FINAL TECHNICAL REPORT

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

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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.

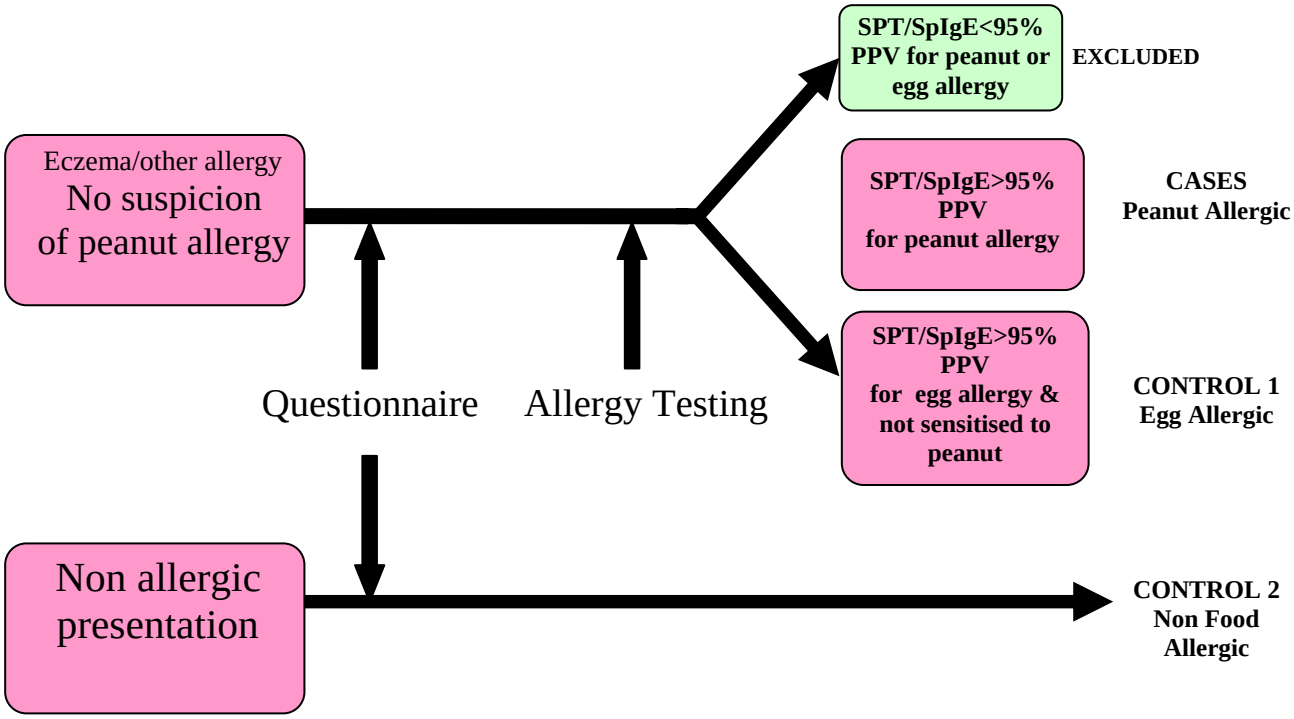

Figure 1 Study Design

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

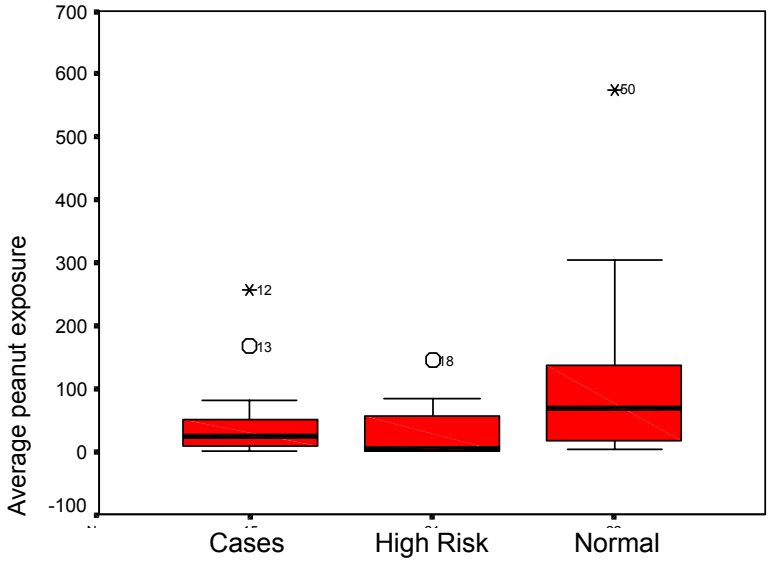

Figure 3: Average weekly household peanut consumption

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

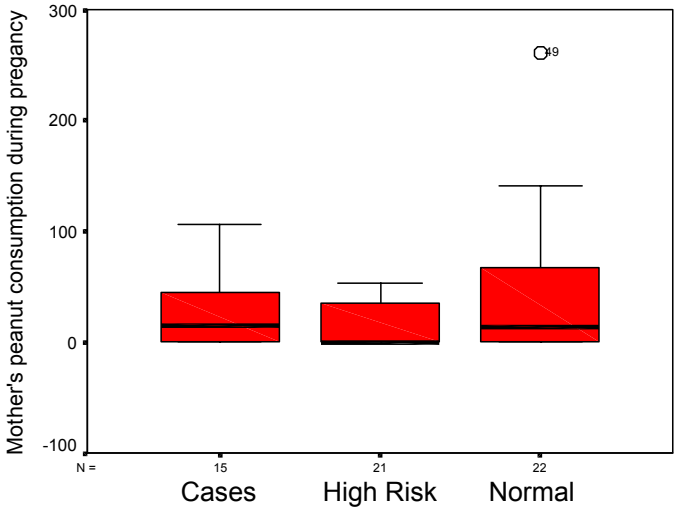

Figure 4: Average weekly maternal peanut consumption during pregnancy.

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

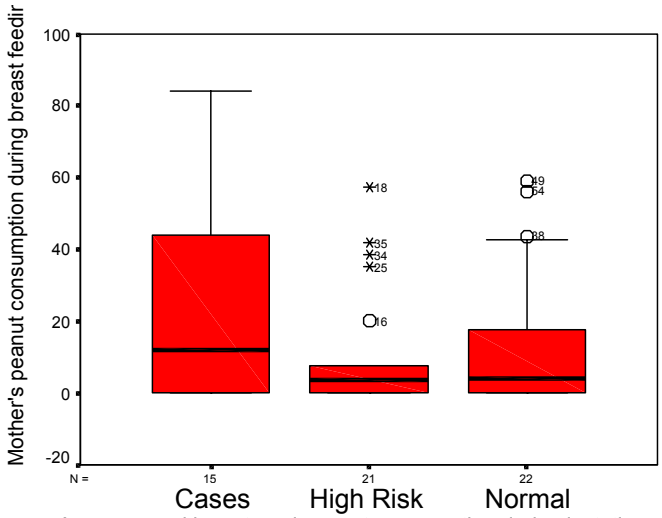

Figure 5: Average weekly maternal peanut consumption during lactation

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.

