire for our proposed uses is therefore not required beyond the 2 year time interval.

Clearly, there is further validation work needed if the FFQ or other tools are to be developed for the accurate assessment of peanut consumption. In the absence of accurate external methods of assessing peanut consumption such as biochemical markers, there value in comparing the FFQ to other equally inaccurate measures of consumption such as food diaries remains limited.

In summary, we have successfully demonstrated high recall accuracy of a FFQ for the retrospective assessment of peanut consumption in pregnant mothers. Despite some minor limitations, this provides a useful tool for investigating the role of relative peanut consumption during pregnancy and early infancy on the later development of peanut allergy in infants.

Discussion of Results of Main Study

Before discussing the specific results relating to each objective, the potential limitations of the main study will be considered.

Potential Limitations

There are a number of potential criticisms of our study design:

1) **Quantification of environmental peanut protein** – we hypothesized that sensitization to peanut is occurring through exposure to environmental allergen yet we are unable to accurately measure the amount of such protein in the environment. Peanut protein is present in foods and creams but also in animal feeds, cosmetics and plastics. Accurately quantifying peanut exposure would be extremely difficult in a current environment. To do so retrospectively is virtually impossible. As a result, we have to settle for surrogate markers of environmental peanut such as household consumption and application of peanut containing creams.

2) **Recall of dietary content** – In order to quantify the peanut content of a diet, accurate details of the content of the diet are required. We asked mothers to recall details not only of their own diet but also that of other family members. In order to limit difficulties in recall, we have only included cases and controls under the age of four. We had designed and piloted the FFQ within the same clinic population that our study population was drawn from as well as demonstrated a high level of recall accuracy as outlined above.

3) **Quantification of dietary peanut protein** – In our study, we are aiming to achieve an estimate of peanut protein consumption by concentrating only on foods with a relatively high peanut content. Although peanut consumption is only a marker of environmental peanut exposure, its precise measurement is also limited. Peanut has become a commonly used ingredient in a variety of foods. Although, in many cases, its presence can be detected from food labeling, this is not always the case. Peanut may be disguised on labels when it appears by generic names such as ‘vegetable oil’

or as part of compound ingredients. At the time of the study, if a compound ingredient constitutes less that 25% by weight of the final product, its ingredients need not be defined on the label. Peanut protein may also exist in trace quantities in a number of foods. However, such small amounts of peanut would not be expected to contribute a significant amount to overall peanut consumption unless eaten in excessive amounts. Another problem related to the quantification of dietary peanut protein is the lack of validation of our FFQ with regards to accuracy of measurement of precise peanut content of the diet. Such a validation would require a 'gold standard' reference measure with which to compare the FFQ. There is no such measure although ongoing validation work will help to determine the accuracy of the FFQ in assessing dietary peanut consumption by comparison with diet diaries as a reference measure.

Although all of the factors discussed here limit our ability to accurately quantify the amount of peanut in the diet, such precise measurement is of less importance than the relative quantities consumed in each group. As long as these factors do not differentially effect the results from Cases and Controls then they should not detract from the significance of our results.

Recall bias – This is the most obvious limitation of a retrospective study. Of particular concern is the possibility that there will be differential recall bias between the mothers of Cases and Controls. We have carefully designed the study to eliminate recall bias. The questionnaire was administered to patients as they arrived at our allergy clinic, before a diagnosis of PA had been made. If PA was specifically suspected by the family, they were excluded due to the potential introduction of recall bias. Nevertheless, this still does not remove the possibility that parents of children who are being referred to an allergy clinic, usually with bad eczema, may suspect food allergies. In our experience, parents of children with eczema nearly always suspect cow's milk as the incriminating allergen and sometimes wheat. They seldom consider peanut as being a possibility, especially as it is seldom in their diet.

Wit our study design, the differences found in familial peanut exposure between Cases and the High Risk Controls simply can not be explained by recall bias or selective bias towards one particular food, as the parents

included do not know or suspect that their children have PA. Recall bias regarding peanut allergy will have operated in the same direction for both the egg and peanut allergic children, not in two completely opposing directions given that parents do not suspect one specific food. The only possibly exception to this would be the High Risk Controls who have eaten (and tolerated) peanut and are thus known not to be allergic. These children were still recruited as their absence would have limited numbers and also prevented study of the relevance of their early peanut exposure to the development (or lack of) later peanut allergy. It is possible that these parents, especially if they were aware of the DoH guidance[28], have been influenced into thinking that they could not have exposed their child to high levels of peanut during pregnancy/breastfeeding as the child did not become peanut allergic. There are two important points to counter this. Firstly, there is no suggestion in the DoH guidance that environmental exposure is important and thus knowing your child is not peanut allergic should not influence recall of any family members prior peanut consumption except the mother. The second point is that an analysis which excludes the High Risk Controls who had tolerated peanut, still returned highly statistically significant differences between all 3 groups (Result of Objective 1 (6ii)). In summary, it is extremely difficult to explain our results simply on the basis of recall bias.

5) Diagnosis of Allergy using SPT/SpIgE –

Our intention in this research is to study children with clinical allergy rather than sensitisation. Ideally, we should be performing DBPCFC on all children included in the peanut allergy Cases to ensure that they are truly clinically allergic. This was not practical within the confines of our budget or time frame. Given that children who have had a reaction to peanut in the community will be considered allergic by their parents and thus subject to bias in their responses, we are only left with the option of using threshold values of specific IgE or SPT wheal size. It has become well established that the magnitude of skin test response /specific IgE levels can predict the outcome

of food challenges[48]. The main limitation of this approach is the difficulty in generalising data from published studies to a particular population[49]. This can be overcome by using threshold values that have been validated in the population under study. The threshold values we have chosen are based on validation of a >95% predictive value in a population of 14000 children (ALSPAC cohort) that have also been validated in our own tertiary clinic population.

Further to this, these values were obtained by examining our entire paediatric cohort whereas this study only includes children under 4 years of age. Cutaneous reactivity is known to vary with age and the histamine-induced wheal diameter increases by 125% from 4 days to 24 months with further increases over childhood and teenage years[50]. Studies of the predictive value of both SPT[51] and Specific IgE[52] in IgE mediated food allergy have shown that threshold values are lower amongst younger children. It is thus reasonable to assume that the threshold values we are using in this study have a greater than 95% predictive value amongst the population we are applying them to. It is also worth noting that when we exclude those cases most likely to be just sensitised, rather than clinically allergic (those with SPT wheal diameter of <10mm), our main outcome measure remains highly statistically significant (Result of Objective 1 (6iii))

Recruitment

Only 133 peanut allergic cases were recruited within the allocated time period. Identification of peanut allergic patients was severely restricted by the absolute requirement that families are not aware that the child is allergic to peanut when they fill out the study questionnaire. As a result, only a small proportion of children with peanut allergy diagnosed in our clinics were suitable for inclusion. Exact records of how many children were excluded as a result of allergy testing, having fulfilled the initial criteria for receiving a questionnaire, were not kept. However, in our special clinics, where children

were selected from our waiting lists if they were under 4 years with no known peanut allergy, 18 peanut allergic cases were yielded for 120 cases seen.
The initial decision to recruit 150 cases was actually in excess of that required according to our power calculations, as outlined in the section relating to Power Calculation above. In consultation with our statistical advisors, it was decided that we could move onto formal analysis of our data with the number of cases we had achieved. Initially, much of our case recruitment was achieved by selecting potential cases from our waiting list and bringing them into the hospital for assessment. However, this resource was eventually exhausted and new cases could then only be drawn from new referrals to the hospital as they came in.
This shortfall in Cases has not compromised our ability to detect the differences between groups for any of our predefined primary endpoints.

Basic Demographics

Whilst no significant differences exist between the groups in terms of age or gender, there was a greater proportion of boys amongst both food allergic groups. This gender bias has been previously documented amongst food allergic children.[53]

Objective 1

Our data clearly indicates that overall peanut consumption during the first year of life, is significantly higher in the households of those infants who went on to develop peanut allergy when compared to the control groups. The median value for average weekly peanut consumption is over 10 times greater in the Cases than the High Risk Controls. Furthermore, the peanut consumption in the High Risk Control group is substantially lower than that of the Normal control group. This difference is explained by the fact that we included only a sub-population of all egg allergic children in our High Risk Control group – those who did not become sensitised to peanut. Egg allergic children are at high risk of developing peanut allergy so this data suggests that the extremely low levels of environmental peanut may have exerted a protective effect over these children.

This effect persists when either total peanut consumption or consumption episodes are taken into account. The number of peanut consumption episodes is calculated in the same manner as the overall consumption but only taking into account how often a peanut containing food was eaten, rather than how much. If the importance of peanut consumption in environmental sensitization is through direct contact after a family member consumes peanut and then touches the infant, then the portion size of the food may well be of much less importance than the simple fact that peanut is being eaten at all. This outcome measure also avoids the potential criticism of our method of assessing portion size. The use of FFQs, particularly retrospectively, in assessing portion size may result in poor recall accuracy. This possibility could be quantified by a validation study of our FFQ, which is currently underway (see above). However, this analysis suggests that the effect of environmental peanut exposure remains apparent even in the absence of this data.

Importance of Peanut Butter

It was initially considered that peanut butter may offer a simple surrogate marker of overall peanut consumption. This was based on our original pilot study, which suggested that peanut butter accounted for the majority of total peanut consumption in all families. Indeed, using peanut butter consumption or episodes of peanut butter consumption as an outcome measure, produces a very similar pattern of results to those found when all peanut consumption is considered. Highly significant differences between all three groups persist. However, on further analysis, peanut butter makes up a significantly different proportion of total peanut consumption in the different groups, with a much higher proportion amongst Cases and virtually none amongst the High Risk Controls. Clearly, if peanut butter represents only a small or non existent proportion of total peanut consumption in many families, particularly in the control groups, it would be of little utility as a surrogate marker of total peanut consumption. However, the apparent ability of peanut butter to reflect so closely the overall peanut consumption in our analyses may be the result of its greater importance relative to consumption of peanut in other forms in relation to it's effect in sensitisation. This importance is most likely due to the high availability of the peanut protein in peanut butter. If environmental exposure involves the transfer of peanut protein from the hands of those who have consumed it, to the skin of the infant, then the likelihood of this happening will differ depending on the nature of the food consumed. For example, peanut butter is extremely high in peanut protein, which is exposed to the environment and is also sticky. This makes it highly amenable to being transferred between surfaces. In contrast, a snickers bar also contains high amounts of peanut protein but the peanut is encapsulated in chocolate. The peanut protein is thus not exposed until after it has been eaten, by which time the opportunity for environmental exposure has passed. This effect was explored by considering differences between the groups, in terms of consumption of peanut in forms other than peanut butter as well as Snickers alone. Snickers was used as it was the most commonly consumed of the food items in which peanut was completely encapsulated by another food.

Analyses of snickers consumption revealed significant differences between the groups, similar but less pronounced than when either total peanut consumption or peanut butter alone were considered.

It remains difficult to extract the relative importance of peanut exposure in different forms, given that most infants live in households where peanut butter, snickers and various other forms of peanut are consumed. The persistence of a statistically significant difference between non peanut butter or snickers consumption in households of Cases and High Risk Controls may simply be the result of a relationship between high consumption of one form of peanut and another. Indeed, there is some correlation between household consumption of peanut butter and household consumption of peanut as non peanut butter (correlation coefficient 0.2). Similarly, there is an association between household consumption of peanut as snickers and household consumption of peanut as non snickers (correlation coefficient 0.13). Therefore, where families eat lots of snickers, they will also be eating more of other forms of peanut. Whilst the snickers consumption may be irrelevant, it's association with other more relevant peanut consumption will make it appear to be important. This possibility is supported by the lack of any significant difference between the proportion of peanut being consumed in the form of snickers in the Cases and High Risk Controls.

A possible way to separate out the importance of different components is to consider snickers consumption in those families where peanut is consumed, but only in forms other than peanut butter. This avoids the risk of confusing the importance of snickers due to it's association with peanut butter consumption.

Group	**Number**	**25th centile**	**Median**	**75th centile**
Cases	17	7.7	15.4	30.8
High Risk Controls	27	3.85	15.4	30.8

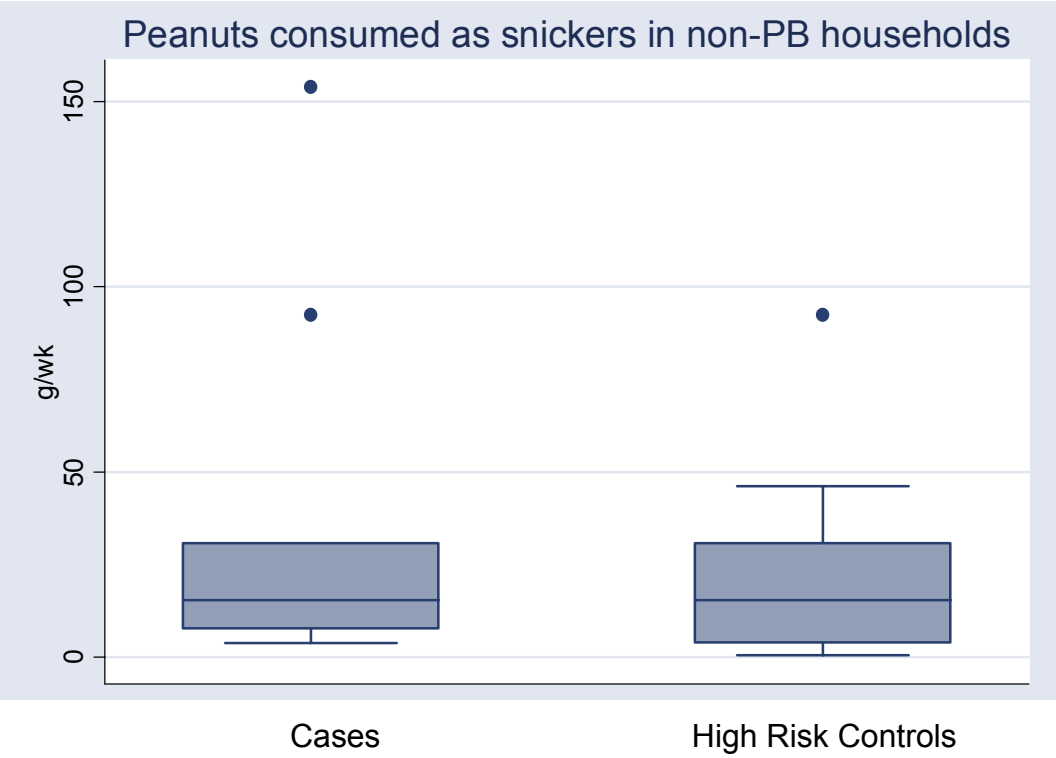


Figure 58: Proportion of total peanut consumption in the form of Snickers, in households where peanut butter is not eaten

There is no significant difference between the 2 groups, in contrast to the previous findings when snickers consumption across the whole group was examined.

Furthermore, the relative importance of different sources of peanut can be considered by separating the total average weekly household consumption in Cases and High Risk Controls in to 3 possible sources-

b) Peanut Butter
c) Snickers
d) Other

The consumption of peanut from these sources was then coded as categorical variables with the following groups (grammes of peanut/week)

1- 0
2- 0.1 - 14.9
3- 15.0 - 29.9
4- greater than 29.9

A logistic regression was performed to see whether these 3 categorical variables predicted whether or not someone was peanut allergic. We found that the snickers-consumption was not significant but the peanut-butter and the other- consumption were highly significant ($p<0.0001$).

If we put all 3 variables in as categorical variables to predict PA, we obtain the following odds-ratios (relative to zero consumption of peanut of that source).

	Odds Ratio	P>z	[95% Conf.Interval]
Snickers Category2	1.26	0.616	0.51 - 3.07
Snickers Category 3	1.21	0.659	0.53 - 2.77
Snickers Category 4	0.97	0.951	0.36 - 2.63
Peanut Butter Category 2	1.58	0.265	0.71 - 3.55
Peanut Butter Category 3	3.88	0.005	1.50 - 10.05
Peanut Butter Category 4	9.15	0.000	4.24 - 19.8
Other Category 2	1.28	0.541	0.58 - 2.80
Other Category 3	2.25	0.073	0.93 - 5.46
Other Category 4	5.10	0.000	2.42 - 10.77

Table 13: Odds Ratio of different amounts of peanut consumption in different forms.

As is apparent from Table 13, with regards to probability of being peanut allergic, Snickers consumption provides little information, other (non Peanut Butter/Snickers) provides some information but peanut butter consumption provides the most. This indicates that the importance of peanut consumption in sensitization is related to the availability of the peanut for environmental exposure in the foods that are consumed. This has implications for the possible advice that may be provided to the parents of children at risk of developing PA. Whilst we have shown that low household peanut consumption may prevent the development of PA, the consumption of peanut by families members in a form that does not bring peanut into contact with the outside environment, may have little influence and thus need not be avoided.