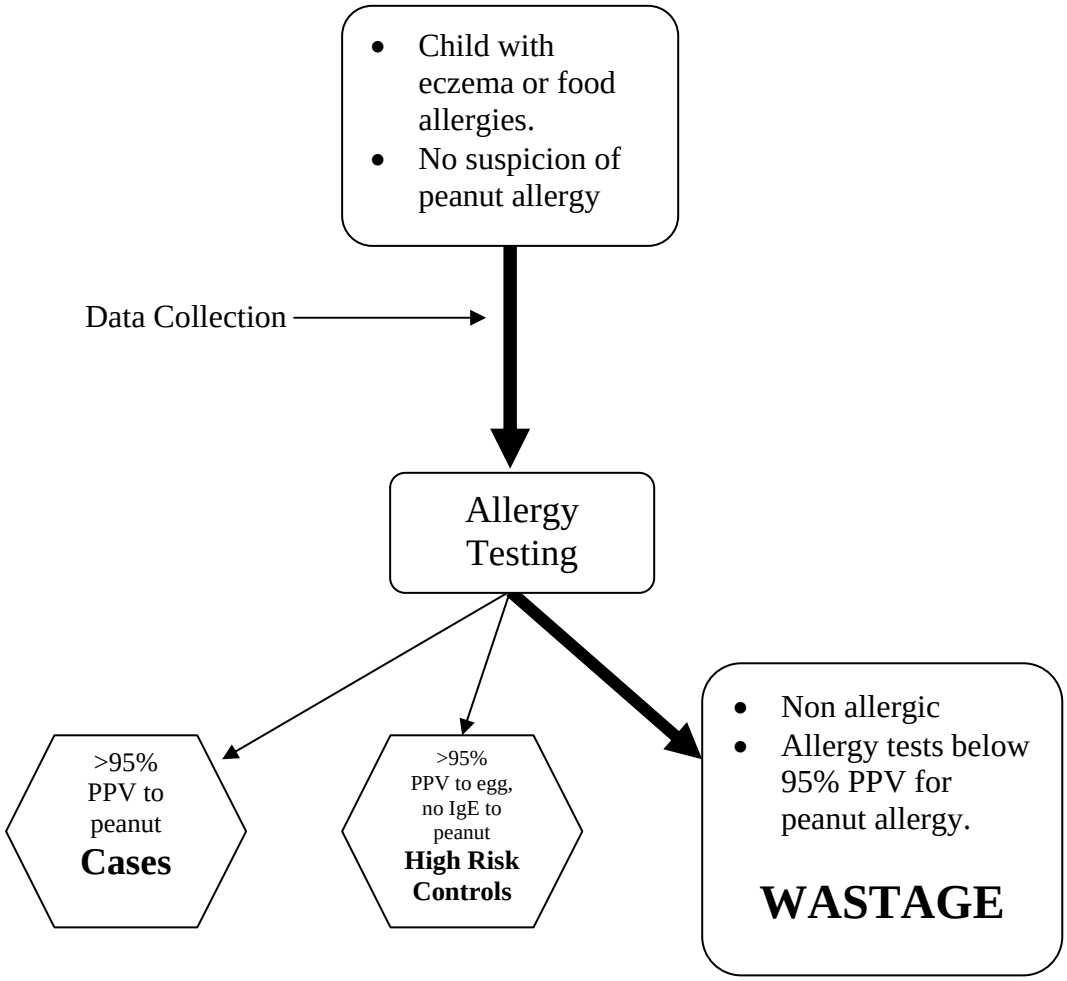

Figure 6: Study Recruitment algorithm

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.

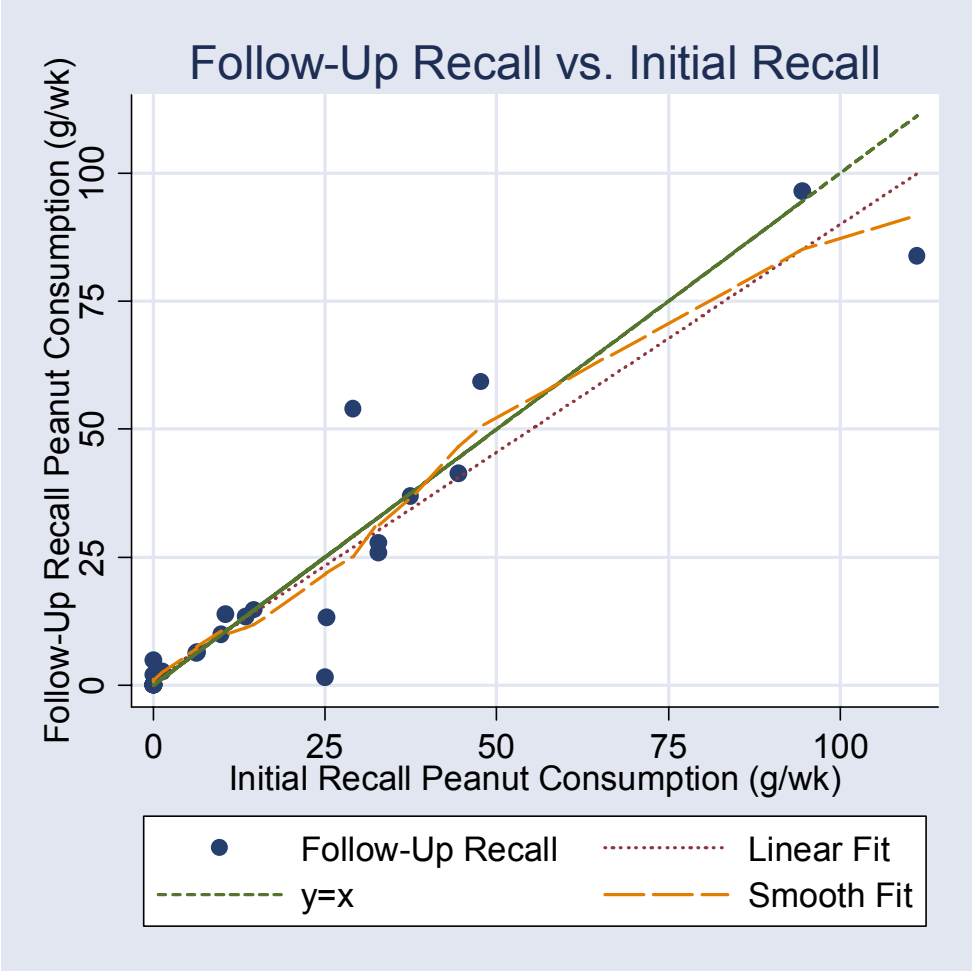

Figure 6 Graph of initial recall against follow up recall

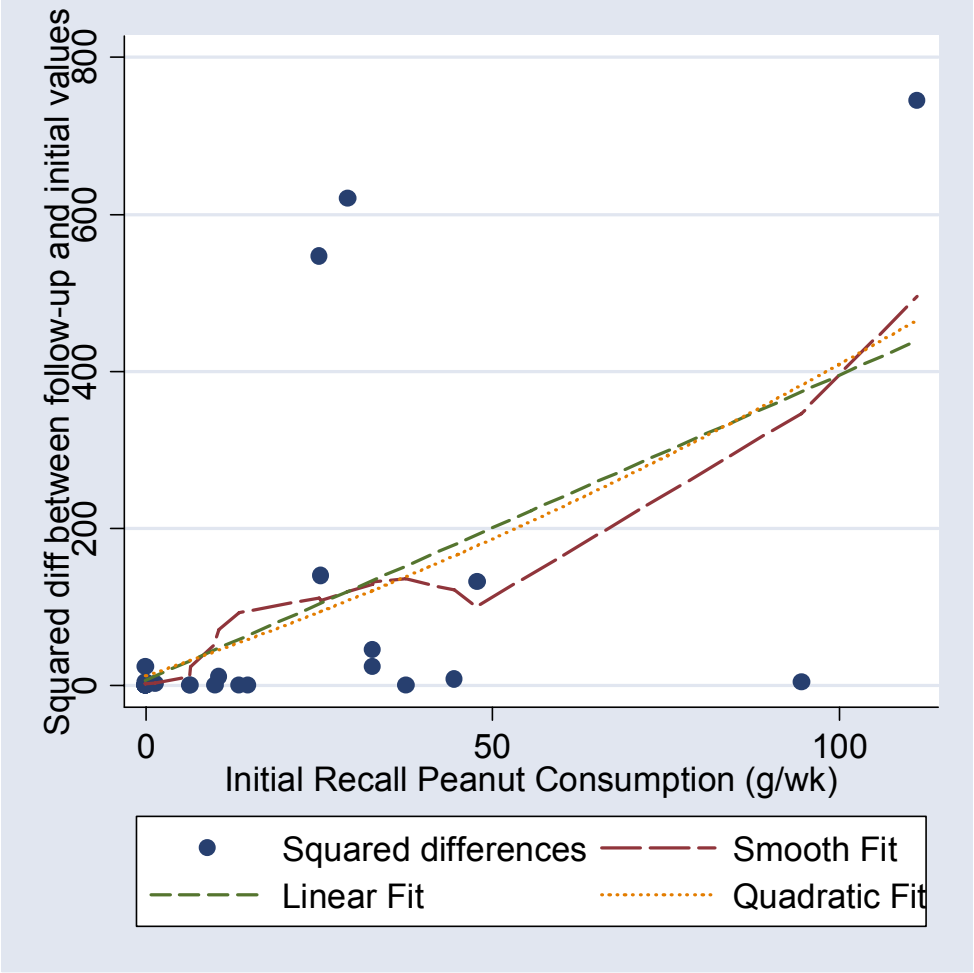

Figure 7 Graph of squared difference between the follow-up and initial values plotted against the initial recall values

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.

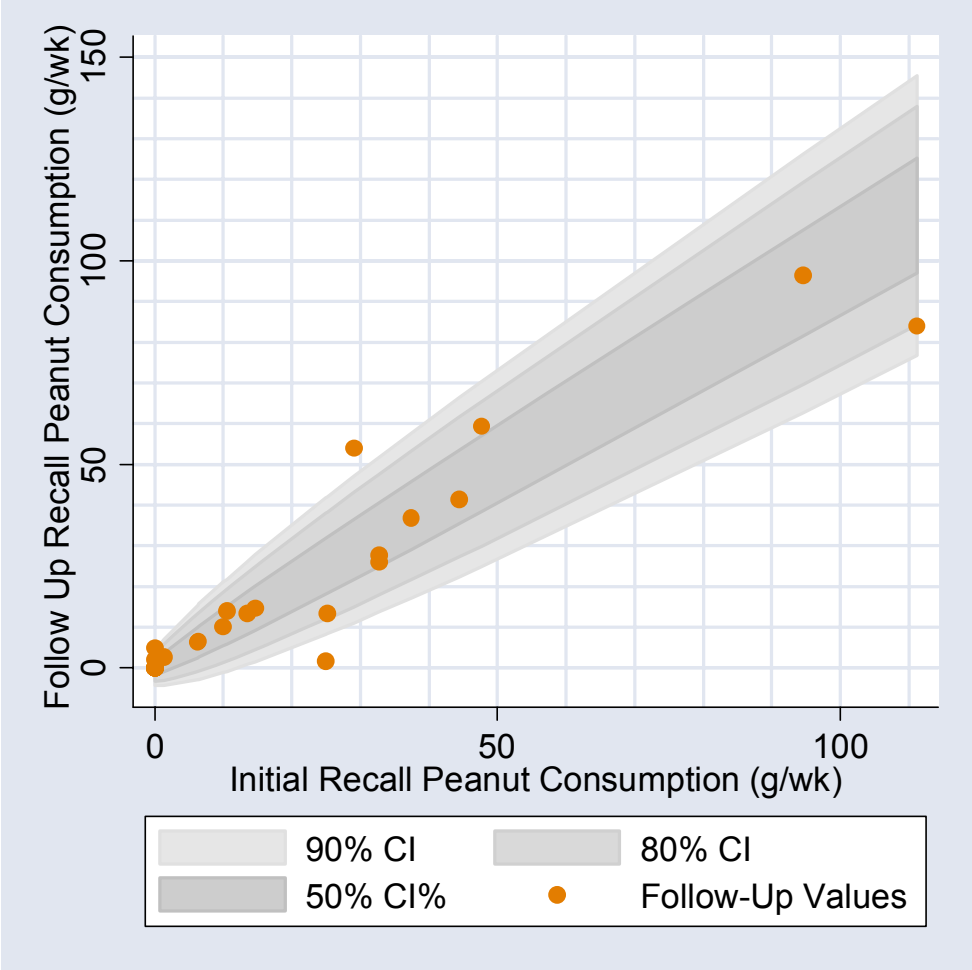

Figure 8 Confidence intervals for prediction of initial recall value from follow up recall value

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%)

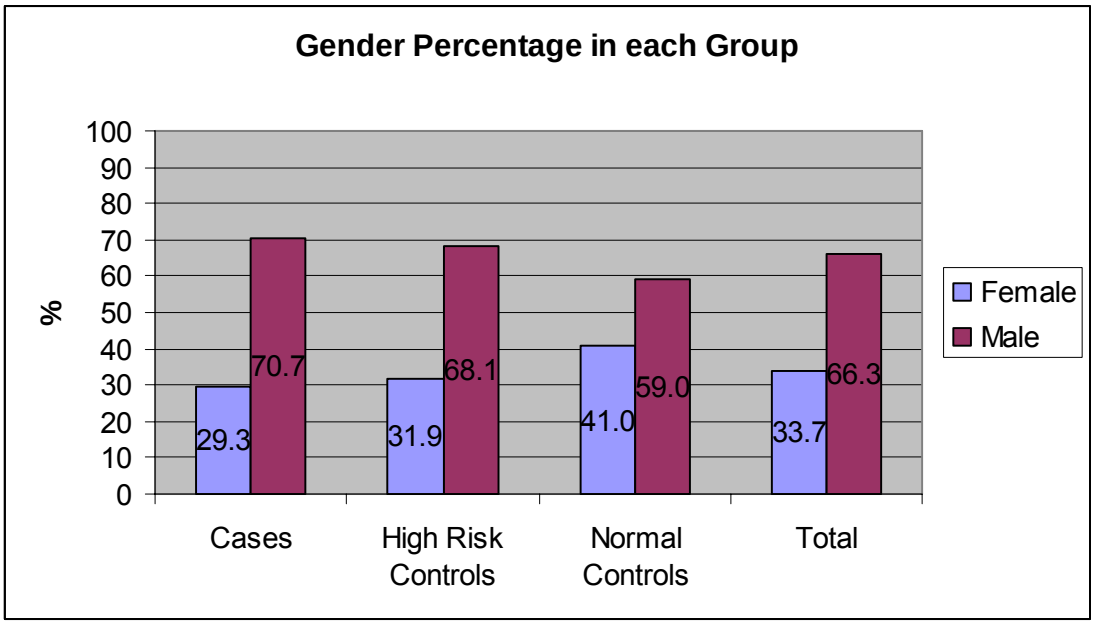

Figure 9: Proportion of males and females in each group

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%)