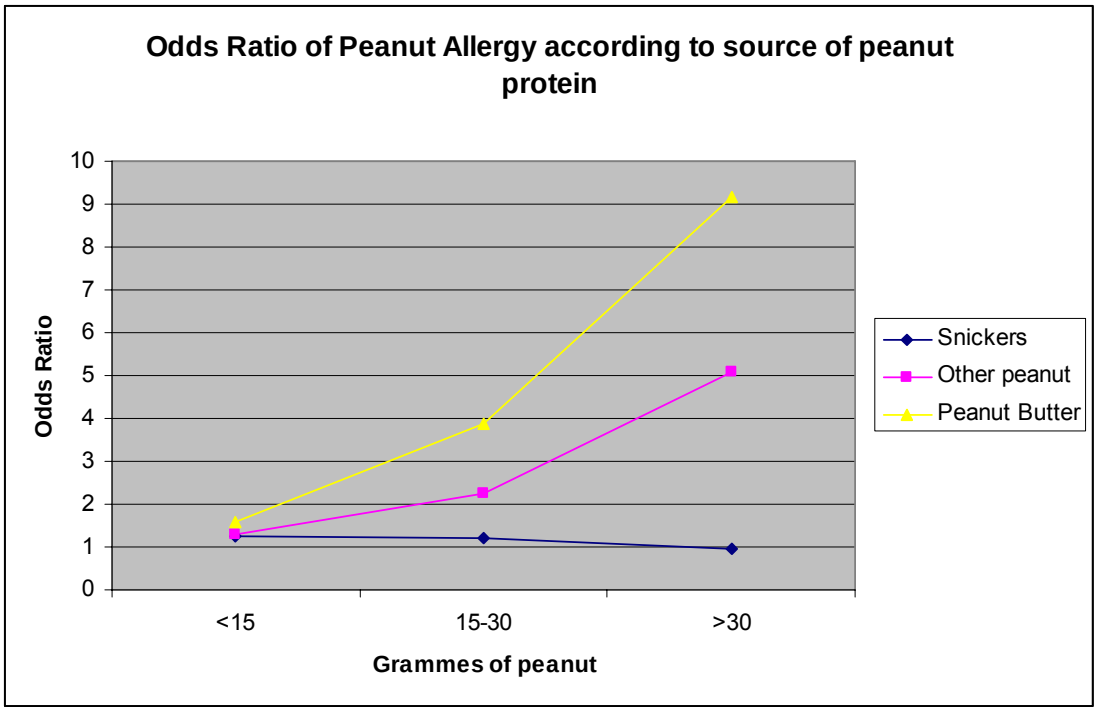


Figure 59: Odds ratio of Peanut Allergy according to source of peanut protein.

Other analyses further indicate the important role of environmental peanut exposure. Although there is no difference in household size between the groups, there were significantly more people eating peanut in the households of the children who developed PA.

Our final analyses were included to address particular issues. The comparison of only Cases who were egg allergic and High Risk Controls was of interest because these two group are extremely similar. They all have egg allergy but differ only in terms of either having peanut allergy (Cases) or not being allergic or sensitised to peanut (High Risk Controls). The fact that the difference in household peanut remains, suggests that this finding is highly specific to the development of PA. There had been concerns that the presence of egg allergy in early life would influence the parents behaviour relating to peanut. If they were aware of a close link between the two, once egg allergy was confirmed they may stop bringing peanut into the home, fearing there child was allergic to that also. This would reduce family peanut consumption, and explain why the levels were so low in the High Risk Controls. However, if this were the case, then we would find that the Cases who had egg allergy, and thus equally party to the same potential effect,

would not have the significantly higher consumption than the High Risk Controls that we demonstrate.

A further possibility is that the knowledge that your child can tolerate peanut, may influence your recall of early behaviours aimed at reducing peanut allergy. If a parent is aware of DoH guidance stating that reduced peanut consumption by the mother during pregnancy and lactation may prevent PA, they may be more likely to recall low consumption during these periods once they know that their child does not have it. However, including only the High Risk Controls who had not eaten peanut (and thus their PA status was unknown at time of data collection), reveals an even more pronounced difference, with an even lower peanut consumption amongst the High Risk Control household. Whilst providing evidence against the possible effect described above, the lower consumption also reflects the increased likelihood of infant peanut consumption in higher peanut consuming families.

The final analysis excluded all the Cases who had their diagnosis of PA based on a SPT of wheal diameter of less than 10mm. As outlined in Limitation 5 in our general discussion above, using specific IgE or SPT wheal size as a surrogate for food challenges introduces the risk of inclusion of children who are just sensitised, rather than clinically allergic to peanut into our Cases. By removing those children included as Cases on the basis of IgE values just above the diagnostic threshold level ie those most likely to be false positives (7-9mm), we demonstrate that reducing the potential number of false positives makes no significant difference to our findings.

It has been noted that in our analyses, a number of individual families showed patterns of exposure that were inconsistent with the rest of the group. Examples would be the Cases who had low household peanut consumption and the High Risk Controls who had high levels of household peanut consumption. These families are considered in greater detail in the discussion of the Additional Results.

In summary, we have demonstrated that children who develop PA have significantly higher household consumption of peanut than those who do not. High Risk Controls appear to be protected by the very low level of consumption in their household during the first year of their life. This effect remains consistent and highly significant throughout our multiple analyses.

The form in which this peanut is eaten also appears to influence the risk of PA developing, with peanut butter consumption being a particularly potent risk. The implication of these results is that PA may be avoidable in high risk children by reducing environmental exposure, although a careful analysis of the other possible routes of sensitisation in the context of these findings is required before conclusions may be drawn. This is the subject of Objective 3.

Department of Health Guidance

It has become generally accepted that food allergies have increased in prevalence over the last 40-50 years. In fact, the evidence base for this is relatively weak and this assumption has largely been based on the well documented increase in respiratory allergy[54]. Indeed, of the small number of studies that have investigated this possibility of a rise in food allergy, two relate to peanut allergy. Grundy et al[39] reported that among two separate birth cohorts, one from 1989 and another from 1994 to 1996 living on the Isle of Wight, the rate of reported peanut allergy for children age 3 to 4 years increased significantly from 0.5% to 1%. An increase in sensitization rates to peanut from 1.1% to 3.3% ($p = .001$) was also noted. Sicherer, using a random digit dial telephone survey in the US[55], revealed the rate of reported peanut allergy in children similarly increased 2-fold over a 5-year period, from 0.4% in 1997 to 0.8% in 2002. This later study was limited in that it relied on history alone, with no skin tests or oral food challenges.

As a response to the apparent rising prevalence of PA, in 1998 the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) produced a report which contained guidance that 'pregnant or breastfeeding women who are themselves atopic, or where another first-degree relative of the child is atopic, may wish to avoid eating peanuts and peanut products during pregnancy and lactation'[28]. Furthermore, avoidance of peanuts by infants up to 3 years of age was recommended. This report acknowledged that there was an absence of data to support the efficacy of maternal exclusion diets in allergy prevention but that infant sensitisation by maternal ingestion of peanut was mechanistically possible. A later review of evidence in 2004 still found no evidence to support the guidance[56]. There has been no published data to date, attempting to establish whether this guidance has been efficacious, either in terms of it's effective dissemination to its intended population, its uptake nor effect on peanut allergy. A recent study used a qualitative approach to gain a deeper understanding of the experiences of mothers' behaviour in light of the DoH guidance[57]. This revealed much interesting data, including evidence of different extents of

avoidance strategies eg total avoidance or just avoiding food where peanut was an obvious ingredient.

Our study has attempted to quantify how well the advice has been disseminated amongst both it's target population as well as to the rest of the population and whether it has led to behavioural change. Rather than simply relying on maternal reporting of adherence to the guidance, data collection for our study allowed a far closer analysis of whether mothers were truly adhering to the guidance.

Analysis of the data relating to the whole sample revealed a low level of awareness of the advice (51%) and interestingly, this was no higher amongst the target population than amongst the low risk mothers. Indeed, 19% of mothers with no personal or family history of atopy not only were aware of the advice but chose to follow it. Such behavioural change during what is already a difficult time may have either arisen from unnecessary anxiety about the risk of PA or led to unnecessary time and expense in trying to avoid peanut[57]. Both of these situations are highly undesirable. A further point of interest is the apparent difficulty of stringent adherence to the advice. Although 30% of mothers reported adhering to the guidance, when stringent adherence based on FFQ data was considered only 15% really had followed the guidance completely. This almost certainly reflects the difficulties in avoiding peanut especially if mother's lack 'clear, consistent factual information and advice about the real risks associated with peanut consumption during pregnancy and lactation'[57]. Of the total sample of 322 high risk mothers, for whom the advice was created, only 17% were aware of the advice, chose to follow it and did so successfully – which was not even significantly more than the proportion of low risk mothers. The DoH guidance is thus poorly disseminated, inadequately targeted and appears to be difficult to adhere to.

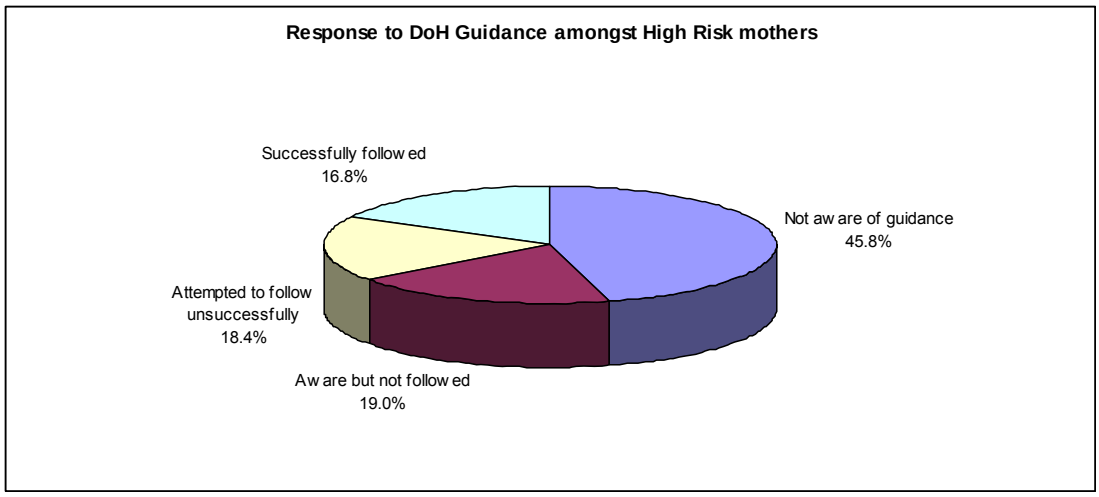


Figure 60: Response to DoH guidance by 'High Risk' mothers

The second issue is whether these guidelines exhibit any evidence of efficacy in the reduction of PA. Although there is evidence of an increase in peanut allergy in the period since the guidance was produced[55], this may not be a reflection of ineffectiveness, given the poor uptake demonstrated here.

When the Cases and High Risks Controls are compared they do reveal significant differences in the uptake of the advice, with higher uptake being associated with not developing PA. Awareness of the guidance is higher (but not significantly so) amongst the Controls whilst larger differences are apparent when actual adherence is considered. A possible explanation for higher reported adherence amongst the High Risk Controls is the influence of having already seen their child tolerate peanut. If, as a proportion of the High Risk Control families do know, that their child has eaten and tolerated peanut, they may be more likely to recall adherence to advice that they can assume was protective. However, no increased report of adherence to guidance is found when the data from children who had eaten peanut was compared with that of those who had not.

A more likely explanation relates to our previous demonstration elsewhere in this study that the Cases have significantly higher peanut consumption by both mothers and by household than the High Risk Controls. This may be relevant for a number of reasons. It may be harder for mothers to avoid peanuts when they are in a high consuming family, although we show that a similar proportion of those who intended to adhere to the advice in either

group, did so successfully. The much lower peanut consumption by Control mothers may simply make it appear that they kept to the advice when in fact it was an unintentional reflection of their normal, low peanut consuming, habits. However, when we only consider the subgroup who intended to avoid peanut and did so successfully, there are again significantly more such mothers in the control group.

There is also a small, and difficult to quantify, element of selection bias that would in fact result in an overestimation of the proportion of Cases who adhered to the advice. Peanut allergic children who had been exposed to peanut for the first time, would in most cases, react. This would exclude them form the study (due to influence of knowing they are allergic on consumption recall) and also indicate that they were not adhering to DoH advice to avoid peanut. Thus amongst children with PA, those who adhere to the advice and have not had any peanut are preferentially being recruited into the Cases group. The presence of significant results despite this possible bias away from it, points towards some efficacy of the advice.

However, some efficacy of this guidance would be expected within our own hypothesis of environmental sensitisation to peanut. The guidance recommends a decrease in maternal peanut consumption during lactation, which, albeit by a different route, also results in a reduction of overall environmental exposure. Furthermore, if, as we suggest, a baseline of very low peanut consumption is protective against environmental sensitisation, then further avoidance will require very little further effort, intentional or otherwise. Thus adherence to the guidance is simply much easier amongst low peanut consuming controls, rather than being an intentional protective intervention.

In summary, a combination of lack of awareness, misunderstanding of their relevance, lack of will or difficulty in following the guidance has resulted in only 17% of the target mothers successfully adhering to DoH advice. The guidance does appear to have some efficacy although this may not be due to the mechanistic theories upon which the advice was based.

Objective 2

Our previous work with the ALSPAC cohort[6] revealed that the application of topical creams containing peanut oil, as well as the presence of eczema or oozing/crusting rashes during infancy were both risk factors for the later development of PA. This data provided the basis of the hypotheses of sensitisation to peanut due to environmental exposure that we have been testing in this project.

Initially, as well as looking at the application of creams to the infants, we had intended to look at creams applied to mother's breasts during lactation. Peanut in breast creams would effectively result in exposure via the oral route which may have very different consequences to cutaneous exposure. However, due to the increased awareness and concern around the use of peanut containing creams, many have had the arachis component removed. Kamillosan, the most popular breast cream amongst our sample of mothers, previously contained peanut oil but no longer does so. In fact, no mother in any group reported applying a cream to her breasts that was known to contain peanut. This made comparison pointless we have thus been unable to pursue this line of inquiry.

We also planned to compare the application of preparations used by the mother on herself, given the possibility of transfer from mother's hands to infant's skin. This raised obvious concerns regarding the difficulty in quantifying such exposure but also proved impractical. A huge variety of creams were reported by mothers and in many cases it proved impossible to elicit enough details of the cream to identify the exact manufacturer. Without this detail we were unable to establish whether the creams contained peanut protein and thus no analysis was possible.

The analyses regarding presence of eczema revealed proportions of children with eczema that were comparable with previous studies. The presence of such high rates of eczema in the first year of life amongst children with food allergy is well documented[58] although the particularly high rates in our sample

are explained by our selection methods in the Cases and High Risk Controls. Children referred to the allergy clinics with eczema were preferentially selected from our waiting list and assessed in our research clinic because of their high yield of food allergy diagnoses. It was the high likelihood of peanut or egg allergy amongst these children that made it practical to obtain information prior to allergy testing (and thus before a diagnosis which would lead to recall bias) without requiring excessive numbers of children to be assessed. The higher rates of eczema, earlier onset and increased severity of the 2 food allergic groups would be expected in comparison to the unselected patients in the Normal Controls. There are no differences in these outcomes between the Cases and High Risk Controls reflecting the similar phenotype that these two groups were drawn from.

It is worth noting that our results relating to severity of eczema should be interpreted with caution. We utilised a severity scale based on the strongest steroid applied during the first year of life. This is not a validated scoring system and takes no account of the use of non steroid anti-inflammatories, differing prescribing practises of doctors or accessibility to healthcare. As our data was collected retrospectively, no validated scoring system, such as SCORAD, was available. A more detailed enquiry into the extent of previous eczema, required treatment and symptomatology would have required use of a more lengthy questionnaire and thus was avoided.

Analysis of the use of all creams applied to the infant's skin reflected the difference in rates of eczema and oozing/crusting rashes between the groups. Again, whilst the Cases (98.5%) and High Risk Controls (96.9%) did not differ from each other, both were significantly greater than the Normal Controls (88%). Interestingly, the proportion of children who applied creams closely mirrored the data from the ALSPAC cohort (100% in peanut allergics, 97% in atopic controls and 84% in an unselected population).

Although a slightly greater proportion of Cases (35.3%) had used creams containing peanut, no significant difference was found between these and the High Risk Controls (33.7%) and the Normal Controls (30.7%). A similar

pattern was found in the mean number of peanut containing creams used per child (over the whole group or just those children using them). The proportion using these creams was markedly lower in all groups when compared with the ALSPAC data (91% in peanut allergics, 53% in atopic controls and 59% in an unselected population).

There are a number of possible explanations for why the ALSPAC findings were not replicated. The most striking difference between the results of the two studies is the considerably lower usage of peanut containing creams in all the groups. This effect seems to be specific to peanut containing creams as the overall cream usage is very similar in the two studies. This decline in use of peanut containing creams would be expected given the decreased availability of such creams after the completion of the ALSPAC study. Serious concerns relating to the use of creams containing peanut appeared in the medical literature in 1998[59] and peanut oil was removed as an ingredient from many creams by manufacturers. Oilatum cream was the most commonly applied peanut containing cream in the ALSPAC study yet this preparation had the peanut oil removed prior to the study period for this project. There is also a degree of parental knowledge regarding the potential risk of peanut containing creams since the ALSPAC data was published, especially as it received considerable media coverage. It would thus be expected that mothers of children with eczema may chose to avoid these products. These two factors would account for the lower peanut containing cream usage, which is also reflected in the lower mean number of peanut containing creams used by the children who applied them. In peanut allergics this fell from 2.1 per child in the ALSPAC study to 1.45 in this study. However, a lesser drop (1.37 to 1.28) is noted in the unselected controls, perhaps reflecting the increased awareness of concerns surrounding the creams in an allergic population.

It is also worth noting that this effect of decreasing cream usage is probably underestimated in this study. The ALSPAC data was based on cream usage in the first 6 months of life, whereas our study asks about the whole first year of life. Furthermore, whilst the ALSPAC study only requested details of

creams for medicinal purposes, our study requested details of all creams used. With such a universal decrease in the use of these creams, it is most likely that the risks of their use would require a much larger sample than we have used in order to detect it.

Use of soya containing creams failed to reveal any significant differences between the Cases and High Risk Controls. Significant lower usage by the Normal Controls will almost certainly have been due to the lower prevalence and severity of eczema amongst this group. Both of the 2 commonly available soya containing topical applications are marketed primarily for the treatment of eczema, rather than for other skin complaints. This is in contrast to many of the peanut containing creams which are more commonly used simply as skin moisturisers rather that as specific treatments for eczema.

There is no previous comparative data to suggest that there has been a decline in the use of soya containing creams. There is no evidence of any move by manufacturers to remove soya oils from creams for children whilst a review of websites for new mothers' did not reveal any suggestion that mother's are trying to avoid these products. It would thus seem most likely that soya containing creams do not have a significant role as a risk factor for peanut allergy, at least at their current levels of usage.

In the Additional Results section above we have carried out some within-group analyses relating to eczema and use of creams. The purpose of these was to explore the interesting observation that some of the Cases have PA despite low levels of exposure to peanut through household consumption whilst some of the High Risk Controls do not have PA despite high levels of exposure. As household peanut consumption is only one element of environmental exposure, the presence of PA in the Cases where there is little household consumption may be a result of increased usage of peanut containing creams. Alternatively, the presence of more severe or earlier onset eczema may make them more sensitive to the relatively lower environmental peanut levels. Conversely, minimal eczema may protect the High Risk Controls from exposure even to high levels of environmental peanut

exposure. These possibilities are explored in more detail in the discussion below.

In summary, the presence of eczema or oozing/crusting rash in the first year of life was more common amongst the food allergics than the normal controls, as was expected. The severity and onset of the eczema followed the same pattern. The application of peanut or soya containing creams was not found to be a specific risk factor for PA although marked decrease in the level of usage of peanut containing creams is the most likely explanation for this.

Objective 3

In the results of Objective 1, we demonstrated that the household peanut consumption in the homes of Cases was significantly higher that that found in the homes of the control groups. Although the impressive size of this effect makes it tempting to link this to causality, there are other possible explanations. The most important of these, is the relative importance of different routes of peanut exposure such as in utero or via lactation. If there is a correlation between maternal consumption during these periods and that of the rest of the household that she is a part of, then high household consumption may simply be a surrogate marker of high maternal consumption, with the latter being causal. Separating out these closely associated factors presents a challenge. We adopted two approaches to this. The first was statistical, use logistical regression techniques to assess the relative importance of consumption by different routes in predicting the likelihood of PA. The other approach was to focus on the families where the link between different routes of exposure had broken down.

Our simple comparisons of maternal peanut consumption during pregnancy and lactation reveal some interesting data. Highly significant differences in both are apparent between the Cases and High Risk Controls, although not quite as significant as those relating to household consumption. This observation has been made before by Frank et al, in South African children[7]. In their work, children with PA were compared to children with other food allergies. It was found that mothers who consumed peanuts more than once a week during pregnancy were more likely to have a peanut-allergic child than mothers who consumed peanuts less than once a week (odds ratio=3.97, 98% confidence interval 0.73-24). The authors did not assess possible environmental peanut exposure and thus did not consider the possibility of this as a confounder. Interestingly, in our data, the maternal consumption during pregnancy or lactation amongst Normal Controls was significantly higher than the High Risk Controls but not significantly different when compared to the Cases. These findings suggest that there is no systematic

effect from recall bias amongst the parents of peanut allergic children, leading to an overestimation of consumption.
The observation that 9% of Cases were never breastfed provides excellent evidence that lactation could not be the sole route of sensitisation in all cases of PA.

Clearly, the different routes of peanut consumption can not be viewed in simple isolation, if we are to obtain clues to their relative importance. We have hypothesised that the greater peanut consumption during pregnancy or lactation amongst mothers of peanut allergic children is not causal, but simply reflects the correlation between maternal consumption and that of the household she lives in.
Indeed, the correlation coefficient between the total weekly household consumption of peanut during infant's first year of life and maternal peanut consumption during pregnancy is 0.45. The correlation coefficient between the total weekly household consumption of peanut during infant's first year of life and maternal peanut consumption during lactation is 0.51.

The presence of such a correlation presents a challenge in the demonstration that the differences we have shown in overall household consumption are independent of maternal peanut consumption during pregnancy and lactation. Our analyses aimed to separate out which of these factors (maternal peanut consumption during pregnancy, maternal peanut consumption during lactation or total household consumption during first year of life) is important in determining the likelihood of PA occurring. Our logistic regression analysis demonstrates that although there initially appears to be a relationship between increasing maternal peanut consumption in pregnancy and lactation and the risk of having PA, this disappears when the household consumption is also considered. This indicates that the maternal peanut consumption in pregnancy and lactation is not adding any further information to the statistical likelihood of an individual having PA or not, once total household consumption values are allowed for. Therefore the observation of higher maternal peanut consumption amongst the mothers of peanut allergic children during pregnancy and lactation, in this data and previous studies[7] is entirely

attributable to the link between this and the household peanut consumption. This is a novel finding.

Our other approach to separating out the different routes of exposure involved considering the families where the relationship between the different routes of exposure had broken down. We find that in families where the only peanut consumption is due to household consumption (because the mother either excluded peanut during pregnancy or lactation), the relationship between increasing household exposure and the likelihood of PA remains. When we consider those families where the only peanut consumption was by the mother during pregnancy or lactation, with no other household exposure, then the relationship disappears, with no discernable association between increasing maternal consumption and the likelihood of PA. Once again, the explanation for this is that the observation of higher maternal peanut consumption amongst the mothers of peanut allergic children during pregnancy and lactation is entirely attributable to the link between this and the household peanut consumption. The marked similarity between figures 35 & 37 are further evidence that it is the overall household exposure that is exerting the causative effect.

In summary, we have shown that further to the highly significant differences between Cases and High Risk Controls in terms of the overall household peanut consumption during the first year of life, similar differences also exist when maternal consumption during pregnancy and lactation are considered. Consumption by these 3 different routes are correlated with each other. In order to establish the relative importance of these different possible routes of exposure in the development of PA, the factors where considered together in a stepwise logistic regression analysis. This demonstrated that the maternal peanut consumption in pregnancy and lactation is not adding any further information to the statistical likelihood of an individual having PA or not, once total household consumption values are allowed for. Furthermore, analysis of families where there was a breakdown of the association between consumption by different routes, revealed that the observation of higher maternal peanut consumption amongst the mothers of peanut allergic children

during pregnancy and lactation is entirely attributable to the link between this and the household peanut consumption. These findings, considered together, provide important evidence for the critical role of environmental exposure to peanut as the primary route of sensitisation and as a risk factor for the development of PA.

Objective 4

The aim of this part of the study was to consider whether other factors that have been linked to increased risk in one or more allergic diseases are important in peanut allergy specifically. Food allergy is almost certainly the result of an interaction between exposure of a genetically susceptible individual to an allergen and various environmental modifying factors[54]. The continuing rise in allergic disease[39,55] has prompted considerable interest in identifying the risk factors for the development of allergic disease. A particular focus has been the search for an association between certain genes and food allergy. Although there is no evidence of specific inheritance of FA, a familial tendency seems to occur in some cases, and a strong hereditability of peanut allergy in twins has been reported[60]. The highest incidence of FA is in atopic children, particularly those with atopic eczema (35%)[61], followed by asthma (6%-8%)[62]. Each atopic phenotype is probably the result of a polygenic inheritance and a complex interaction between genes and environmental factors[63]. Two recent studies[64,65] have found some interesting association between certain genes and increased incidence of food allergy, but these appear to be of limited clinical significance. The hunt for genes with a strong association to food allergy continues.

There has also been much research into the possible influence of environmental factors on the development of food allergy. The most established of these risk factors are bottle feeding[66] and early introduction of solid foods during the first 4 months of life[67].

Environmental factors which are perceived to have changed over a similar timescale to the increased prevalence of allergic disease have received much attention. Factors such as delivery by Caesarean Section[68] and high maternal age[69] have been demonstrated as independent risk factors in some studies. However, none of these factors provide us with a reliable method of predicting which children will develop food allergy.

There has been little work which has focused on risk factors specifically for peanut allergy, by comparing children with this condition to a high risk control group. This study aimed to identify which factors may be important in the development or prevention of PA by such a comparison.

If significant differences are limited to comparison between Cases and Normal Controls and between High Risk Controls and Normal Controls then this suggests a risk factor for food allergy. Risk factors specific to peanut allergy will lead to significant differences between Cases and High Risk Controls also.

1) Socio-economic status – no significant differences between the groups was detected. This could well be a reflection of the geographical homogeneity of the population from which all 3 groups were drawn. The SOC 2000 classification that was used does have some limitations. Status is based entirely on employment and takes no account of education level, earnings or living conditions. This scale may therefore not be detecting the relevant socio-economic factors which most effect the likelihood of allergic disease developing.

2) Ethnicity of child based on parental ethnicity – Caucasian ethnicity had been identified as a possible risk factor for sensitisation to foods in previous FSA funded work. There is also anecdotal evidence, currently being investigated, that suggests that PA is becoming more prevalent amongst non Caucasian groups in the UK. Our results show no differences between the two food allergic groups, but significantly more Caucasians in the Normal Controls than in the food allergic groups. This is consistent with the previous findings mentioned above although the reasons for this remain unclear. A recruitment bias seems unlikely. In fact, we expected to see greater ethnic diversity in the Normal Controls due to differences in the population from which our groups are drawn. A proportion of children attending allergy clinics (and thus in the Cases and High Risk Controls) are referrals from outside the area for which the hospital provides General Paediatric services. These patients are referred from around the South East (95% Caucasian as of Census 2001) where there is a less ethnically diverse population than London. All the Normal Controls were recruited from General Paediatric clinics and thus represent the highly diverse population around Paddington (73%

Caucasian as of Census 2001). It may be the case that some systematic bias entered into the recruitment at General Paediatric Clinics, with parents less likely to fill in a voluntary questionnaire if English was not their first language. This may have been less likely to occur in a specialist clinic, where it may be perceived that the questionnaire was of greater relevance to their child and more worthwhile in completing.

The higher proportion of non Caucasian families in the food allergic groups also runs contrary to observations that there is a the lower incidence of allergy to some foods, including peanut, previously documented in Asian populations relative to Caucasian ones[70]. Changing disease patterns in migrant populations are well described and an increasing prevalence of allergy in non Caucasian populations in the UK could be the result of altered environmental factors. This drift towards disease rates of the host population alone could not explain why they should be more prevalent than in an unselected control group. The hypothesis of peanut sensitisation via cutaneous routes may explain this, if children from ethnic families are exposed to higher environmental peanut levels due to cultural dietary issues, relative to their Caucasian peers. We were unable to find such differences between the Caucasian and non Caucasian families in our study.

The finding that food allergy is more common amongst children with parents of mixed parentage would be expected within a population with a greater proportion of non Caucasians. However, although there is no previous data on mixed parentage, the high proportion of children of mixed ethnicity dying from fatal anaphylaxis to food has been noted previously (Anaphylaxis Campaign, personal communication). A mechanism for this increased susceptibility remains unclear but this association warrants further investigation.

3) Nationality of Patient Based on Parental Nationality – this outcome was considered because of an anecdotal increase in population mobility resulting in increased mixing of different Caucasian populations. Although a greater proportion of Caucasian couples of mixed nationality

were found amongst the Cases, this difference did not reach statistical significance.

4) Age of initial peanut consumption by infant – whilst early ingestion of peanut protein (in milk formula) had previously been linked to peanut sensitisation[35], there is no evidence of this in other larger studies[6]. Although there appear to be significant differences between the groups in our study, this needs to be interpreted with caution. Any peanut consumption in those with PA would, in the majority of cases, lead to an allergic reaction and thus exclusion from this study. As a result, we do not have useful data on age of first peanut consumption in those with PA as most have never eaten it.

   The exception to this were the minority of children who reported no reaction to peanut on exposure yet were still diagnosed with PA on the basis of later allergy testing. 14 (11%) of those diagnosed with PA in this study reported such exposure to peanut. This is consistent with previous research[1] demonstrating that approximately 10-15% of children with reactions to peanut have had previous oral exposure without reactions. Given the exclusion of those who reacted to peanut on ingestion, further analysis on our data set with regards to age of consumption will be of no value.

   There is also a significantly lower proportion of children in the High Risk Control group, relative to the Normal Controls, who had eaten peanut. This is best explained by the intentional avoidance of peanut in the diet of infant's with a family history of allergy, as advised by the DoH[28]. The age of introduction of peanut in the High Risk and Normal Controls did not differ significantly.

5) Soy consumption in infancy – In the ALSPAC data, infant soya consumption was independently associated with peanut allergy[6]. Entering milk allergy, egg allergy or rashes into a regression analysis did not alter this association, which could thus not be explained as a dietary response to other conditions. It was considered a possibility that the reason for this was cross-sensitisation through common epitopes,

although there is a low prevalence of clinical reactivity to soya amongst infants with peanut allergy[71]. We were unable to replicate this finding in our study. No significant differences were found between the Cases and High Risk Controls in terms of proportion of children who had ingested soya at all or just in the first year of life. The significantly lower proportion of children in the Normal Control group (relative to both Cases and High Risk Controls) who used soya milk can be accounted for by the lower levels of eczema and family history of atopy in this group. Amongst the children who took soya milk, the age at which it was first taken and length of time they had it for did not differ between the groups.

6) Prematurity – Low Gestational age has been shown to be related to a higher risk of asthma in later life[72,73,74]. One study relating gestational age to asthma in adulthood reported a similar result[75], but other studies did not find this relation[76,77]. Kuehr[78] reported an increased sensitization to aeroallergens in children born with a low gestational age. High gestational age was found to be associated with the risk of atopic dermatitis[79] and atopy[77], whilst low gestational age was reported to be protective for rhinitis[75].

   In our study we did not find a difference between Cases and High Risk Controls although prematurity was significantly more common in the Normal Control group. The rate of prematurity in these food allergic groups also appears low compared to the 5-11% of infants born before 37 weeks in industrialised countries[80]. Whilst this may reflect a true association with food allergy, a more likely explanation is a degree of selection bias. Our Normal Control group were recruited from our General Paediatric clinic population, where children born prematurely are likely to be overrepresented due to the many long term sequelae of preterm birth.

7) Pet Ownership – Exposure to pets has been a subject of considerable interest especially with regards a possible role in both the exacerbation and possible protective role of high exposure levels in respiratory

allergy[36]. There has been no previous association made with food allergy. The Cases and High Risk Controls have a very similar proportion of pet (both cat and dog) ownership. However, our data reveals a significantly lower rate of dog ownership amongst the combined food allergics when compared to the Normal Controls. There are also higher rates of cat ownership amongst the Normal Controls although this did not reach significance. Overall pet ownership is also significantly higher in the Normal Controls. The most likely explanation for these differences would be the tendency of atopic families to not keep pets, due to the effects of their dander on their allergies. Indeed, family history of atopy is significantly lower in the Normal Controls although a stepwise logistic analysis would be of value in determining whether the effect of pet ownership is independent of this.

8) Bronchiolitis, wheeze and asthma –

There is growing evidence that RSV infection may also predispose some children to the development of asthma. This is based on the observation that children who wheeze with RSV-induced bronchiolitis are more likely to develop into allergic asthmatics[81]. There has been no previous evidence of an association between food allergy and RSV bronchiolitis in the literature. There is no evidence to suggest otherwise from the data in this study.

As would be predicted when comparing an atopic to an uselected group of children, rates of wheeze and parental reports of asthma were higher amongst the food allergic groups. No differences for any factors relating to wheeze, asthma or bronchiolitis were significantly different between Cases and High Risk Controls.

9) Passive smoking - Exposure to tobacco smoke has previously been demonstrated to be an important risk factor for a number of health problems related to the respiratory tract in children. This includes lower respiratory infections, asthma and middle ear disease[82,83]. The evidence favouring exposure to passive smoking as a risk factor for atopic sensitization in children is much less convincing[84]. Studies of skin prick

reactivity and/or atopic disorders in Sweden[85], Estonia[86] and Italy[87] have suggested that perinatal and postnatal exposure to tobacco smoke increases the risk for atopic sensitization. This detrimental effect of environmental tobacco smoke (ETS) has also been demonstrated in non respiratory allergy as children are at a higher risk of developing atopic eczema when exposed to ETS and genetically predisposed children are at higher risk of sensitization to house dust mites[88]. There has been less work looking specifically at ETS in relation to sensitisation to foods. In the prospective German Multicentre Allergy Study, at the age of 3, children who were pre- and postnatally exposed to tobacco smoke had a significantly higher risk of sensitization to food allergens (odds ratio: 2.3, 95% C.I.: 1.1-4.6) than unexposed children. Children who were only postnatally exposed by a smoking mother also had a 2.2 times higher risk (95% C.I.: 0.9-5.9) of sensitization than unexposed children. These two categories (pre- and/or postnatal exposure) contribute to the significant overall effect of the tobacco smoke exposure (P< or =0.02). No significant association between tobacco smoke exposure and specific sensitization to inhalant allergens was observed[89].

In contrast, studies in Poland[86] and Norway[90] favour a protective effect. A more recent Swedish study demonstrated an association between exposure to tobacco smoke and a low risk for atopic disorder in adult smokers and their children alike[91]. There are several different mechanisms that could explain a preventive effect of tobacco smoke for atopic disorders. Tobacco smoke has been shown to affect the number and function of T lymphocytes[92]. A significantly reduced immune response to inhaled antigens has been suggested as a consequence of this altered T lymphocyte system[92]. It is also possible that the chronic changes of the respiratory tract, with mucosal oedema and mucus hypersecretion[83], can cause a relative barrier for allergens. Despite this, there is no apparent difference detected within our study in terms of exposure to passive smoking. This is consistent with more recent prospective studies in children at high risk of allergic disease (both parents atopic) that suggest that ETS has little or no effect on the development of atopy[93].