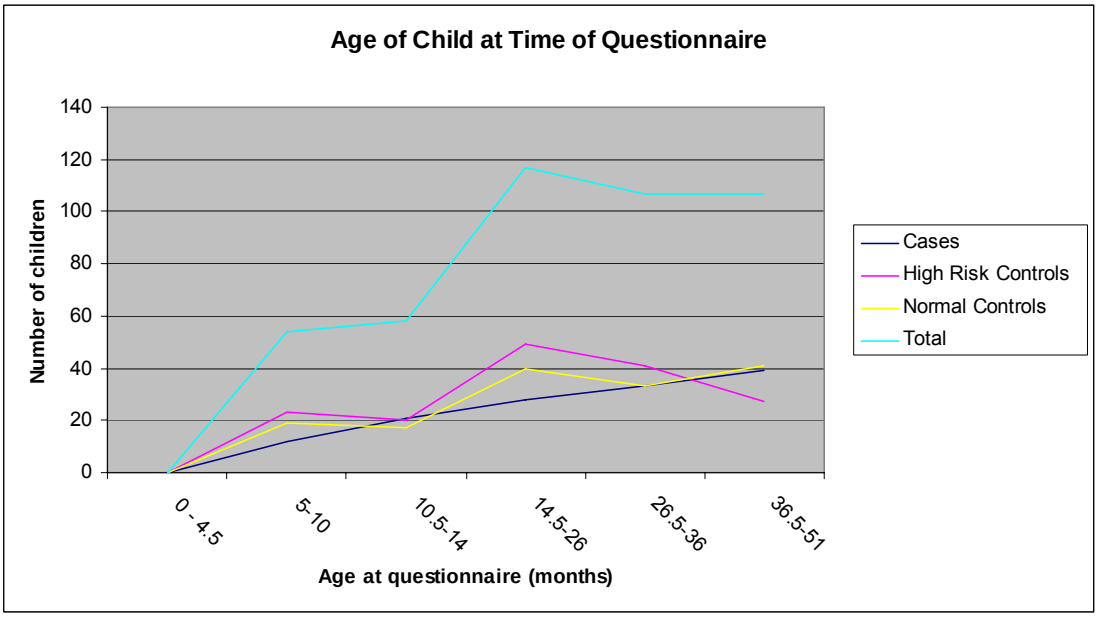

Figure 10: Age of child at time of questionnaire

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

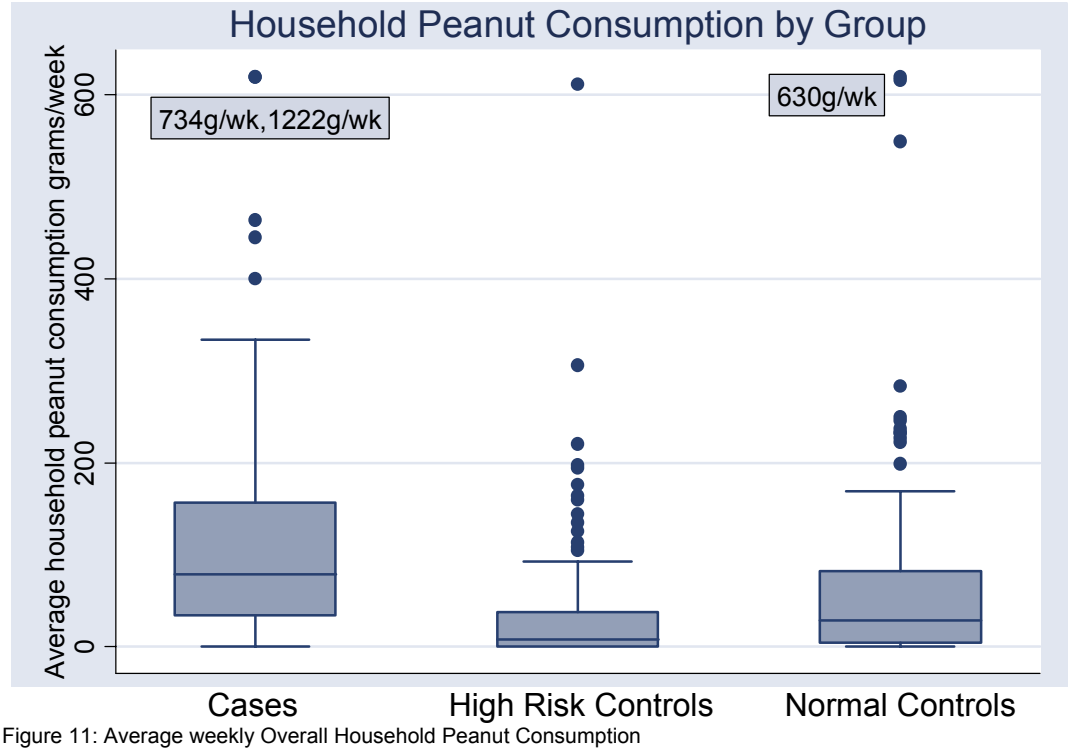

Figure 11: Average weekly Overall Household Peanut Consumption

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

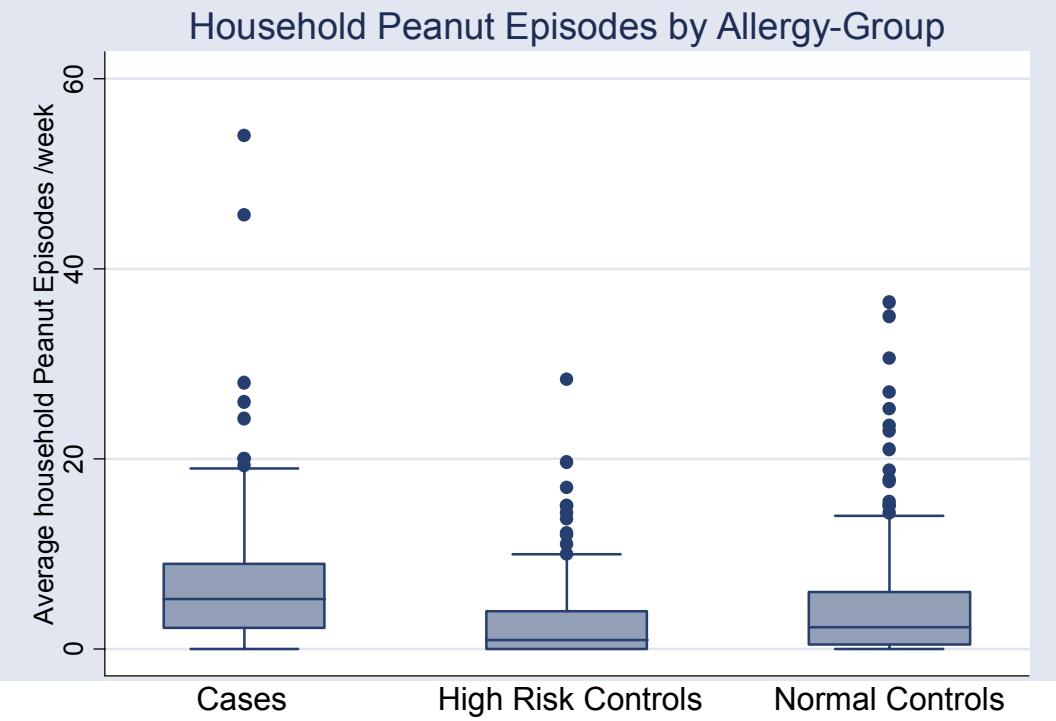

Figure 12: Average weekly Overall Household Episodes of Peanut Consumption