10) Family History of Allergy – significant differences were expected between the Cases/High Risk Controls and the Normal Controls for any marker of atopy, given the selection criteria. No differences between the Cases and High Risk Controls were apparent and these would have been difficult to explain had they appeared. A significant difference between these two groups was apparent for the presence of food allergy in other family members. This was due to the discrepancy between reports of food allergy in older siblings. Reports of food allergy by other family members needs to be interpreted with care. There was no formal assessment of these allergies or any detailed history or diagnostic testing. It is well established that the perception of allergy does not correlate with true allergy[94]. It is for this reason that food allergy was considered separately from the other atopic conditions in our analyses. Although still included in the data, many descriptions of food allergies were more consistent with food intolerances such as lactose intolerance or scromboid poisoning. Whilst not explaining why this discrepancy occurred, it means that we can not ascribe this to a genuine difference in allergy.

11) Breast feeding is discussed with the results of Objective 3 above.

12) Number of Older Siblings – The association of hay fever with family structure and the apparent protective effect of having increasing numbers of older siblings[95] formed the basis of the Hygiene Hypothesis. The finding that there is a lower risk of allergy amongst second and third children is supported by a firm evidence base[96] although the mechanisms that underlie this phenomena remain unclear[97]. However, we found no evidence of a difference in the number of older siblings when comparing our food allergic group to normal controls. This is a small sample to look for this outcome compared to the cited studies, especially in a population where only 17 of the 443 children had more than 2 older siblings.

In summary, our study of a number of possible risk factors failed to reveal differences between our Cases and High Risk Controls. The only exception was a markedly lower rate of reported allergies amongst siblings of the Cases. When these two groups were considered together as a single group of food allergic children, some interesting differences in comparison to our Normal Control Group arose. Higher rates of eczema, asthma, wheeze, use of soya milk and a family history of atopy amongst the food allergic group were found, as expected. However, a lower proportion of Caucasians, prematurity and dog ownership as well as a higher proportion of children of mixed ethnicity were also found in the food allergic group relative to Normal Controls. Findings regarding ethnicity have been noted in previous data. Although a formal logistical analysis will be required to assess whether these are independent risk factors, their presence demands further enquiry.

Additional Results

This groups of analyses were performed in order to investigate the observation that:

1) Some of the Cases have PA despite apparently relatively low levels of exposure to peanut through household consumption

2) Some of the High Risk Controls do not have PA despite apparently relatively high levels of exposure.

The presence of these groups challenges our hypothesis that PA is the result of environmental exposure to peanut in early life. However, there are many possible explanations for their existence, that are not inconsistent with our proposals. These two groups will be discussed separately although the possible explanations are similar for both groups:

a) Lack of sensitivity in detecting relevant household peanut consumption – the limitations of using an FFQ to retrospectively quantify peanut consumption have been detailed above. Further to these, there are also issues relating to the relevance of peanut consumption by different family members. For example, parents estimate their weekly peanut consumption but do not differentiate between what they eat at home and what is eaten whilst away from the home. The latter will have a much lower chance of leading to environmental exposure to an infant who is not present. This may explain why some High Risk children appear to have avoided PA despite high levels of exposure, because in fact they were not in reality being exposed to these high levels.

b) Relative importance of different forms of peanut consumption – as outlined in the results to Objective 1, the form in which peanut has been consumed may be highly relevant to the resulting environmental exposure. Whilst peanut butter is sticky and a source of pure exposed peanut, the peanut content in Snickers is entirely encapsulated by chocolate. If the high peanut exposure of some High Risk controls is predominantly due to high family consumption of peanut in the form of Snickers, then this may not translate to high levels of exposure in reality. Similarly, if the relatively low peanut exposure of some Cases is predominantly due to high family consumption of peanut in the form of peanut butter, then this may have provided as much exposure to

peanut as the levels found in families where more peanut is consumed, but in other less available forms.

c) Household peanut consumption is only one component of total environmental exposure – infants may be exposed to peanut in the environment by the application of topically applied creams as well as by the vapour from peanut products. The Cases who have PA despite low levels of household peanut consumption, may have been exposed to relatively high levels of peanut through topically applied creams

d) Presence of other risk/protective factors – the effect of environmental exposure to peanut will depend not only on the amount of allergen present but also on host factors. Cutaneous exposure to peanut has been demonstrated in animal models only where the skin has been abraded, to mimic the effects of eczema[19]. The immune system of an infant with severe eczema, of early onset and wide distribution would be effectively exposed to more environmental peanut that a child with no eczema, in the same household. Other risk factors might include genetic factors such as atopy.

e) Importance of other routes of exposure – the relative importance of exposure to peanut protein in utero and via breast milk have been discussed at length in the discussion of Objective 1 & 3 above. However, another important factor in considering what may have protected children in the High Risk Controls from PA despite the presence of high levels of environmental peanut is the possibility that these children had developed oral tolerance. There is some evidence that early oral exposure may be required to prevent the development of allergy. Oral tolerance induction is well recognised in murine models and to a lesser degree, in the human literature[98,99].

There is one adult study showing that feeding keyhole limpet hemocyanin (KLH) results in immunological tolerance to KLH antigen[98]. The only study that attempted to induce tolerance to a food allergen [100] was conducted in patients who already had established milk allergy. The result of this study were promising: 71% of highly allergic children were able to tolerate a daily intake of 200 mL of milk after treatment. However, this was an uncontrolled study and therefore the possibility

that these children would have shown spontaneous resolutions cannot be discounted.

There is some evidence that oral exposure to nickel results in tolerance. Numerous studies, both prospective and retrospective, show that early cutaneous exposure to jewelry, particularly through ear piercing, is a risk factor for the development of contact dermatitis to nickel. Three independent studies[101,102,103], including one prospective birth cohort study, show that the early application of orthodontic braces made of nickel strongly protects against the development of contact dermatitis to nickel (in one study there was an odds ratio of 0.07). Indeed, the level of nickel in both saliva and serum of individuals increases significantly after the insertion of fixed orthodontic appliances and this is thought to result in oral tolerance. Similarly, parents exposed to pancreatic extract by inhalation or contact develop IgE mediated allergic reactions but not the patients who were exposed to the extract by oral route[104].

Clinical observations from countries in south-east Asia and Africa where peanuts are consumed in high amounts in different snack forms during infancy, suggest the rate of peanut allergy to be low. As these differences could be due to genetics, we have examined these geographical variations more carefully by comparing the prevalence of peanut allergy in Jewish children in the UK and Israel. The relative risk of peanut allergy in the UK is fifteen fold higher than in Israel (unpublished data).

Data on peanut consumption has also been prospectively obtained for Jewish infants aged 8-14 months in both UK and Israel. Preliminary data suggest that most Israeli infants (81%) had eaten peanut by a year of age (median 10 months of age), with a median peanut protein consumption of 6.0g peanut protein/week. In contrast, the majority of UK infants (78%) had not been exposed to peanut protein by a year of age with significantly lower peanut consumption patterns recorded, median 0g/week. There is thus a statistically significant difference between peanut protein consumption in infants in Israel and the UK. These data are consistent with the notion that high-dose, first-time

peanut exposure may lead to oral tolerance. Indeed, if single oral exposure to peanut were to have no effect on promoting or preventing peanut allergy then one might expect to see numerous children react on subsequent exposures. This is not the case.

Animal models demonstrate that a high early dose of oral protein antigen is highly effective in inducing tolerance to the respective antigen, even in the case of subsequent administrations of antigen in the presence of potent immune-adjuvants. A literature search on oral tolerance induction in animal models has revealed 33 publications over the last 35 years in which a single oral dose of antigen was sufficient to induce tolerance. The phenomenon has been demonstrated for different antigens in different experimental models. The data is consistent, uniformly showing that a single dose of oral protein administration effectively causes immunological tolerance and prevents the expression of related clinical disease. Oral tolerance induction in animal models is most potent in its effects on delayed type I hypersensitivity responses; prevention of antibody responses through induction of oral tolerance is less consistent. However, numerous publications point to the fact that a single dose of food allergen in mice (beta-lactoglobulin, ovalbumen, peanut) is particularly effective in preventing the development of subsequent IgE mediated responses. A recent study[105] showed that naïve mice orally tolerised to beta-lactoglobulin were unable to mount significant IgE responses after subsequent sensitization with beta-lactoglobulin injected with alum (intraperitoneally). Similarly, there were no significant T-cell responses to beta-lactoglobulin in the pre-tolerised animals.

Later in 2004, Strid and colleagues[106] fed mice a single intragastric feed of defatted peanut flour at doses varying from 0.2mg to 100mg per mouse. Seven days after the feed, animals were immunised with 100mcg of peanut antigen emulsified with Complete Freunds Adjuvant. Three weeks later animals were given a recall immunisation with 100mcg antigen. Mice were assayed for T cell proliferation to peanut, cytokine production, delayed type hypersensitivity responses and antibody responses. Tolerizing doses of 100mg of peanut protein

resulted in significant reduction in delayed-type hypersensitivity responses and inhibition of proliferative responses to peanut. Animals tolerised to 100mg of peanut protein showed significantly reduced interferon gamma and IL4 production. Specific IgE responses to peanut following sensitization were almost completely prevented by the single tolerizing dose. However, very low “tolerizing” doses of peanut below 2 mg per animal resulted in enhanced delayed-type hypersentisitivity responses, T-cell proliferative responses, cytokine production and IgE production. Doses between 2 and 20 mg of peanut protein induced no difference in T- and B-cell responses compared to sham-tolerized animals. Tolerance to peanut was only achieved at doses of 100 mg per animal. Oral tolerance to peanut was shown to be antigen specific. Tolerizing doses of peanut did not promote tolerance to ovalbumen and vice versa.

Thus, comparing the oral peanut exposure of the infants in the High Risk Control group may offer an explanation as to how children exposed to high levels of environmental peanut did not become sensitised. However, the retrospective nature of this study makes it difficult to establish temporal relationships and thus the induction of oral tolerance will remain a possibility requiring further examination in prospective, interventional studies.

Given the possibilities outlined above, the groups will be discussed in turn:

1) Cases with low household peanut consumption: A sub-group analysis was carried out using Cases who come from households where the overall household peanut consumption (HPC) was in the lowest quartile for the group (low HPC). Considering the possible explanations above as to why these children became allergic to peanut, they were compared to the rest of the Cases (high HPC) in terms of presence, severity and onset of eczema, use of soy/peanut containing creams, consumption of soya milk, oral exposure to peanut and the proportion of peanut consumed by the family in the form of peanut butter.

No significant difference between the lowest quartile (for average total weekly HPC) and the upper 3 quartiles was found for any of these factors. A

significantly lower proportion of peanut consumed by the family in the form of peanut butter was found in the low HPC families. This is most likely due to the high peanut content of peanut butter, which ensures that any family with anything other than minimal peanut butter consumption will have enough total HPC to place then above the upper limit for the low HPC group.

Although our extra analyses did not reveal an explanation for PA in these low consuming household, the possibility of underestimation of environmental peanut exposure or underestimation of eczema severity using our unvalidated score remain. It should be noted that peanut consumption in the households of these 'low' household consuming Cases, is still markedly higher than the consumption in the households of the High Risk Controls. In fact, only 13 Cases have an average total household consumption less than 7.83g/week - the median for the High Risk Group. Therefore the most likely explanation for the lack of a difference between the 1st and the other 3 quartiles is that many of those in this lower quartile are still exposed to enough environmental peanut to account for their PA. Analysis only with the 13 Cases where family consumption was lower than the mean for the High Risk Controls would provide too small a sample for useful analysis.

2) High Risk Controls with high household peanut consumption: A sub-group analysis was carried out using High Risk Controls who come from households where the overall household peanut consumption was in the highest quartile for the group (high HPC). Considering the possible explanations above as to why these children had not become allergic to peanut, they were compared to the rest of the High Risk Controls (low HPC) in terms of age, the presence, severity and onset of eczema, oral exposure to peanut and the proportion of peanut consumed by the family in the form of peanut butter.

No significant differences were shown between the presence, onset or severity of eczema between the low and high consuming families, suggesting that the children from the high peanut consuming families had not been protected from sensitisation to peanut by the absence of eczema.

The age of the two groups did not differ significantly. It is possible that the reason some children were not peanut sensitised was simply that the process

was yet to occur. If this were the case we would have expected the children with high environmental peanut to be significantly younger and were they to be followed up as they grew up, peanut sensitisation would have been apparent.

A significantly lower proportion of peanut consumed by the family in the form of peanut butter was found in the low HPC families. This is most likely due to the reasons outlined for this same difference found between the low and high HPC Cases.

Significant differences were found when the low and high HPC groups were compared in terms of early oral peanut exposure of the child. A significantly greater population of the children in the high HPC group had eaten peanut themselves. This difference was apparent when infant peanut consumption was considered regardless of the age of consumption or when only consumption by the age of 2 or 3 is considered. The effect disappears if consumption by 18 months is considered, where the number of children having eaten peanut are very small. Further to this, there is a significantly higher consumption of peanut amongst those who had eaten it, in the high HPC. Although 19 children had reported oral exposure to peanut in the low HPC, of the 18 who provided details, only 3 of these children had a regular intake (compared to 13 of 15 in the high HPC). Indeed, most had simply had an accidental exposure on a single occasion. This is similar to the 14 of the Cases who had oral exposure to peanut, which did not cause a reaction. In all of these, the exposure was a single incident of accidental consumption.

There are two possible explanations for our findings. The first is simply that with increasing household consumption, there is a greater likelihood that the infant in that home will have eaten peanut. This may be as an accident, which would be more likely in a home where more peanut is consumed by other family members. Alternatively, peanut may have been introduced into the diet, again which would be more likely in a family who consumes a lot of peanut. If this association were the reason, then we would expect it to hold for the other groups. However, when the Cases are divided along similar lines and the upper quartile of HPC compared to the lower 3 quartiles, there is no difference in the proportion of children who have consumed peanut themselves (4/33 vs 10/100). Similarly with the Normal Controls (16/38 vs 62/122) there is no

difference in the proportion of children eating peanut in the high or the low HPC group. The other possibility is that the early exposure to oral peanut, especially with regular consumption, has led to oral tolerance induction and it is this which is protecting the High Risk Control children in the high HPC group from PA. Obviously, our retrospective design is not the ideal way to demonstrate this phenomenon and prospective interventional trials will be needed to see whether the early introduction of peanut into the diet prevents later onset of PA. This could well be the phenomena we are observing in Israel, as mentioned above, where there is high infant peanut consumption and low rates of PA.

In summary, the presence of Cases who had PA despite only relatively low levels of exposure to household peanut during the first year of life could not be explained by an increased severity or earlier onset of eczema nor by an increased exposure to peanut or soy containing creams, ingestion of soy milk or increased exposure to peanut in the form of peanut butter. A number of other possible explanations have been outlined. However, the presence of High Risk Controls, who did not develop sensitisation to peanut despite high household consumption, revealed a significantly higher rate of infant peanut consumption relative to other High Risk Controls. This suggests a possible role for oral tolerance induction as a result of early high dose exposure.

Conclusions

In this project we have demonstrated:

- The presence of eczema or oozing/crusting rash in the first year of life was more common amongst the food allergic children than the normal controls.

- Children who develop PA are exposed to significantly higher household consumption of peanut during the first year of their life than those who do not.

- High Risk Controls have relatively low levels of peanut consumption in their household during the first year of their life.

- There is higher maternal peanut consumption amongst the mothers of peanut allergic children during pregnancy and lactation but this is entirely attributable to the link between this and the overall household peanut consumption during the first year of life.

- The form in which peanut is eaten also appears to influence the risk of PA developing, with peanut butter consumption being a particularly potent risk.

- High Risk Controls, who did not develop sensitisation to peanut, despite high household consumption, are significantly more likely to have eaten peanut themselves, relative to other High Risk Controls.

Taken together, these observations strongly support our proposed model of sensitisation to peanut. The exposure of genetically predisposed children, coupled with eczema, to increasing levels of peanut in their environment results in an increasing risk of developing peanut allergy.

Low levels of environmental peanut appear to protect children at high risk of PA from developing sensitisation. Furthermore, early oral exposure to peanut also appears to exert a protective effect possibly by the induction of tolerance, even in the presence of high levels of environmental exposure.

This model of sensitisation to peanut has profound implications for the development of strategies to prevent future cases. The possible strategic options for children at high risk of developing PA would include:

1) Treat early eczema very aggressively. By maintaining a normal skin barrier, the risk of sensitisation by cutaneous exposure could be minimized.

2) Implement measures to reduce the infant's exposure to environmental peanut allergy. This may prove impractical although our data suggests that not all peanut need be removed from the householder's diet, if the peanut is covered (such as in chocolate bars) and does not come into contact with the environment.

3) Introduction of high dose peanut orally to children, in order to induce tolerance. Environmental measures will then be unnecessary.

All of these strategies require prospective, randomised controlled interventional trials to establish their efficacy, before changes in public health policy can be altered. In the Further Work section below, we have also outlined some of the work required to obtain in vitro evidence of cutaneous sensitisation to peanut, as well as the possible role of gut immunity in promoting immune tolerance.

Our other findings include:

- A combination of lack of awareness, misunderstanding of their relevance, lack of will or difficulty in following the DoH guidance has resulted in only 17% of the target mothers successfully adhering to it.

- DoH guidance does appear to have some efficacy in preventing PA although this may not be due to the mechanistic theories upon which the advice was based.

- The application of peanut or soya containing creams was not found to be a specific risk factor for PA although marked decrease in the level of usage of peanut containing creams is the most likely explanation for this.

- Investigation of a number of possible risk factors failed to reveal any factors specific to PA

- Comparison of food allergic children to non food allergic controls revealed higher rates of eczema, asthma, wheeze, use of soya milk, family history of atopy and mixed ethnicity amongst the food allergic group but a lower proportion of Caucasians, prematurity and dog ownership.

Acknowledgements

The authors wish to acknowledge the Food Standards Agency for their support of this work.

The authors would also like to acknowledge the assistance of Dr George DuToit in questionnaire design and validation, as well as Rosan Meyer for her expertise in the development of the Food Frequency Questionnaire. We would also like to acknowledge Tina Torr and the other members of the Department of Paediatric Dietetics at St Mary's Hospital for their help in obtaining information on the peanut content of a variety of foods.

We would like to acknowledge the expert statistical assistance of Prof Peter Sasieni and Dr Huma Syed of the Cancer Research UK Centre of Epidemiology,Mathematics and Statistics, Wolfson Institute of Preventive Medicine, London.

We would finally like to acknowledge all the staff of the Department of Paediatric Allergy at St Mary's Hospital for their considerable assistance in identifying potentially suitable cases and controls from already busy clinics.

References

1. Hourihane JO'B, Kilburn SA, Dean TP, Warner JO. Clinical characteristics of peanut allergy. *Clin Exp Allergy* 1997;**27**:634-9.
2. Ewan PW. Clinical study of peanut and nut allergy in 62 consecutive patients: new features and associations. *BMJ*. 1996;**312**(7038):1074-8.
3. Blyth T, Lack G. Are we generating peanut allergy? *The Asthma Journal* 2002;**3**:120-1.
4. Zeiger RS, Heller MSN, Mellon MH, Forsythe AB, O'Connor RD, Hamburger RN et al. Effect of combined maternal and infant food allergen avoidance on development of atopy in early infancy: A randomised study. *J Clin Allergy Immunol* 1989;**84**:72-89.
5. Zeiger RS, Heller MSN. The development and prediction of atopy in high-risk children: follow-up at age seven years in a prospective randomized study of combined maternal and infant food allergen avoidance.*J Allergy Clin Immunol* 1995;**95**(6):1179-90.
6. Lack G, Fox D, Northstone K, Golding J. Factors associated with the development of peanut allergy. *N Engl J Med* 2003;**348**(11):977-85.
7. Frank L, Marian A, Visser M, Weinberg E, Potter PC. Exposure to peanuts in utero and in infancy and the development of sensitization to peanut allergens in young children. *Pediatr Allergy Immunol* 1999;**10**(1):27-32.
8. Tariq SM, Stevens M, Matthews S, Ridout S, Twiselton R, Hide DW. Cohort study of peanut and tree nut sensitisation by age of 4 years. *BMJ*. 1996;**313**(7056):514-7.
9. Jarvinen KM, Makinen-Kiljunen S, Suomalainen H. Cow's milk challenge through human milk evokes immune responses in infants with cow's milk allergy.*J Pediatr*. 1999;**135**(4):506-12.
10. Vadas P, Wai Y, Burks W, Perelman B. Detection of peanut allergens in breast milk of lactating women.*JAMA.* 2001;**285**(13):1746-8.
11. Dybendal T, Elsayed S. Dust from carpeted and smooth floors. VI. Allergens in homes compared with those in schools in Norway. *Allergy* 1994 Apr;**49**(4):210-6.
12. Witteman AM, van Leeuwen J, van der Zee J, Aalberse RC. Food allergens in house dust. *Int Arch Allergy Immunol.* 1995 Aug;**107**(4):566-8.
13. Platts-Mills T, deWeck AL. Dust mite allergens and asthma – a worldwide problem. *JACI* 1989;**83**:416-427.
14. Perry TT, Conover-Walker MK, Pomes A, Chapman MD, Wood RA. Distribution of peanut allergen in the environment. *J Allergy Clin Immunol.* 2004 May;**113**(5):973-6.
15. Jensen CS, Lisby S, Baadsgaard O, Volund A, Menne T. Decrease in nickel sensitization in a Danish schoolgirl population with ears pierced after implementation of a nickel-exposure regulation. *Br J Dermatol.* 2002 Apr;**146**(4):636-42.
16. Saloga J, Renz H, Larson G, Gelfand EW. Increased airway responsiveness in mice is dependent on local challenge with antigen despite systemic sensitisation.. *Am J Respir Crit Care* 1994;**149**:65-70.
17. Renz H, Smith HR, Henson JE, Ray BS, Irvin CG, Gelfand EW. Aerosolised antigen exposure without adjuvant causes increased IgE production and increased airway responsiveness in the mouse. *J Allergy Clin Immunology* 1992;**89**:1127.
18. Hsieh K-Y et al. Epicutaneous exposure to protein antigen and food allergy. *Clin Exp Allergy* 2003;**33**:1067-75.
19. Strid et al. Disruption of the stratum corneum allows potent epicutaneous immunization with protein antigens resulting in a dominant systemic Th2 response. *Eur J Immunol. 2004* Aug;34(8):2100-9.
20. Roberts G, Golder N, Lack G.Bronchial challenges with aerosolized food in asthmatic, food-allergic children.*Allergy.* 2002 Aug;**57**(8):713-7.
21. Roberts G, Lack G. Relevance of inhalational exposure to food allergens. *Curr Opin Allergy Clin Immunol* 2003;**3**.
22. Bernstein et al. Clinical and Immunological studies among egg processing workers with occupational asthma. *J Allergy Clin Immunol* 1987;**80**:791-797.
23. Jyo, Kohmoto K, Katsutmai T, Otsuka T, Oka SD, Mitsui S. Hoya (sea squirt) asthma. In: Von Nostrand Reinhold, editor. Occupational Asthma. London: 1980:209-228.
24. Cartier A, Malo J-L, Forest F. Occupational asthma in snow crab- processing workers. *J Allergy Clin Immunol* 1984;**78**:344-348.

25. Armentia A, Martin-Santos JM, Quintero A, Fernandez A, Barber D, Alonso E, Gil I. Bakers' asthma: prevalence and evaluation of immunotherapy with a wheat flour extract. *Ann Allergy* 1990 Oct;**65**(4):265-72.
26. van Reijsen FC, Felius A, Wauters EA, Bruijnzeel-Koomen CA, Koppelman SJ.T-cell reactivity for a peanut-derived epitope in the skin of a young infant with atopic dermatitis. *J Allergy Clin Immunol*. 1998 Feb;**101**(2 Pt 1):207-9.
27. Legendre C, Caillat-Zucman S, Samuel D, Morelon S, Bismuth H, Bach JF, Kreis H. Transfer of symptomatic peanut allergy to the recipient of a combined liver-and-kidney transplant. *N Engl J Med*. 1997 Sep 18;**337**(12):822-4.
28. Committee on Toxicity of Chemicals in Food Consumer Products and the Environment. Peanut Allergy. Department of Health, 1998.
29. Ewan PW. Prevention of Peanut Allergy. *Lancet* 1998;**352**:4-5.
30. Lack G, Golding J. Reduced exposure might increase allergic sensitisation. *BMJ* 1996;**313**:300
31. Strobel S, Mowat AM. Immune responses to dietary antigens: oral tolerance. *Immunology Today* 1998; **19**(4):173-181
32. Dalal I, Binson I, Reifen R, Amitai Z, Shohat T, Rahmani S et al. Food allergy is a matter of geography after all: sesame as a major cause of severe IgE-mediated food allergic reactions among infants and young children in Israel. *Allergy*. 2002;**57**(4):362-5.
33. Hourihane JO, Dean TP, Warner JO. Peanut allergy in relation to heredity, maternal diet, and other atopic diseases: results of a questionnaire survey, skin prick testing, and food challenges. *BMJ*. 1996 Aug 31;**313**(7056):518-21.
34. Zimmerman B, Forsyth S, Gold M.Highly atopic children: formation of IgE antibody to food protein, especially peanut. *J Allergy Clin Immunol* 1989;**83**(4):764-70.
35. Moneret-Vautrin DA, Hatahet R, Kanny G.Risks of milk formulas containing peanut oil contaminated with peanut allergens in infants with atopic dermatitis. *Pediatr Allergy Immunol* ;**5**(3):184-8.
36. Perzanowski MS, Ronmark E, Platts-Mills TA, Lundback B.Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. *Am J Respir Crit Care Med* 2002;**166**(5):696-702.
37. Poyser MA, Nelson H, Ehrlich RI, Bateman ED, Parnell S, Puterman A, Weinberg E.Socioeconomic deprivation and asthma prevalence and severity in young adolescents. *Eur Respir J* 2002;**19**(5):892-8.
38. Lemanske RF Jr.The childhood origins of asthma (COAST) study. *Pediatr Allergy Immunol* 2002;**13** Suppl 15:38-43.
39. Grundy J, Matthews S, Bateman B, Dean T, Arshad SH. Rising prevalence of allergy to peanut in children: Data from 2 sequential cohorts. J Allergy Clin Immunol. 2002 Nov;110(5):784-9.
40. Margetts BM & Nelson M. Validation of Dietary Assessment. In Margetts BM, Nelson M (Eds). Design Concepts in Nutritional Epidemiology. 2nd ed. Oxford University Press 2001.
41. Byers T, Marshall J, Anthony E, Fiedler R, Zielezny M. The reliability of dietary history from the distant past. *Am J Epidemiol.* 1987 Jun;**125**(6):999-1011.
42. Lee RD, Nieman DC. Nutritional Assessment.3rd ed. London, McGraw Hill.2003
43. Rohan TE, Potter JD. Retrospective assessment of dietary intake. *Am J Epidemiol.* 1984 Dec;**120**(6):876-87.
44. Hourihane JO, Bedwani SJ, Dean TP, Warner JO**.** Randomised, double blind, crossover challenge study of allergenicity of peanut oils in subjects allergic to peanuts.*BMJ*. 1997 Apr 12;**314**(7087):1084-8.
45. Hunter DJ, Sampson L, Stampfer MJ, Colditz GA, Rosner B, Willett WC. Variability in portion sizes of commonly consumed foods among a population of women in the United States. *Am J Epidemiol.* 1988 Jun;**127**(6):1240-9.
46. Cade J, Thompson R, Burley V, Warm D. Development, validation and utilisation of food-frequency questionnaires - a review.*Public Health Nutr.* 2002 Aug;**5**(4):567-87.
47. Kroke A et al. Validation of a self-administered food-frequency questionnaire administered in the European Prospective Investigation into Cancer and Nutrition Study: comparison of energy, protein, and macronutrient intakes estimated with the doubly labeled water, urinary nitrogen, and repeat 24-h dietary recall methods. *Am J Clin Nutr* 1999;**70**:439-47.
48. Roberts G, Lack G. Food allergy-getting more out of your skin prick tests. Clin Exp Allergy. 2000 Nov;30(11):1495-8.

49. Eigenmann PA.Are specific immunoglobulin E titres reliable for prediction of food allergy? *Clin Exp Allergy.* 2005 Mar;**35**(3):247-9.
50. Menardo JL, Bousquet J, Rodiere M, Astruc J, Michel FB. Skin test reactivity in infancy.*J Allergy Clin Immunol.* 1985 Jun;**75**(6):646-51.
51. Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. *Clin Exp Allergy.* 2000 Nov;**30**(11):1540-6.
52. Celik-Bilgili S, Mehl A, Verstege A, Staden U, Nocon M, Beyer K, Niggemann B. The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges.*Clin Exp Allergy*. 2005 Mar;**35**(3):268-73.
53. Pumphrey R, Stanworth SJ. The clinical spectrum of anaphylaxis in north-west England. *Clin Exp Allergy* **26** (1996); **26**(12):1364-70.
54. Bjorksten B. Genetic and environmental risk factors for the development of food allergy. *Curr Opin Allergy Clin Immunol.* 2005 Jun;**5**(3):249-53.
55. Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: a 5-year follow-up study. *J Allergy Clin Immunol.* 2003 Dec;**112**(6):1203-7.
56. Muraro A, Dreborg S, Halken S, Host A, Niggemann B, Aalberse R, Arshad SH, Berg Av A, Carlsen KH, Duschen K, Eigenmann P, Hill D, Jones C, Mellon M, Oldeus G, Oranje A, Pascual C, Prescott S, Sampson H, Svartengren M, Vandenplas Y, Wahn U, Warner JA, Warner JO, Wickman M, Zeiger RS. Dietary prevention of allergic diseases in infants and small children. Part III: Critical review of published peer-reviewed observational and interventional studies and final recommendations. *Pediatr Allergy Immunol.* 2004 Aug;**15**(4):291-307.
57. Turke J, Venter C, Dean T. Maternal experiences of peanut avoidance during pregnancy/lactation: an in-depth qualitative study. *Pediatr Allergy Immunol.* 2005 Sep;**16**(6):512-8.
58. Cantani A. The growing genetic links and the early onset of atopic diseases in children stress the unique role of the atopic march: a meta-analysis. *J Investig Allergol Clin Immunol.* 1999 Sep-Oct;**9**(5):314-20.
59. Fox DE, Lack G. Peanut allergy. *Lancet*. 1998 Aug 29;**352**(9129):741.
60. Sicherer SH, *et al.*: Genetics of peanut allergy: a twin study. *J Allergy Clin Immunol* 2000; **106**:53-56.
61. Eigenmann PA, *et al.*: Prevalence of IgE-mediated food allergy among children with atopic dermatitis. *Pediatrics* 1998; **101**:e8.
62. Novembre E: Food and respiratory allergy. *J Allergy Clin Immunol* 1998;**81**:1059-1065.
63. Schuller DE. Risk factors for food allergy. *Curr Allergy Asthma Rep.* 2004 Nov;**4**(6):433-8.
64. Woo JG, Assa'ad A, Heizer AB, Bernstein JA, Hershey GK. The -159 C-->T polymorphism of CD14 is associated with nonatopic asthma and food allergy. *J Allergy Clin Immunol.* 2003 Aug;**112**(2):438-44.
65. Hand S, Darke C, Thompson J, Stingl C, Rolf S, Jones KP, Davies BH. Human leucocyte antigen polymorphisms in nut-allergic patients in South Wales. *Clin Exp Allergy*. 2004 May;**34**(5):720-4.
66. Saarinen KM, *et al.*: Supplementary feeding in maternity hospitals and the risk of cow's milk allergy: a prospective study of 6209 infants. *J Allergy Clin Immunol* 1999;**104**:457-461.
67. Fergusson DM: Early solid feeding and recurrent childhood eczema: a 10-year longitudinal study. *Pediatrics* 1990; **86**:541-546.
68. Laubereau B, Filipiak-Pittroff B, von Berg A, Grubl A, Reinhardt D, Wichmann HE, Koletzko S; GINI Study Group. Caesarean section and gastrointestinal symptoms, atopic dermatitis, and sensitisation during the first year of life. *Arch Dis Child.* 2004 Nov;**89**(11):993-7.
69. Dioun AF, Harris SK, Hibberd PL. Is maternal age at delivery related to childhood food allergy? *Pediatr Allergy Immunol.* 2003 Aug;**14**(4):307-11.
70. Hill D.J.; Hosking C.S.; Zhie C.Y.; Leung R.; Baratwidjaja K.; Iikura Y.; Iyngkaran N.; Gonzalez-Andaya A.; Wah L.B.; Hsieh K.H. The frequency of food allergy in Australia and Asia. *Envir Tox and Pharmacol*. 1997;**4**:101-110.
71. Bock SA, Atkins FM. The natural history of peanut allergy. *J Allergy Clin Immunol.* 1989 May;**83**(5):900-4.
72. Bernsen RM, de Jongste JC, Koes BW, Aardoom HA, van der Wouden JC. Perinatal characteristics and obstetric complications as risk factors for asthma, allergy and eczema at the age of 6 years. *Clin Exp Allergy.* 2005 Sep;**35**(9):1135-40.