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

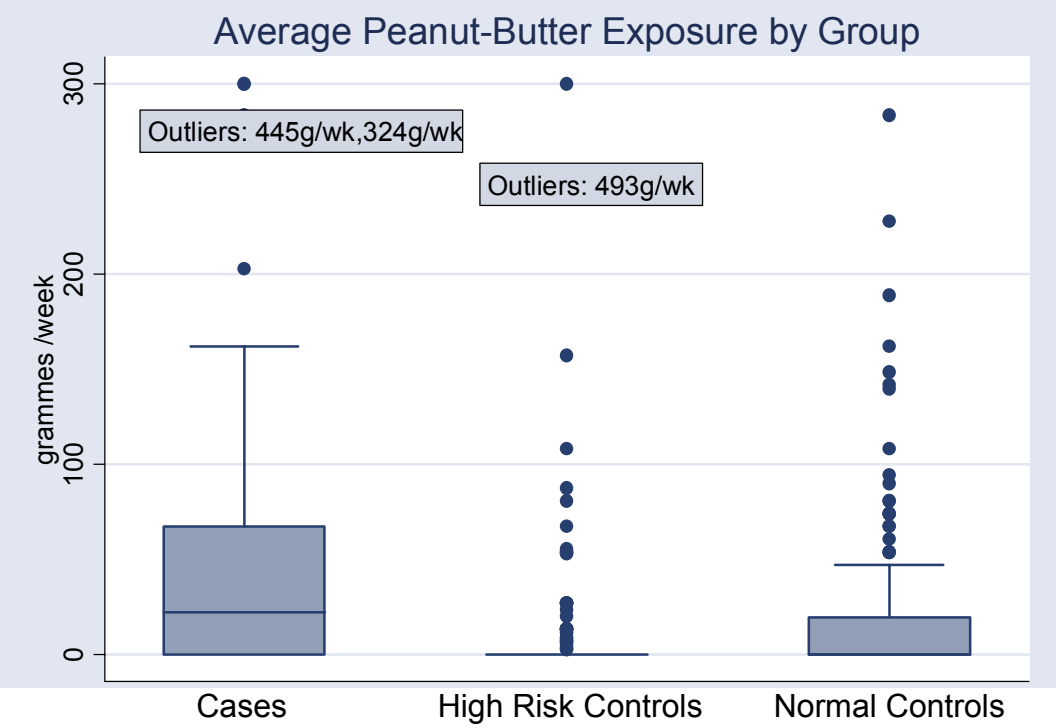

Figure 13: Average weekly Overall Household Peanut Butter Consumption

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

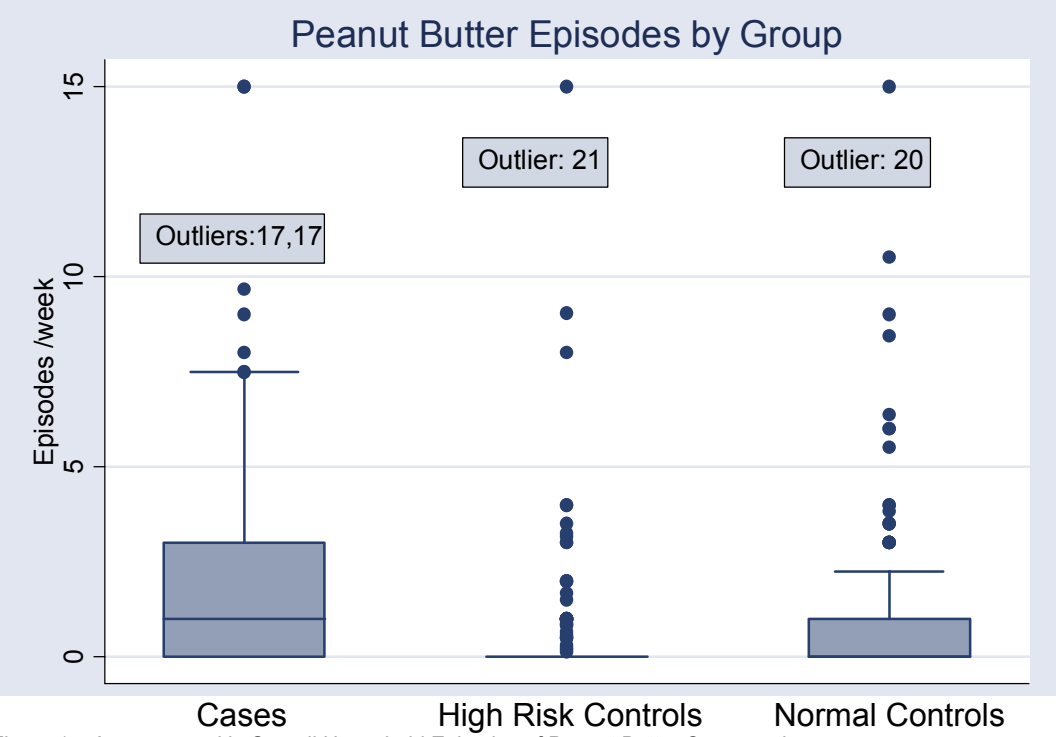

Figure 14: Average weekly Overall Household Episodes of Peanut Butter Consumption

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.