73. von Mutius E, Nicolai T, Martinez FD. Prematurity as a risk factor for asthma in preadolescent children. *J Pediatr* 1993; **123**: 223–9.
74. Schaubel D, Johansen H, Dutta M, Desmeules M, Becker A, Mao Y. Neonatal characteristics as risk factors for preschool asthma. *J Asthma* 1996; **33**: 255–64.
75. Braback L, Hedberg A. Perinatal risk factors for atopic disease in conscripts. *Clin Exp Allergy* 1998; **28**: 936–42.
76. Hagstrom B, Nyberg P, Nilsson PM. Asthma in adult life – is there an association with birth weight? *Scand J Prim Health Care* 1998; **16**: 117–20.
77. Pekkanen J, Xu B, Jarvelin MR. Gestational age and occurrence of atopy at age 31 – a prospective birth cohort study in Finland. *Clin Exp Allergy* 2001; **31**: 95–102.
78. Kuehr J, Frischer T, Karmaus W *et al*. Early childhood risk factors for sensitization at school age. *J Allergy Clin Immunol* 1992; **90** (Part 1): 358–63.
79. Olesen AB, Ellingsen AR, Olesen H, Juul S, Thestrup-Pedersen K. Atopic dermatitis and birth factors: historical follow up by record linkage. *Br Med J* 1997; 3**14**: 1003–8.
80. Wen SW, Smith G, Yang Q, Walker M. Epidemiology of preterm birth and neonatal outcome. *Semin Fetal Neonatal Med.* 2004 Dec;**9**(6):429-35.
81. Kalina WV, Gershwin LJ. Progress in defining the role of RSV in allergy and asthma: from clinical observations to animal models. *Clin Dev Immunol*. 2004 Jun;**11**(2):113-9.
82. DiFranza JR & Lew RA. Morbidity and mortality in children associated with the use of tobacco products by other people. *Pediatrics* 1996; **97**:560–8.
83. Cook DG & Strachan DP. Summary of effects of parental smoking on the respiratory health of children and implications for research. *Thorax* 1999; **54**:357–66.
84. Strachan DP & Cook DG. Health effects of passive smoking. 5. Parental smoking and allergic sensitisation in children. *Thorax* 1998; **53**:117–23.
85. Norrman E, Rosenhall L, Nyström L, Jonsson E, Stjernberg N. Prevalence of positive skin prick tests, allergic asthma, and rhinoconjunctivitis in teenagers in northern Sweden. *Allergy* 1994; **49**:808–15.
86. Bråbäck L, Breborowicz A, Julge K et al. Risk factors for respiratory symptoms and atopic sensitisation in the Baltic area. *Arch Dis Child* 1995; **72**:487–93.
87. Ronchetti R, Bonci E, Cutrera R et al. Enhanced allergic sensitisation related to parental smoking. *Arch Dis Child* 1992; **67**:496–500.
88. Kramer U, Lemmen CH, Behrendt H, Link E, Schafer T, Gostomzyk J, Scherer G, Ring J. The effect of environmental tobacco smoke on eczema and allergic sensitization in children. *Br J Dermatol.* 2004 Jan;**150**(1):111-8.
89. Kulig M, Luck W, Lau S, Niggemann B, Bergmann R, Klettke U, Guggenmoos-Holzmann I, Wahn U. Effect of pre- and postnatal tobacco smoke exposure on specific sensitization to food and inhalant allergens during the first 3 years of life. Multicenter Allergy Study Group, Germany. *Allergy*. 1999 Mar;**54**(3):220-8.
90. Soyseth V, Kongerud J, Boe J. Postnatal maternal smoking increases the prevalence of asthma but not of bronchial hyperresponsiveness or atopy in their children. *Chest* 1995; **107**:389–94.
91. Hjern A, Hedberg A, Haglund B, Rosen M. Does tobacco smoke prevent atopic disorders? A study of two generations of Swedish residents. *Clin Exp Allergy*. 2001 Jun;**31**(6):908-14.
92. Holt P. Immune and inflammatory function in cigarette smokers. *Thorax* 1987; **42**:241–9.
93. Murray CS, Woodcock A, Smillie FI, Cain G, Kissen P, Custovic A; NACMAAS Study Group. Tobacco smoke exposure, wheeze, and atopy. *Pediatr Pulmonol.* 2004 Jun;**37**(6):492-8.
94. Young E, Stoneham MD, Petruckevitch A, Barton J, Rona R. A population study of food intolerance. *Lancet*. 1994 May 7;**343**(8906):1127-30.
95. Strachan DP, Taylor EM, Carpenter RG. Family structure, neonatal infection, and hay fever in adolescence. *Arch Dis Child*. 1996 May;**74**(5):422-6.
96. Matricardi PM, Franzinelli F, Franco A, Caprio G, Murru F, Cioffi D, Ferrigno L, Palermo A, Ciccarelli N, Rosmini F. Sibship size, birth order, and atopy in 11,371 Italian young men. *J Allergy Clin Immunol*. 1998 Apr;**101**(4 Pt 1):439-44.
97. Bodner C, Godden D, Seaton A. Family size, childhood infections and atopic diseases. The Aberdeen WHEASE Group. *Thorax.* 1998 Jan;**53**(1):28-32.
98. Husby S, Mestecky J, Moldoveanu Z, Holland S, Elson CO. Oral tolerance in humans. T cell but not B cell tolerance after antigen feeding. *J Immunol.* 1994; **152**(9):4663-4670.
99. Ngan J, Kind LS. Suppressor T cells for IgE and IgG in Peyer's patches of mice made tolerant by the oral administration of ovalbumin. *J Immunol.* 1978; **120**(3):861-865.

100. Meglio P, Bartone E, Plantamura M, Arabito E, Giampietro PG. A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. *Allergy.* 2004; **59**(9):980-987.
101. Kerosuo H, Kullaa A, Kerosuo E, Kanerva L, Hensten-Pettersen A. Nickel allergy in adolescents in relation to orthodontic treatment and piercing of ears. *Am J Orthod Dentofacial Orthop.* 1996; **109**(2):148-154.
102. Mortz CG, Lauritsen JM, Bindslev-Jensen C, Andersen KE. Nickel sensitization in adolescents and association with ear piercing, use of dental braces and hand eczema. The Odense Adolescence Cohort Study on Atopic Diseases and Dermatitis (TOACS). *Acta Derm Venereol.* 2002; **82**(5):359-364.
103. Van Hoogstraten IM, Andersen KE, Von Blomberg BM, Boden D, Bruynzeel DP et al. Reduced frequency of nickel allergy upon oral nickel contact at an early age. *Clin Exp Immunol.* 1991; **85**(3):441-445.
104. Twarog FJ, Weinstein SF, Khaw KT, Strieder DJ, Colten HR. Hypersensitivity to pancreatic extracts in parents of patients with cyctic fibrosis. *J Allergy Clin Immunol.* 1977; **59**(1):35-40.
105. Frossard CP, Tropia L, Hauser C, Eigenmann PA. Lymphocytes in Peyer patches regulate clinical tolerance in a murine model of food allergy. *J Allergy Clin Immunol.* 2004; **113**(5):958-964.
106. Strid J, Thomson M, Hourihane J, Kimber I, Strobel S. A novel model of sensitization and oral tolerance to peanut protein. *Immunology.* 2004; **113**(3):293-303.
107. Eigenmann PA. T lymphocytes in food allergy: overview of an intricate network of circulating and organ-resident cells. *Pediatr Allergy Immunol.* 2002 Jun;**13**(3):162-71.
108. Santamaria Babi LF, Picker LJ, Perez Soler MT, Drzimalla K, Flohr P, Blaser K, Hauser C. Circulating allergen-reactive T cells from patients with atopic dermatitis and allergic contact dermatitis express the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen. *J Exp Med.* 1995 May 1;**181**(5):1935-40.
109. Jensen CS, Lisby S, Baadsgaard O, Volund A, Menne T. Characterization of lymphocyte subpopulations and cytokine profiles in peripheral blood of nickel-sensitive individuals with systemic contact dermatitis after oral nickel exposure. *Contact Dermatitis.* 2004 Jan;**50**(1):31-8.
110. Schweighoffer T, Tanaka Y, Tidswell M, Erle DJ, Horgan KJ, Luce GE, Lazarovits AI, Buck D, Shaw S. Selective expression of integrin alpha 4 beta 7 on a subset of human CD4+ memory T cells with Hallmarks of gut-trophism. *J Immunol.* 1993 Jul 15;**151**(2):717-29.
111. Kantele A et al. Differential homing commitments of antigen-specific T cells after oral or parenteral immunization in humans. *Journal of Immunology* 1999;**162**:5173-5177.
112. Rott LS et al. Expression of mucosal homing receptor $\alpha 4\beta 7$ by circulating CD4+ cells with memory for intestinal rotavirus. *J Clin Invest* 1997;**100**:1204-8.
113. Fox AT, Lack G. High Environmental Exposure to Peanut in Infancy as a Risk Factor for Peanut Allergy. *Journal of Allergy & Clinical Immunology* 2005; **115**(2) Suppl:34.
114. Turcanu V, Maleki S, Lack G. Characterization of lymphocyte responses to peanuts in normal children, peanut-allergic children, and allergic children who acquired tolerance to peanuts. *J Clin Invest* 2003;**111**:1065-72.

Appendix A

Study Questionnaire

Are Children with Peanut Allergy Sensitised by Indirect Exposure during Infancy?

Most of these questions relate to how much peanut either you or your family were eating in the past. We understand that we are asking for detailed information about events that may have occurred a long time ago so we do appreciate any time you are spending in order to provide us with the best answers you can. If you don't know the answer then please indicate this, rather than leaving it blank.

Please note that any reference to 'Your child' is referring to your child with peanut allergy that you have brought to see the doctor today.

The questionnaire should take about 30 minutes to complete.

Thank you very much for your time.

About You (your child's Mother)

1. Please circle any of these conditions that you suffer from:

ASTHMA ECZEMA HAYFEVER

2. Please circle your ethnic background:

Caucasian Black

Asian Indian Asian Chinese

Arabic/Middle Eastern Other_____

What is your country of birth?_____

3. Do you have any allergies to food & if so, to what food(s)? NO YES_____

4. Do you dislike peanuts? NO YES

5. Do you dislike other nuts? NO YES

6. What is your main occupation? _____

7. **Try and remember back to when you were pregnant with your child**.

How many times did you eat the foods listed in a normal week and how much of the food did you eat each time?

Example:

If you ate 1 snickers bar on 2 occasions in a normal week then you would fill in:

Snickers 2 times 1 bars

and if you ate 2 slices of bread & peanut butter on 4 occasions during a normal week:

Peanut butter 4 times 2 slices

A sandwich of 2 slices of bread filled with peanut butter would only count as 1 slice of peanut butter.

Type of food	**Times eaten in a week**	**Amount eaten each time**
Peanut butter		slices
Snickers		bars
Peanut M&Ms		packs
Whole peanuts		handfuls
Crunchy Nut Cornflakes		bowls
Crunchy Nut Cornflakes Red		bowls
Revels		bars
Tracker Roasted nut		bars
Rowntree's Lion Bar		bars
Cadbury's Star Bar		bars
Cadbury's Fuse		bars
Cadbury's Picnic		bars
Reese's Peanut Butter Cups		cups
Satay sauce		servings
Peanut soup		servings
Bamba snack		packs
Hazelnut spread eg Nutella		slices

Please write down any other sources of peanuts you may have eaten, and how much per week:

_____

_____

_____

Did you eat more, less or the same amount of peanut that you normally would, whilst you were pregnant with your child? LESS MORE SAME

Please write down any other nuts or foods containing other nuts that you may have eaten, and how much you ate in a normal week. Examples include walnut, hazelnut, pine nut, brazil nut, pistachios, macademia nuts, almonds and chocolate containing them:

_____

_____

8. Did you breastfeed your child? YES NO

If you did not breastfeed your child, please ignore questions 8, 9 and 10 and go straight to question 11.

9. How long did you breast feed for? _____months

10. **Try and remember back to when you were breast-feeding your child**.
How many times did you eat the foods listed in a normal week and how much each time?

Type of food	**Times eaten in a week**	**Amount eaten each time**
Peanut butter		slices
Snickers		bars
Peanut M&Ms		packs
Whole peanuts		handfuls
Crunchy Nut Cornflakes		bowls
Crunchy Nut Cornflakes Red		bowls
Revels		bars
Tracker Roasted nut		bars
Rowntree's Lion Bar		bars
Cadbury's Star Bar		bars
Cadbury's Fuse		bars
Cadbury's Picnic		bars
Reese's Peanut Butter Cups		cups
Satay sauce		servings
Peanut soup		servings
Bamba snack		packs
Hazelnut spread eg Nutella		slices

Please write down any other sources of peanuts you may have eaten, and how much per week:

_____

_____

Please write down any other nuts or foods containing other nuts that you may have eaten, and how much you ate in a normal week. Examples include walnut, hazelnut, pine nut, brazil nut, pistachios, macademia nuts, almonds and chocolate containing them:

_____

_____

11. Did you use any creams on your breasts, whilst you were breastfeeding?

YES **NO**

If so, what were the creams called?

_____

Did you apply any creams to your body, such as moisturisers, whilst your child was under the age of one?
YES NO

If you did, could you name them (please give as much detail as possible)?
1.
2.
3.

12. **Try to think back to when your child was under the age of one**, and **not** being breastfed. How many times did you eat the foods listed in a normal week and how much each time?

Type of food	**Times eaten in a week**	**Amount eaten each time**
Peanut butter		slices
Snickers		bars
Peanut M&Ms		packs
Whole peanuts		handfuls
Crunchy Nut Cornflakes		bowls
Crunchy Nut Cornflakes Red		bowls
Revels		bars
Tracker Roasted nut		bars
Rowntree's Lion Bar		bars
Cadbury's Star Bar		bars
Cadbury's Fuse		bars
Cadbury's Picnic		bars
Reese's Peanut Butter Cups		cups
Satay sauce		servings
Peanut soup		servings
Bamba snack		packs
Hazelnut spread eg Nutella		slices

Please write down any other sources of peanuts you may have eaten, and how much per week:

_____

_____

Please write down any other nuts or foods containing other nuts that you may have eaten, and how much you ate in a normal week. Examples include walnut, hazelnut, pine nut, brazil nut, pistachios, macademia nuts, almonds and chocolate containing them:

_____

_____

About your child

1. How old is your child today? ___years___months

2. Was your child born early and if so, how many weeks? NO YES ___weeks

3. About your child:

Does your child have allergies to any foods? YES NO

If your child does have allergy to other foods, which foods are they?_____

Has your child ever had food containing peanut? YES NO

If so, how old was your child when he/she first had food with peanut in it?___years___months

If your child has had peanut before, try to think back to when they first started to eat it. How many times did your child eat the foods listed in a normal week and how much each time?

Type of food	**Times eaten in a week**	**Amount eaten each time**
Peanut butter		slices
Snickers		bars
Peanut M&Ms		packs
Whole peanuts		handfuls
Crunchy Nut Cornflakes		bowls
Crunchy Nut Cornflakes Red		bowls
Revels		bars
Tracker Roasted nut		bars
Rowntree's Lion Bar		bars
Cadbury's Star Bar		bars
Cadbury's Fuse		bars
Cadbury's Picnic		bars
Reese's Peanut Butter Cups		cups
Satay sauce		servings
Peanut soup		servings
Bamba snack		packs
Hazelnut spread eg Nutella		slices

Please write down any other sources of peanuts they may have eaten, and how much per week:

_____

_____

Please write down any other nuts or foods containing other nuts that they may have eaten, and how much you ate in a normal week. Examples include walnut, hazelnut, pine nut, brazil nut, pistachios, macademia nuts, almonds and chocolate containing them:

_____

_____

Has your child ever had food containing egg? YES NO

If so, how old was your child when he/she first had food with egg in it? ___years___months

4. About your child's skin

Does your child have eczema? YES NO

If so, how old was your child when it first appeared? ___years ___months

Have you used any creams on the eczema ? YES NO

Did your child have an oozing or crusting rash before 1 year of age eg cradle cap, nappy rash?
YES NO

If they did, please describe where it was_____

When did it first appear? _____months

Did you use any creams on the rash?
YES NO

If so, what were they called? _____

Did you ever use any of the following creams on your child's skin before he/she was 1 years old?

Hydrocortisone NO YES Why?_____		Betnovate NO YES Why?_____	
Elocon NO YES Why?_____		Eumovate NO YES Why?_____	
Zinc Cream BP NO YES Why?_____		Zinc & Castor Oil oint NO YES Why?_____	
Oilatum Cream NO YES Why?_____		Hydromol cream NO YES Why?_____	
Polytar Plus NO YES Why?_____		Polytar Shampoo NO YES Why?_____	
Polytar Liquid NO YES Why?_____		Polytar Emollient NO YES Why?_____	
Kamillossan NO YES Why?_____		Arachis Oil NO YES Why?_____	
Diprobase Cream NO YES Why?_____		Oily Calamine Lotion NO YES Why?_____	
Almond Oil NO YES Why?_____		Balneum NO YES Why?_____	
Balneum plus NO YES Why?_____		Calendula Baby Moistr NO YES Why?_____	
Calendula Nappy cream NO YES Why?_____			

Any other creams?

_____

5. About your child's breathing:

Has your child ever wheezed? YES NO

How old was your child when he/she first wheezed? ___years ___months

Has your child ever been diagnosed with bronchiolitis? YES NO

If your child has had bronchiolitis, how old was he/she? ___months

Were you ever told that your child's bronchiolitis was due to the RSV virus? YES NO DON'T KNOW

Has your child been diagnosed with asthma? YES NO

If your child is asthmatic, what medication is he/she on for this? _____

6. About your child's diet:

Was your child ever given soy milk eg Wysoy, Infasoy? YES NO

If so, why was this started? _____

How old was your child when he/she first had soy milk? ___years ___months

How long did your child keep having soya milk for? ___years ___months

7.Was there a cat or dog in your home, when your child was born (please circle):

CAT DOG NEITHER

8. How many people were smoking in your house when your child was first born? _____

How many cigarettes was each person smoking per day? Person 1 ____

Person 2 ____

Person 3 ____

About your child's father

1. Please circle his ethnic background:

Caucasian Black

Asian Indian Asian Chinese

Arabic/Middle Eastern Other_____

What is his country of birth?_____

2. Was he living with you when your child was born? YES NO

3. Please circle any of these conditions that he suffers from:

ASTHMA ECZEMA HAYFEVER

4. Does he have any allergies to food & if so, to what food(s)?NO YES _____

5. What is his main occupation?_____

6. **Try and remember back to when your child was under the age of one**. How many times did your partner eat the foods listed in a normal week and how much each time?

Type of food	**Times eaten in a week**	**Amount eaten each time**
Peanut butter		slices
Snickers		bars
Peanut M&Ms		packs
Whole peanuts		handfuls
Crunchy Nut Cornflakes		bowls
Crunchy Nut Cornflakes Red		bowls
Revels		bars
Tracker Roasted nut		bars
Rowntree's Lion Bar		bars
Cadbury's Star Bar		bars
Cadbury's Fuse		bars
Cadbury's Picnic		bars
Reese's Peanut Butter Cups		cups
Satay sauce		servings
Peanut soup		servings
Bamba snack		packs
Hazelnut spread eg Nutella		slices

Please write down any other sources of peanuts he may have eaten, and how much in a normal week:

_____

_____

Please write down any other nuts or foods containing other nuts that he may have eaten, and how much he ate in a normal week. Examples include walnut, hazelnut, pine nut, brazil nut, pistachios, macademia nuts, almonds and chocolate containing them:

_____

_____

About your child's brothers and sisters

Please list all of your other children's name, sex, age and any conditions they may have:

Name	Sex	Age	Please circle if they have any of these conditions
			ASTHMA ECZEMA HAYFEVER
			ASTHMA ECZEMA HAYFEVER
			ASTHMA ECZEMA HAYFEVER
			ASTHMA ECZEMA HAYFEVER
			ASTHMA ECZEMA HAYFEVER

Did any of your child's **older** brothers or sisters live away from the family home, when your child was born? YES NO

If so, whom?_____

Do any of these children have allergies to any foods? YES NO

If they do, please give details_____

Try and remember back to when your child was under the age of one.
For each of your child's **older** brothers or sisters, please fill in how many times they ate the foods listed in a normal week and how much each time?

SIBLING 1:
Name:

Type of food	Times eaten in a week	Amount eaten each time
Peanut butter		slices
Snickers		bars
Peanut M&Ms		packs
Whole peanuts		handfuls
Crunchy Nut Cornflakes		bowls
Crunchy Nut Cornflakes Red		bowls
Revels		bars
Tracker Roasted nut		bars
Rowntree's Lion Bar		bars
Cadbury's Star Bar		bars
Cadbury's Fuse		bars
Cadbury's Picnic		bars
Reese's Peanut Butter Cups		cups
Satay sauce		servings
Peanut soup		servings
Bamba snack		packs
Hazelnut spread eg Nutella		slices

Please write down any other sources of peanuts they may have eaten, and how much in a normal week:

_____
_____
_____

Please write down any other nuts or foods containing other nuts that they may have eaten, and how much they ate in a normal week. Examples include walnut, hazelnut, pine nut, brazil nut, pistachios, macademia nuts, almonds and chocolate containing them:

_____
_____
_____

SIBLING 2:
Name:

Type of food	Times eaten in a week	Amount eaten each time
Peanut butter		slices
Snickers		bars
Peanut M&Ms		packs
Whole peanuts		handfuls
Crunchy Nut Cornflakes		bowls
Crunchy Nut Cornflakes Red		bowls
Revels		bars
Tracker Roasted nut		bars
Rowntree's Lion Bar		bars
Cadbury's Star Bar		bars
Cadbury's Fuse		bars
Cadbury's Picnic		bars
Reese's Peanut Butter Cups		cups
Satay sauce		servings
Peanut soup		servings
Bamba snack		packs
Hazelnut spread eg Nutella		slices

Please write down any other sources of peanuts they may have eaten, and how much in a normal week:

_____
_____
_____

Please write down any other nuts or foods containing other nuts that they may have eaten, and how much they ate in a normal week. Examples include walnut, hazelnut, pine nut, brazil nut, pistachios, macademia nuts, almonds and chocolate containing them:

_____
_____
_____

SIBLING 3:

Name:

Type of food	Times eaten in a week	Amount eaten each time
Peanut butter		slices
Snickers		bars
Peanut M&Ms		packs
Whole peanuts		handfuls
Crunchy Nut Cornflakes		bowls
Crunchy Nut Cornflakes Red		bowls
Revels		bars
Tracker Roasted nut		bars
Rowntree's Lion Bar		bars
Cadbury's Star Bar		bars
Cadbury's Fuse		bars
Cadbury's Picnic		bars
Reese's Peanut Butter Cups		cups
Satay sauce		servings
Peanut soup		servings
Bamba snack		packs
Hazelnut spread eg Nutella		slices

Please write down any other sources of peanuts they may have eaten, and how much in a normal week:

_____
_____
_____

Please write down any other nuts or foods containing other nuts that they may have eaten, and how much they ate in a normal week. Examples include walnut, hazelnut, pine nut, brazil nut, pistachios, macademia nuts, almonds and chocolate containing them:

_____
_____
_____

If your child has any further older brothers or sisters, please complete their details on a separate sheet of paper.

About other people who lived with you when your child was under the age of one.

This may include grandparents, relatives or lodgers but only if your family home was their main residence.

For each person, please indicate how many portions of the foods listed they ate on an average week **during the time that your child was under the age of one.**

Person 1:
Relationship to affected child:

Type of food	Times eaten in a week	Amount eaten each time
Peanut butter		slices
Snickers		bars
Peanut M&Ms		packs
Whole peanuts		handfuls
Crunchy Nut Cornflakes		bowls
Crunchy Nut Cornflakes Red		bowls
Revels		bars
Tracker Roasted nut		bars
Rowntree's Lion Bar		bars
Cadbury's Star Bar		bars
Cadbury's Fuse		bars
Cadbury's Picnic		bars
Reese's Peanut Butter Cups		cups
Satay sauce		servings
Peanut soup		servings
Bamba snack		packs
Hazelnut spread eg Nutella		slices

Please write down any other sources of peanuts they may have eaten, and how much in a normal week:

_____
_____
_____

Please write down any other nuts or foods containing other nuts that they may have eaten, and how much they ate in a normal week. Examples include walnut, hazelnut, pine nut, brazil nut, pistachios, macademia nuts, almonds and chocolate containing them:

_____
_____
_____

Person 2:

Relationship to affected child:

Type of food	Times eaten in a week	Amount eaten each time
Peanut butter		slices
Snickers		bars
Peanut M&Ms		packs
Whole peanuts		handfuls
Crunchy Nut Cornflakes		bowls
Crunchy Nut Cornflakes Red		bowls
Revels		bars
Tracker Roasted nut		bars
Rowntree's Lion Bar		bars
Cadbury's Star Bar		bars
Cadbury's Fuse		bars
Cadbury's Picnic		bars
Reese's Peanut Butter Cups		cups
Satay sauce		servings
Peanut soup		servings
Bamba snack		packs
Hazelnut spread eg Nutella		slices

Please write down any other sources of peanuts they may have eaten, and how much in a normal week:

_____
_____
_____

Please write down any other nuts or foods containing other nuts that they may have eaten, and how much they ate in a normal week. Examples include walnut, hazelnut, pine nut, brazil nut, pistachios, macademia nuts, almonds and chocolate containing them:

_____
_____
_____

If there were any further people living with you, please complete their details on a separate sheet of paper.

Thank you very much for your time.

Please return the questionnaire to the doctor who gave it to you.

Appendix B

Information for Parents

Patient Information

Environmental Peanut Exposure as a Risk Factor for Peanut Allergy

We would like permission to use information about you and your family as part of our research. This document explains about our study and why we would like to know this information.

1. The aim of the study
To ascertain if increased exposure of a child to tiny amounts of peanut in the home can cause allergy to peanut in later life.

2. Why is the study being done?
Peanut allergy is becoming increasingly common in this country but there is still a lot of debate over its causes. We know that some things, such as eczema, increase the chances of peanut allergy developing. However, unlike most allergies, severe reactions tend to occur on the first occasion that a child has eaten peanut. This suggests that the child has actually had enough contact with peanut in the past to allow them to develop an allergy. It has proved very difficult to show that such contact is coming from breast milk or a mother's diet during pregnancy. We are wondering if peanut in a child's home environment, is causing sensitisation through skin contact.

3. How is the study to be done?
We plan to compare the amount of peanut being eaten in the families of children who developed peanut allergies to the families of children who did not. The reason we have approached you is that it is possible that your child has peanut allergy although we do not yet know if this is the case. We are particularly interested in the foods eaten in your household around the time that the child you have brought to clinic was under the age of one.
This will involve you filling out questionnaires about the eating habits of yourself and your household to allow us to build a picture of how much exposure to peanut could have occurred in your child's infancy.

4. What exactly do you need to do to take part?
Fill out the questionnaire about you and your household's diet. Questions mainly relate to food eaten around the time that the child you have brought to clinic was under the age of one.
This should only take about 20 minutes of your time and is much appreciated. A researcher will be with you to help you fill the questionnaire out.

5. Are there any risks or discomforts?
The only effort is the time taken to fill in the questionnaires. This should be about 10 to 20 minutes. No risk can be foreseen as all the questionnaires are strictly confidential.

6. What are the potential benefits?
This study could potentially give us new insight in to the factors involved in the development of peanut allergy. Once we understand this process better, we can start to devise strategies to combat it.

7. Who will have access to the completed questionnaires?
You will notice a number on the top of your questionnaire. The only people to have access to the list of which child each number relates to, will be the principal researcher (for administrative purposes) and a representative of the St Mary's Hospital Ethics Committee. The study is, thus, strictly confidential and the information obtained will not be used for any purpose other than this study, without your permission.

8. Do I have to take part in the study?
No. If you decide, now or at a later stage, that you do not wish to participate in this research project, that is entirely your right, **and will not in any way prejudice any present or future treatment.**

9. How do I contact the researchers?
The principal researcher is Dr Adam Fox, the current Specialist Registrar in Paediatric Allergy at St Mary's Hospital. The other person involved is Dr Gideon Lack, consultant Paediatric Allergist, who is overseeing the study.
If you have any questions or any difficulties filling out the questionnaires then contact Dr Adam Fox
(020) 7886 6666 bleep 4432 during working hours
07958 409048 mobile
adam.fox2@virgin.net

10. Who do I speak to if problems arise?
If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in any other way, please contact the Chairman of the Research Ethics Committee, by post via the Research and Development Office, St Mary's Hospital, Praed Street, London W2 1NY, or if urgent, by telephone on 020 7886 6666 and the Committee administration will put you in contact with him.

Many thanks for all your help

Appendix C

Further Work

Further Work

After initial exposure to any food, the immune system can respond in a number of ways. In most cases, tolerance results. In a minority of cases, especially in atopic children, the exposure leads to the deviant response of allergy. It remains unclear how the route by which antigen exposure occurs influences the underlying immune response.

In the introductory section of this report, we outlined much of the current evidence to support the pivotal role that environmental allergen exposure plays in sensitization to peanut. The work we have outlined in this report considerably strengthens this evidence. It is also worth noting that there is also much evidence with regards to the development of tolerance as a result of early oral exposure. This has also been touched on in the discussion of the Additional Results above.

To recap, mouse models have shown that oral tolerance induction using high doses of food antigens, including peanut, can prevent subsequent allergic sensitisation to these foods[105]. Whilst there is no such direct evidence of oral tolerance induction in man, it has been observed in countries where allergenic foods are included early in the infant diet (Israel, Southern Africa and China), that food allergies are less prevalent[32]. Furthermore, sensitisation to Nickel, which occurs by the cutaneous route, is less common amongst those who have had oral exposure to Ni in teeth braces[15], suggesting that oral tolerance induction prevents later cutaneous sensitisation.

Despite the evidence outlined above, it is only with closer observation of the immune response itself that a greater understanding of the relative importance of difference routes of antigen exposure will be obtained.

<u>Background to proposed project</u>

To date, the mechanisms directing 'trafficking' cells have only scarcely been explored in food allergy but events potentially responsible for directing T cells toward allergy sites may be extrapolated from experimental works[107]. Memory T lymphocytes express homing receptors on their surface that reflect the site where they were initially sensitized. The presence of these homing receptors facilitates the circulation of the T cell, guided by counterpart vascular

addressins, through lymph nodes, lymphatics and the blood before returning to the tissue where it is most likely to encounter the allergen again. There are two homing receptors of particular interest for this project - the Cutaneous Lymphocyte-associated antigen (CLA) - a skin homing receptor, the expression of which implies sensitization in the skin and $\alpha4\beta7$ integrin - a gut homing receptor, the expression of which implies sensitization in the gut.

Cutaneous Lymphocyte-associated antigen (CLA) - a skin homing receptor

In peanut allergic patients, allergen specific T cells to peanut have been discovered in the skin[26]. In one case report, a previously non-allergic patient suffered anaphylaxis to peanut after receiving a liver and kidney transplant from a peanut allergic donor[27]. Chimerism was only detected in the skin of the recipient suggesting that allergen specific T cells had a homing commitment to the site of sensitization. This homing commitment is thought to be due to the expression on cutaneous lymphocyte antigen (CLA) on the surface of the T cells. In contact dermatitis due to Nickel allergy there is a clear correlation between increased skin exposure and increased likelihood of sensitization[15]. In patients allergic to nickel, the Ni-dependent memory T cell response is largely confined to T cells that express CLA[108], reflecting the initial route of sensitization. Such Ni-sensitive individuals also have a rise in serum IL-5 levels after challenge with Ni, suggesting a Th2 skewed immune response[109]. A similar restriction of the T cell proliferative response to the CLA+ memory subset is observed for House Dust Mite (HDM) in patients with atopic dermatitis (AD).

$\alpha4\beta7$ integrin - a gut homing receptor

Initially, $\alpha4\beta7$ was described as an integrin expressed in activated T cells with homing properties to the gut[110].

Further evidence that $\alpha4\beta7$ expression reflects sensitization in the gut came from Kantele et al[111] who immunized human volunteers with KLH (Keyhole Limpet Hemocyanin) first orally and then parenterally. Expression of $\alpha4\beta7$ integrin on T cells specific for KLH were compared to those who

received only parenteral KLH immunization. A significantly higher proportion of the mucosally primed T cells compared to parenterally primed cells were found to express $\alpha4\beta7$ integrin, demonstrating that expression of homing receptors depends of the site of antigen encounter. Similarly, Rott et al[112] demonstrated rotavirus-induced proliferation of $\alpha4\beta7$+ T cells from rotavirus-sensitized individuals, while the same cells proliferated only weakly to mumps antigen, which is first encountered following intramuscular administration.

Using this knowledge of homing receptors, this proposed project would investigate the in vitro evidence that sensitization to peanut occurs via the cutaneous route whereas tolerance is induced by gut exposure to peanut. If sensitization occurs in the skin, then this will be reflected in the homing receptor profile of peanut specific memory T cells. We know from our previous work that peanut specific T cells responses in allergic children are characterized by a Th2 pattern of cytokines. We thus hypothesize that immune responses to peanut in the allergic child will occur predominantly in skin homing (CLA+) lymphocytes with a Th2 phenotype. If tolerance to peanut is the result of high dose exposure through the gut, then again the homing receptor profile of peanut specific memory T cells will reflect the site of initial encounter. Thus we further hypothesize that in non allergic children tolerogenic Th1/regulatory responses to peanut will be found predominantly in the gut homing, $\alpha4\beta7$+ T cells.

Hypothesis 1:

Allergic sensitization to peanut occurs through the skin, leading to Th2 responses and peanut allergy.

Specific Aims:

a) To measure and compare proliferative T cell responses to peanut in peanut allergic children specifically amongst the Cutaneous

Lymphocyte-associated Antigen (CLA) expressing (skin homing) and $\alpha 4\beta 7$ integrin expressing (gut homing) memory T cells subpopulations.

b) To study the functional phenotype of peanut specific T cells in peanut allergic children.

Expected Findings:

a) Proliferative T cell responses to peanut in peanut allergic children will be greater in Cutaneous Lymphocyte-associated Antigen (CLA) + than in $\alpha 4\beta 7$ integrin + memory T cells.

b) The functional phenotype of peanut specific T cells will be different in the CLA+ population of peanut allergic individuals with a skew towards Th2 cytokine production.

Hypothesis 2:

Early exposure to peanut protein through the gut leads to regulatory and Th1 responses to peanut and oral tolerance.

Specific Aims:

a) To measure and compare proliferative T cell responses to peanut in peanut tolerant children specifically amongst the Cutaneous Lymphocyte-associated Antigen (CLA) expressing (skin homing) and $\alpha 4\beta 7$ integrin expressing (gut homing) memory T cells subpopulations.

b) To study the functional phenotype of peanut specific T cells in peanut tolerant children.

Expected Findings:

a) Proliferative T cell responses to peanut in peanut tolerant children will be greater in $\alpha 4\beta 7$ integrin + than in Cutaneous Lymphocyte-associated Antigen (CLA) + memory T cells.

b) The functional phenotype of peanut specific T cells in non allergic children will be characterized by both a Th1 response and regulatory T cell responses.