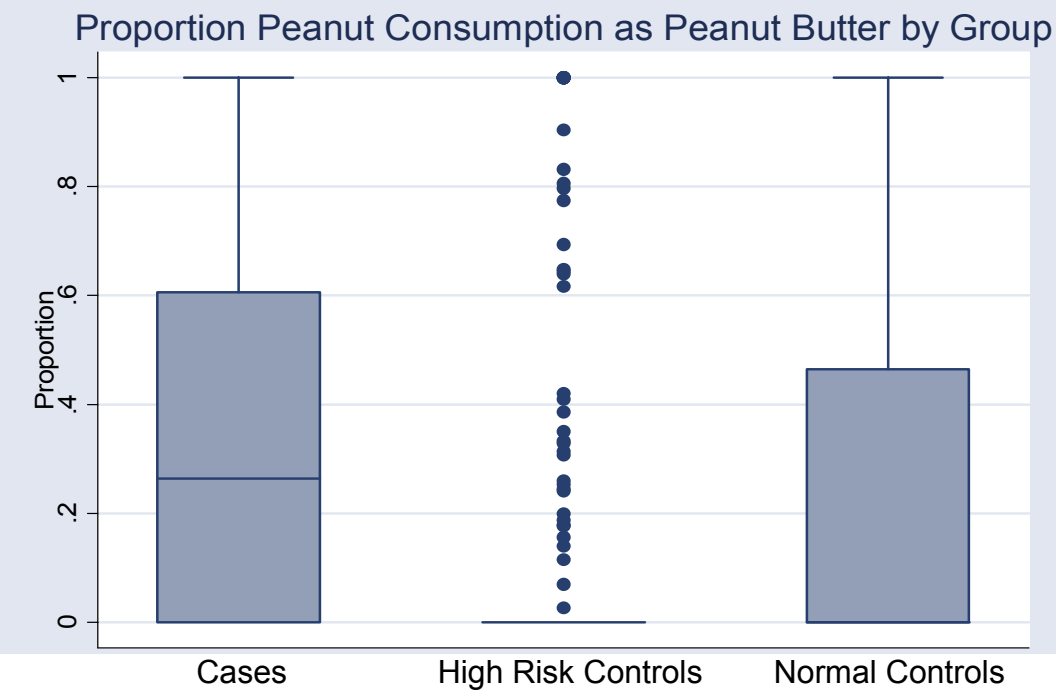

Figure 15: Proportion of Household Peanut Consumption in form of Peanut Butter

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

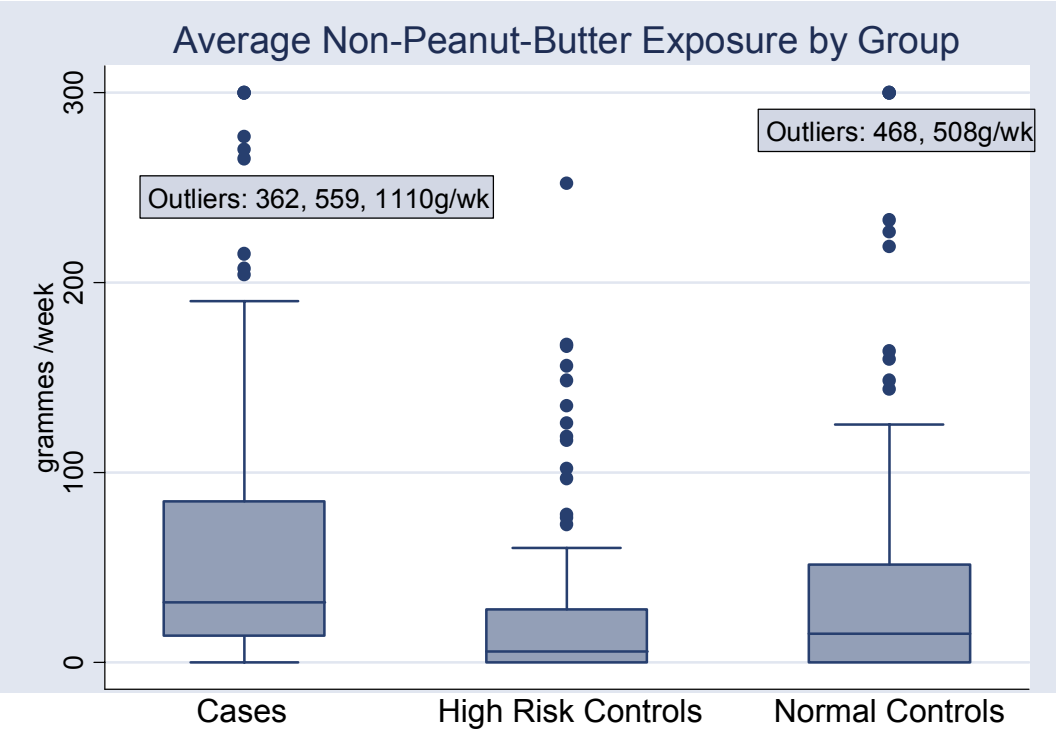

Figure 16: Average weekly Overall Household Consumption of Peanut excluding Peanut Butter

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

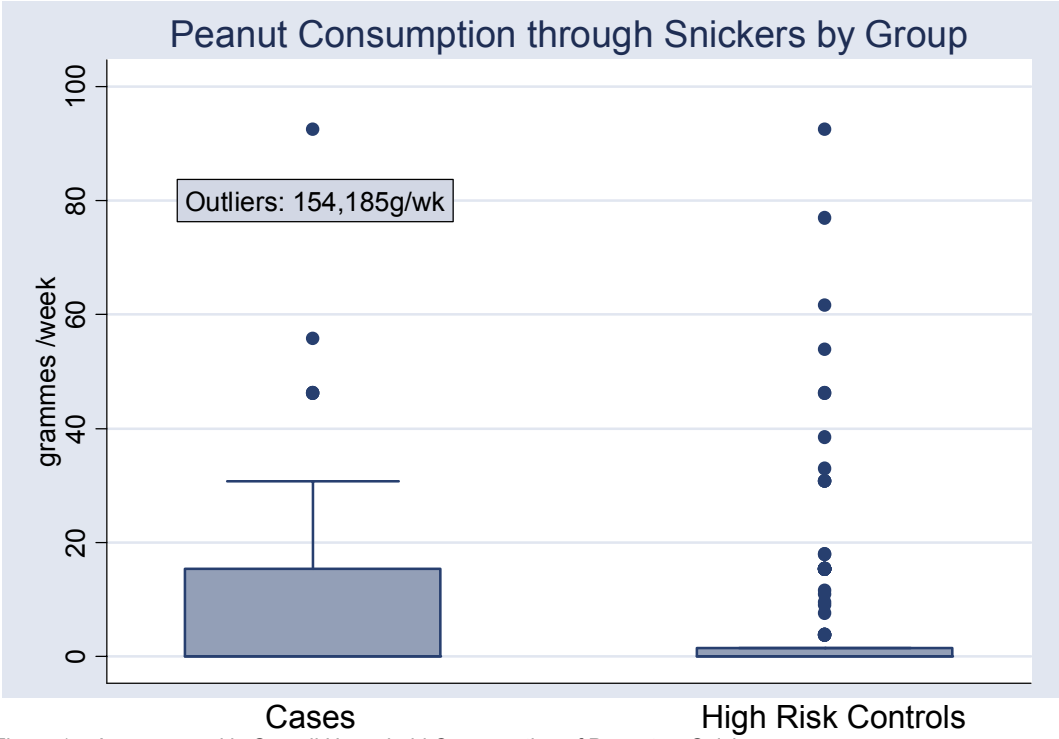

Figure 17: Average weekly Overall Household Consumption of Peanut as Snickers

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

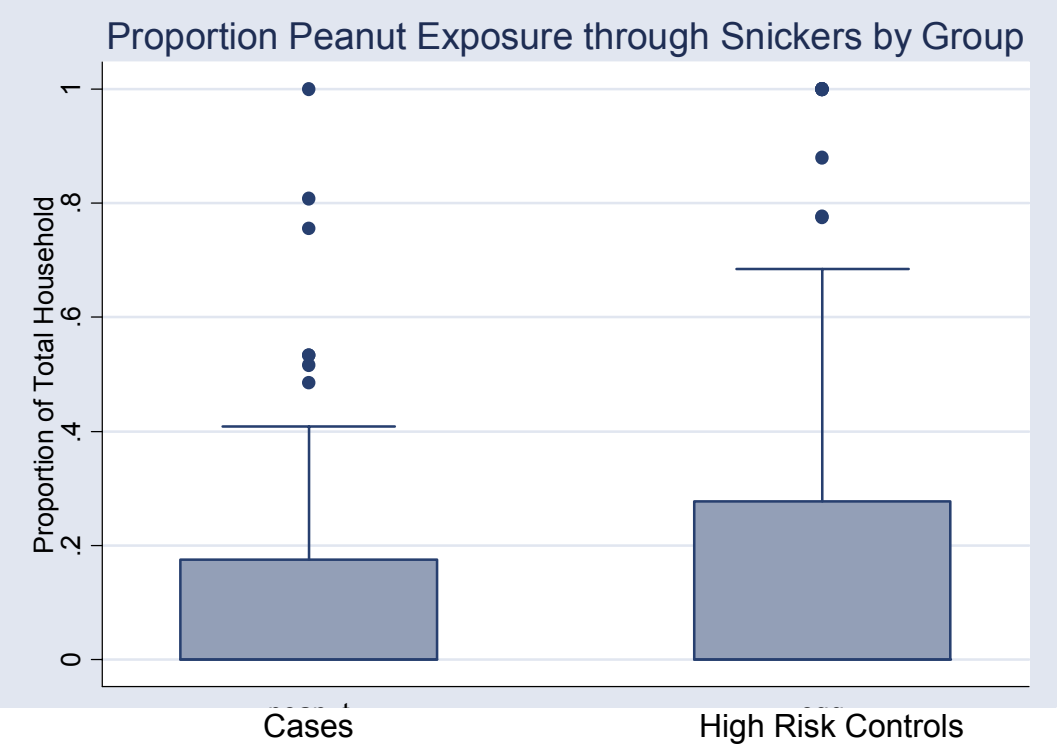

Figure 19: Proportion of Average weekly Overall Household Peanut Consumption as Snickers

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

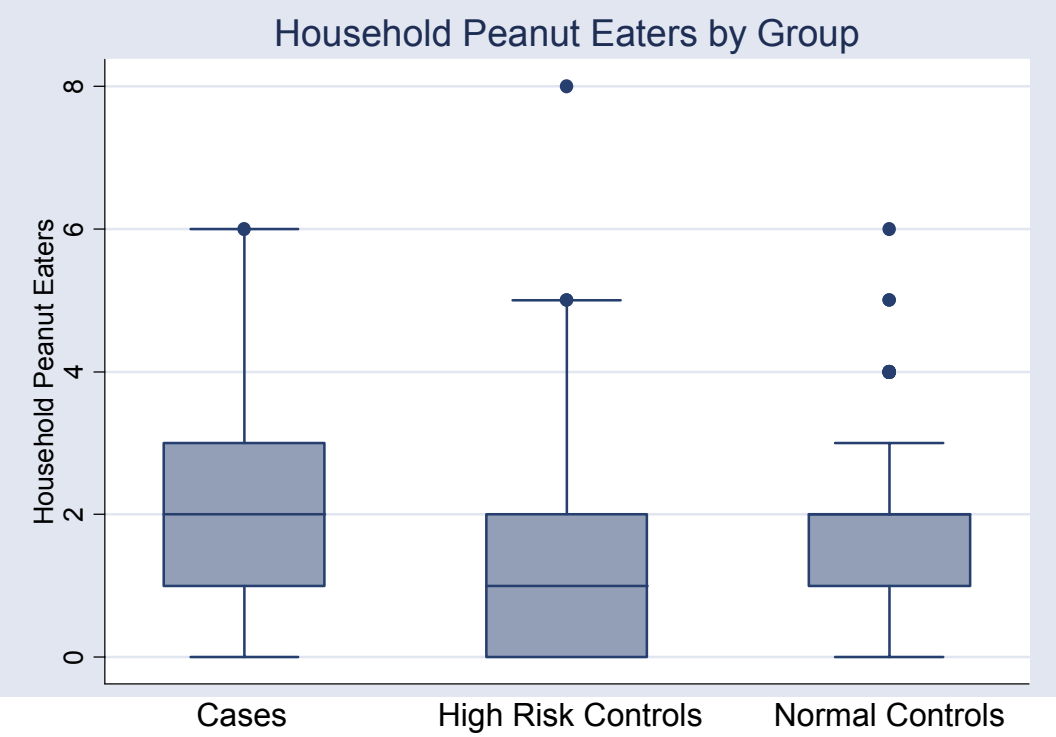

Figure 20: Number of Peanut Eaters in Household

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

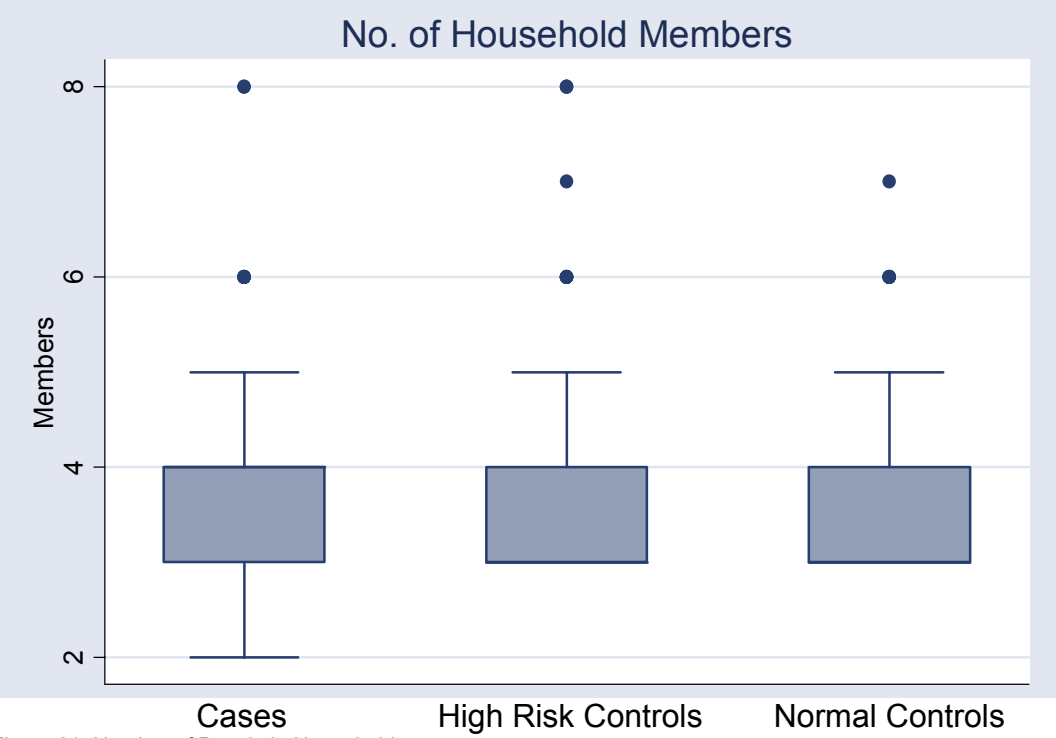

Figure 21: Number of People in Household

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

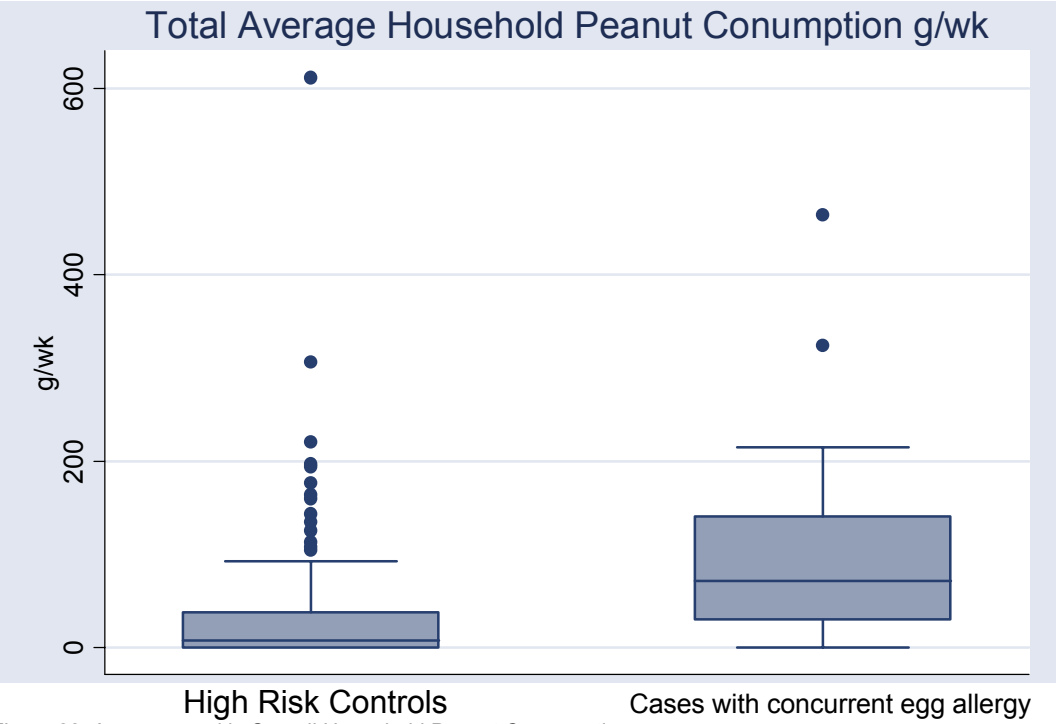

Figure 22: Average weekly Overall Household Peanut Consumption

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