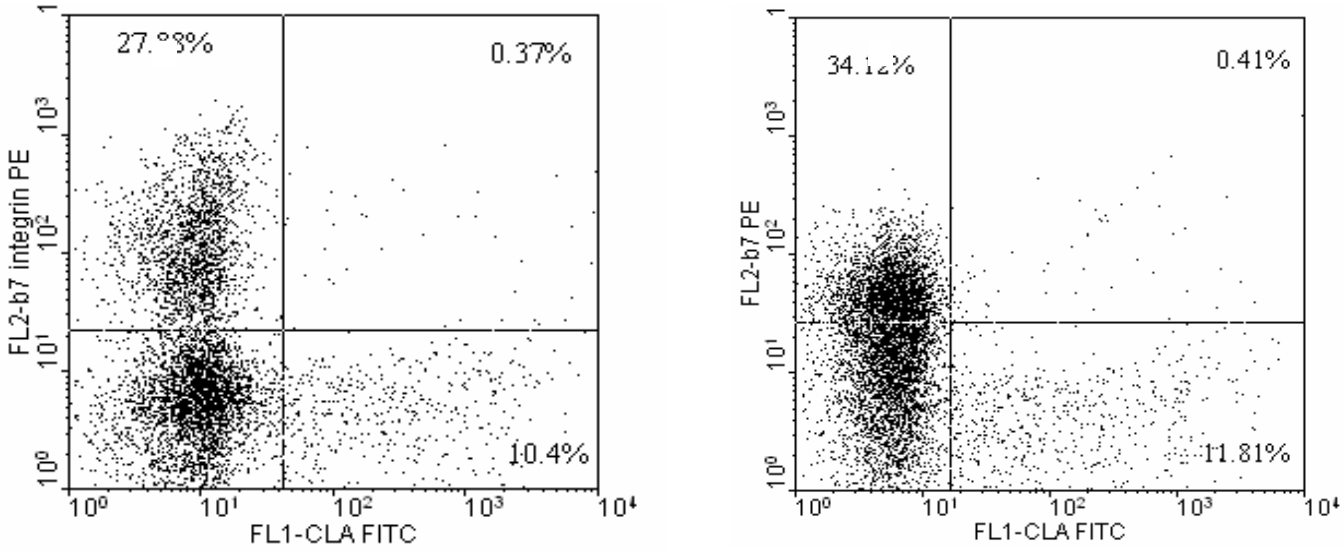
Preliminary Studies

Our group has been investigating the evidence for environmental allergen exposure as the route of sensitization in peanut allergy as well as the possible role of high dose oral exposure in tolerance induction. We have been able to show that exposure to peanut containing skin creams[6] or high levels of peanut in the home environment during infancy are risk factor for the development of PA[113]. Conversely, low levels of environmental peanut during infancy appear to protect against PA even amongst children at high risk (those with egg allergy). Furthermore, we have preliminary data demonstrating that early oral exposure to peanut is associated with a decreased prevalence of PA. An explanation for the findings in our work is that sensitization to peanut is occurring as a result of environmental exposure in the absence of early high dose oral exposure, which induces oral tolerance. As described above, it is known that the initial site of sensitization can be identified by the presence of homing receptors (CLA, $\alpha 4\beta 7$) expressed on the surface of T cells. Therefore the investigation of homing receptor expression on peanut specific T cells from peanut allergic and peanut tolerant children will provide an important clue regarding the site of initial allergic sensitization in PA.

Whilst work has been done to characterize the lymphocyte responses to peanut in normal, peanut allergic, and allergic children who acquire tolerance to peanut, there has been little work looking specifically at lymphocyte trafficking in peanut allergy. Investigating immune responses against foods is hindered by the fact that circulating food antigen–specific lymphocytes are very rare. In a novel approach we used carboxyfluorescein succinimidyl ester (CFSE) to identify peanut-specific lymphocytes by flow cytometry. We confirmed that these cells are indeed peanut specific by cloning. Peanut-

allergic donors showed Th2 polarization of cytokine production by peanut-specific cells, whilst non allergic children had a Th1 skew[114].

We have experience in our laboratory of separation of RO/RA as well as CLA+ and $\alpha 4\beta 7$ subpopulations of T cells. We have observed from this work that approximately 10-15% of circulating T helper cells are CLA+ in children (figure 60), allowing us to isolate this population and assess their response to peanut.



Peanut allergic donor, M, 9yrs old

Peanut non-allergic donor, M, 11yrs old

Fig 61 Percentages of CLA+ and β7 integrin cells amongst PBMC

The dot plots in Figure 61 also demonstrate that expression of CLA and $\beta 7$ integrin is mutually exclusive, with very few double positive cells. This should allow the isolation of highly pure cell subsets.

We had attempted to look at homing receptor characteristics of peanut specific T cells in vitro using CFSE, comparing the expression of homing receptors in peanut allergic and peanut tolerant subjects. This was unsuccessful as culture conditions and antigen stimulation altered the CLA and $\alpha 4\beta 7$ expression. A different approach is thus required.

However, in our preliminary work, we were able to demonstrate separation of T cell subsets from a non allergic donor using magnetic beads and were able to achieve relatively high levels of purification. Peripheral blood mononuclear cells (PBMC) were isolated from blood, incubated with the appropriate magnetic beads bound to monoclonal antibodies and subsets were separated

by passing the cells through a capture column placed in a magnetic field (figure 62).

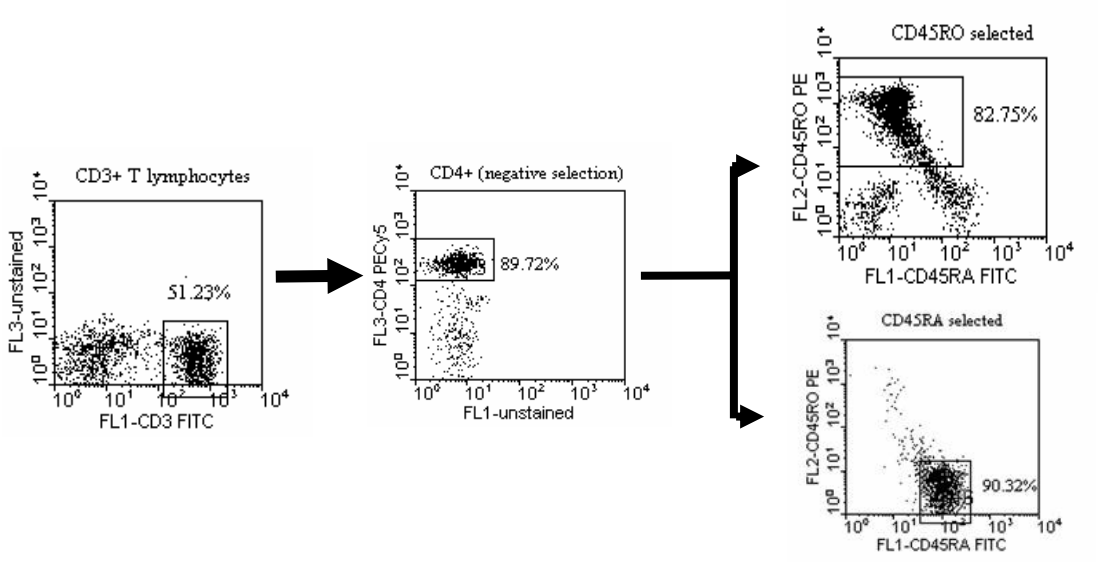


Fig 62 Separation of memory and naïve CD4+ T cell subsets using magnetic beads.

Experimental Design and Methods

On the basis of the specific aims outlined above, the project can be broken down into a 6 discrete tasks.

1) Phenotypic characterization of potential recruits

2) Patient recruitment and donation of blood

3) Separation of PBMC into CLA+ & $\alpha 4\beta 7$+ memory T cell subsets

4) Measurement of proliferation of specific T cells to peanut and to control antigen (ovalbumin)

5) Assessment of cytokine production by the CLA+ & $\alpha 4\beta 7$+ memory T cell subsets cultured in the presence of peanut or control antigen

6) Analysis and interpretation of data

1) Phenotypic characterization of potential recruits

Our projects requires the recruitment of 2 groups of children: those who are allergic to peanuts (PA), and those who are not allergic to peanut (NA). We will also aim to recruit a number of children who are known to have previously

been allergic to peanut but have since outgrown this allergy. It is difficult to predict what we will find in this fascinating group of outgrowers (OG).
In both PA and NA recruits, we will also add an inclusion criteria relating to egg allergy. In order to ensure that any differences seen in peak proliferation in the different T cell subpopulations is specific to peanut, we require the use of a control antigen. We will use ovalbumin for this purpose and thus require children who are tolerant to egg. The use of this control will ensure that any increase in response amongst CLA+ T cells to peanut, if it is absent to ovalbumin, cannot simply be put down the effect of eczema on the homing receptor profile of allergen specific cells.

2) Patient recruitment and donation of blood

Patients from our Paediatric Allergy clinic population who fulfil the inclusion criteria for any of the groups above, will be approached and asked to donate a sample of blood. The Paediatric Allergy service at St Mary's Hospital is a tertiary referral centre for West London and surrounds. With up to 8 clinics per week, over 750 new patients, as well as many more follow up patients, are seen each year. A register of children with PA is already in place, facilitating patient identification. Many of our patients have regular bloods tests for measurement of specific IgE and extra blood for this project, once informed consent has been obtained, can be drawn at the same time.
Clinical assessment of peanut allergy status (PA, NA and OG), recruitment of donors, obtaining the informed consent for the participation in the study and collecting blood will be done by research staff according to the established diagnostic criteria above and the guidelines of the Local Research Ethics Committee. Venous blood will be anti-coagulated using citrate dextrose solution. Peripheral blood mononuclear cells (PBMC) separation from the peripheral blood of PA, NA and OG donors will be done by centrifugation over a density gradient (Histopaque).

3) Separation of PBMC into CLA+ & $\alpha 4\beta 7$+ memory T cell subsets

As in our preliminary work, CD4+ T helper cells will be negatively separated using the CD4+ T Cell Negative Isolation Kit II (from Miltenyi Biotech, Bisley, UK). Negative separation of memory T helper lymphocytes will be done using CD45RA MicroBeads (Miltenyi Biotec). We will then separate this memory T cell subset into CLA+ and CLA- using FITC-labelled anti-CLA antibody (clone HECA-452) (BD Biosciences) and Anti-FITC Microbeads (Miltenyi Biotech). Furthermore, $\beta 7$ integrin expressing T cells will be purified from amongst the CLA- T cell population, using PE-labelled anti-$\beta 7$ integrin (clone FIB504) and Anti-PE Microbeads (Miltenyi Biotech). Briefly, PBMC will be incubated with MACS beads, then unbound beads will be washed and the cells that bound beads will be captured on a column placed in a strong magnetic field while the cells that are negative for the respective marker will not be retained on the column. This procedure allows for both positive and negative selection of T lymphocyte subsets reaching 90-95% purity.

We expect to start with 100 million PBMC from 50ml of blood. Given our previous experiments on purification of subsets, we expect to obtain at least 1 million CLA+ memory T cells and at least as many $\alpha 4\beta 7$+ memory T cells.

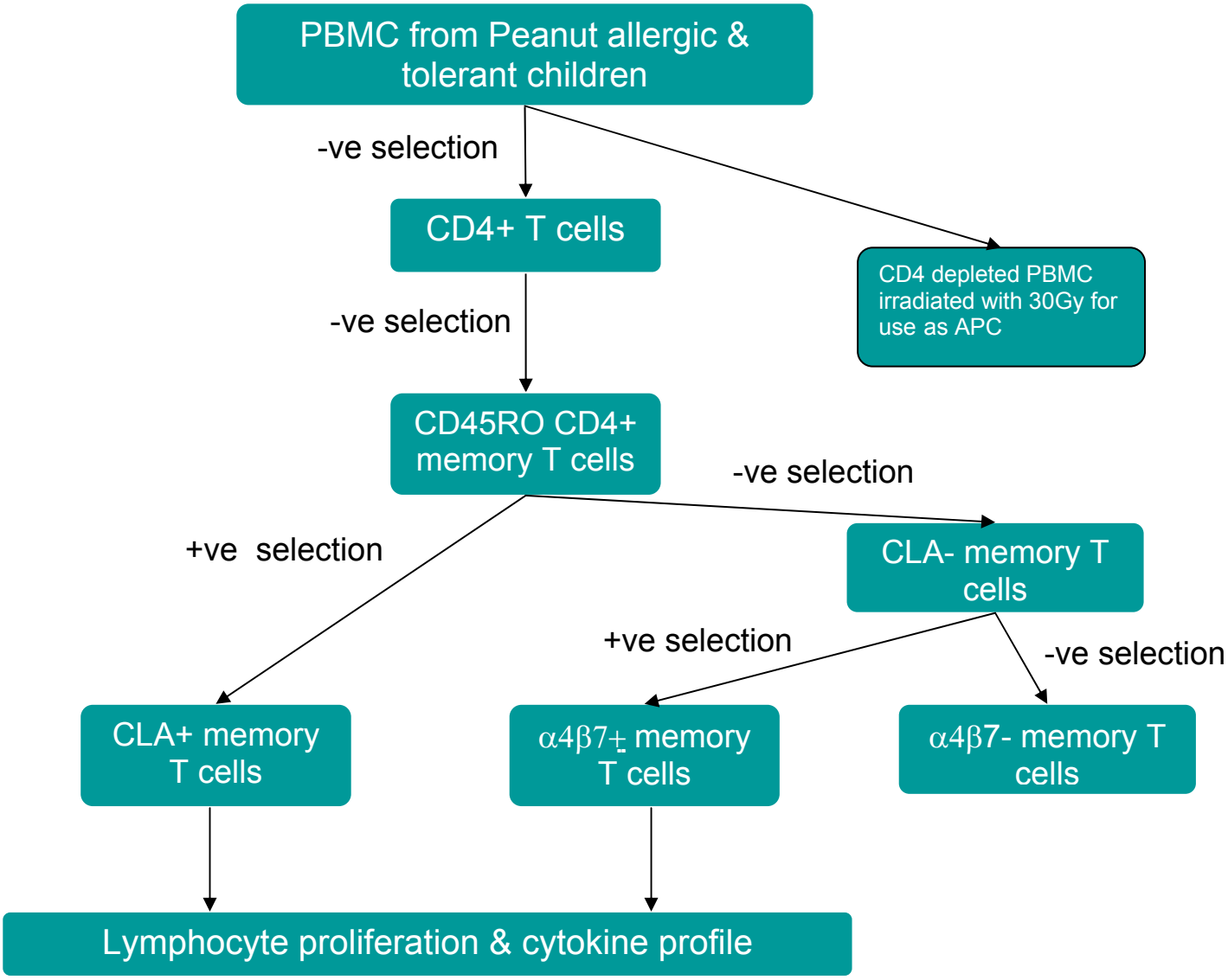


Figure 63: Experimental design

4) Measurement of proliferation of specific T cells to peanut and to control antigen (ovalbumin)

The purified CLA+ and α4β7+ subsets will be stimulated with whole peanut extract (ALK) (at a final concentration of 100 micrograms / ml) in the presence of irradiated PBMCs as antigen presenting cells. Similarly, part of the the purified subsets will also be stimulated with ovalbumin, as a control antigen. Cultures will be set in RPMI medium supplemented with 5% autologous plasma. Peanut-specific PBMC proliferation will be determined by measuring [3H]-methyl thymidine incorporation into DNA during cell division. Briefly, at different time points (days 3, 5 and 7) after setting the PBMC cultures, [3H]-methyl thymidine (0.5 microCi/well) will be added to 100 microlitres cell

aliquots and [3H]-methyl thymidine incorporation into DNA will be measured after a 6h-incubation period. Stimulation indices to peanut in the CLA+cells will be compared to those in the $\alpha4\beta7$cells in both peanut allergic and peanut tolerant children. If the in vitro peanut-specific response (proliferation) of peanut allergic individuals occurs predominantly in the CLA+ skin homing subset while the response in non-allergic individuals occurs in the $\alpha4\beta7$subset, this would be strongly suggestive that it is indeed the cutaneous route of sensitization that is involved in the pathogenesis of PA and the oral route involved in tolerance.

5) Assessment of cytokine production by the CLA+ & a4b7+ memory T cell subsets cultured in the presence of peanut or control antigen

Cytokine production (including IL4, IL5, IFN-γ, TNF-α, IL10 and TGF-β) by the CLA / $\beta7$ integrin positive memory T helper lymphocyte subsets cultured in the presence of peanut antigens will be assessed using cytobeads. Briefly, cell culture supernatant will be collected at different time points and incubated with cytobeads in order to determine the peanut-specific cytokine production phenotype of the isolated T cell subsets. Given our knowledge that the peanut specific T cell response in children with PA is Th2 skewed[55], if this same response is shown to be arising from the CLA+ subset, and not from the $\alpha4\beta7$subset, then this would support our hypothesis of initial cutaneous sensitization. Conversely, in peanut tolerant individuals, if the T cell responses in the $\alpha4\beta7$ population are characterized by production of IL10 and TGF$-\beta$ this will strongly suggest that regulatory T cells are involved in oral tolerance induction in the human.

6) Analysis and interpretation of data

Relative peak proliferation in the CLA+ and $\alpha4\beta7$ T cell subsets for each individual case will be expressed as a ratio ([CLA+]/[$\alpha4\beta7$]). The ratios from children in the PA group will then be compared to those from the NA group. We intend to take logarithmic transformation of PSI-5 (proliferation stimulation

index to peanut on day 5) because of the skewness of its values among peanut allergic children and non-peanut allergic children. We expect that the data will not be normally distributed and will thus use a non parametric test for unpaired data (Wilcoxon rank sum test).

Peanut/egg specific cytokine production in T cell subsets of each donor will be measured using cytobeads and expressed as quantity of each cytokine produced in response to the respective antigen (picograms/million cells). We will compare production of different cytokines from the subsets of individual patients. We expect again, that the data will not be normally distributed and will thus use a non parametric test for unpaired data (Wilcoxon rank sum test). Differences between peak proliferation indices and cytokine production to peanut in the different T cell subsets will then be interpreted in the light of our working hypotheses. We anticipate a requirement of 15 cases in each group. This estimation of the appropriate sample size for the study was made by examining preliminary data produced by our research group including proliferation and cytokine production in children aged between 6 and 16 who are either peanut allergic or non-allergic. We detected statistically significant differences in log(PSI-5) proliferation (unpublished data) and cytokine production[55] between PBMCs isolated from 9 peanut allergic and 9 non allergic subjects. We assume that when we measure proliferation in highly purified T cell subsets, these differences will be similar or more apparent. Therefore, a conservative estimate would suggest that we would need to investigate a similar number of patients. Statistical support will be provided by the Imperial College Statistical Advisory Service.

Ethical Considerations: Ethical approval has been obtained for this project through St Mary's NHS Trust Local Research Ethics Committee. Reference 02.92.

Timetable:

The total running time for the project would be 1 year. This will include 3 phases:

1) Preparation of assays and optimization of technical performance (4 weeks).

2) Recruitment of patients and performing of experiments as outlined above, which will be carried out concurrently (40 weeks). This will require no more than 1 recruit per week to complete the project.
3) Analysis and interpretation of data (8 weeks).