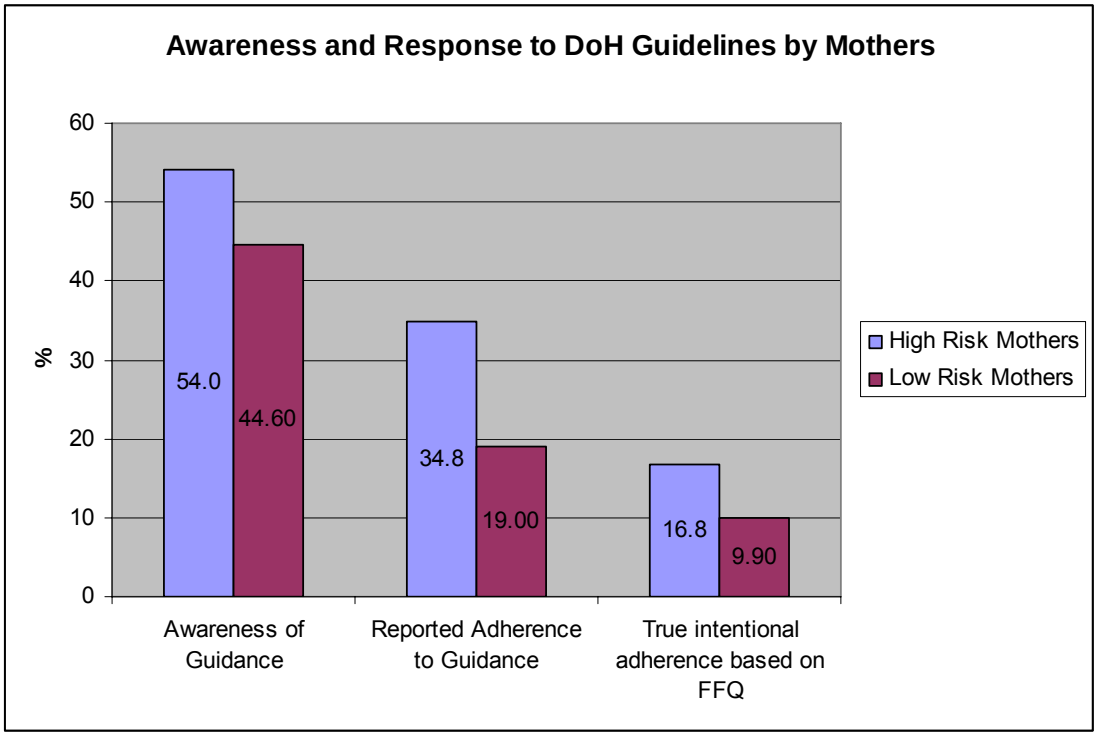

Figure 23: Awareness and response to DoH guidelines

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

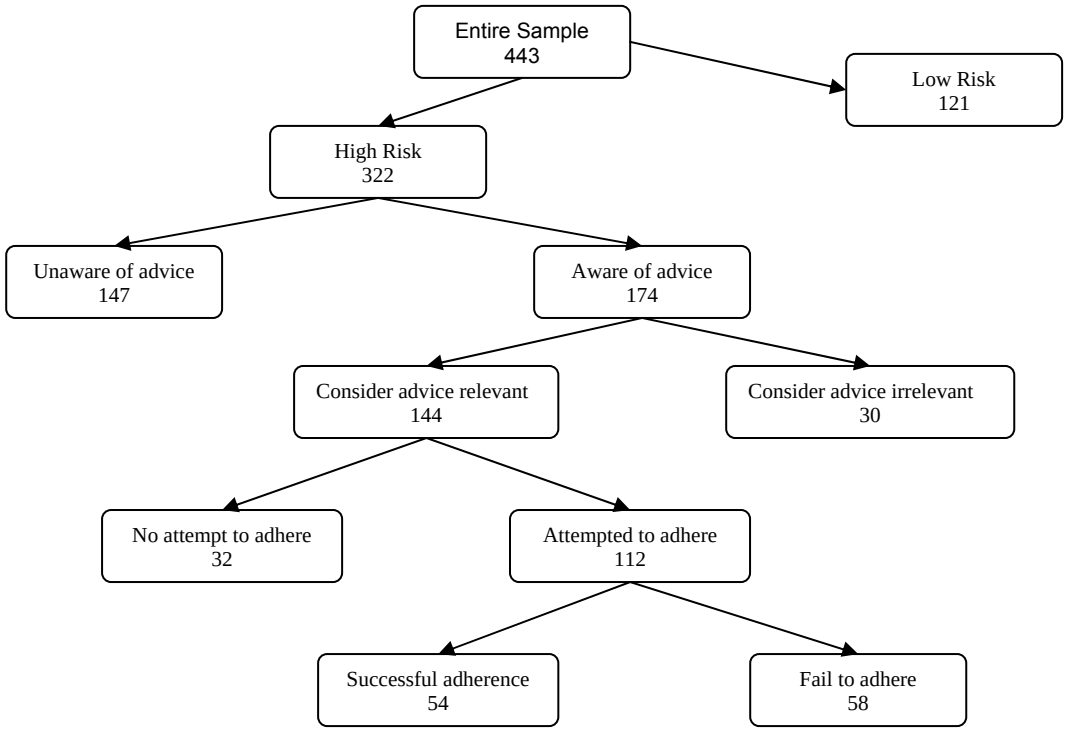

Figure 24: Awareness and response to DoH guidelines by 'High Risk' mothers

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.

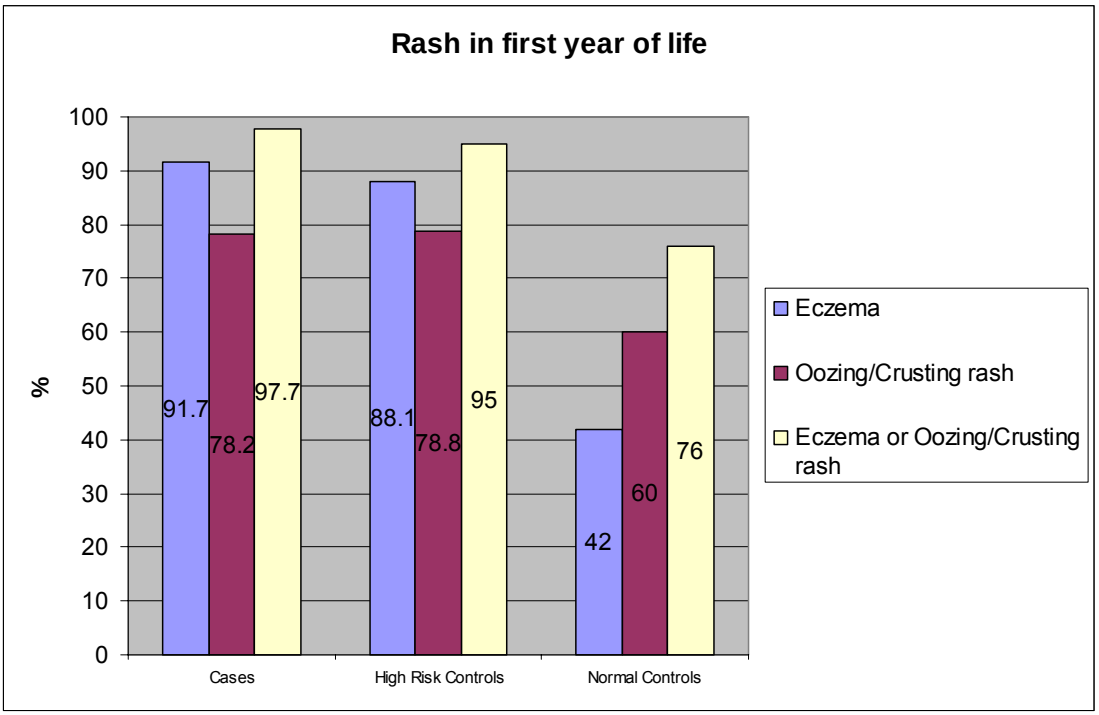

Figure 25: Presence of rashes in first year of life

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

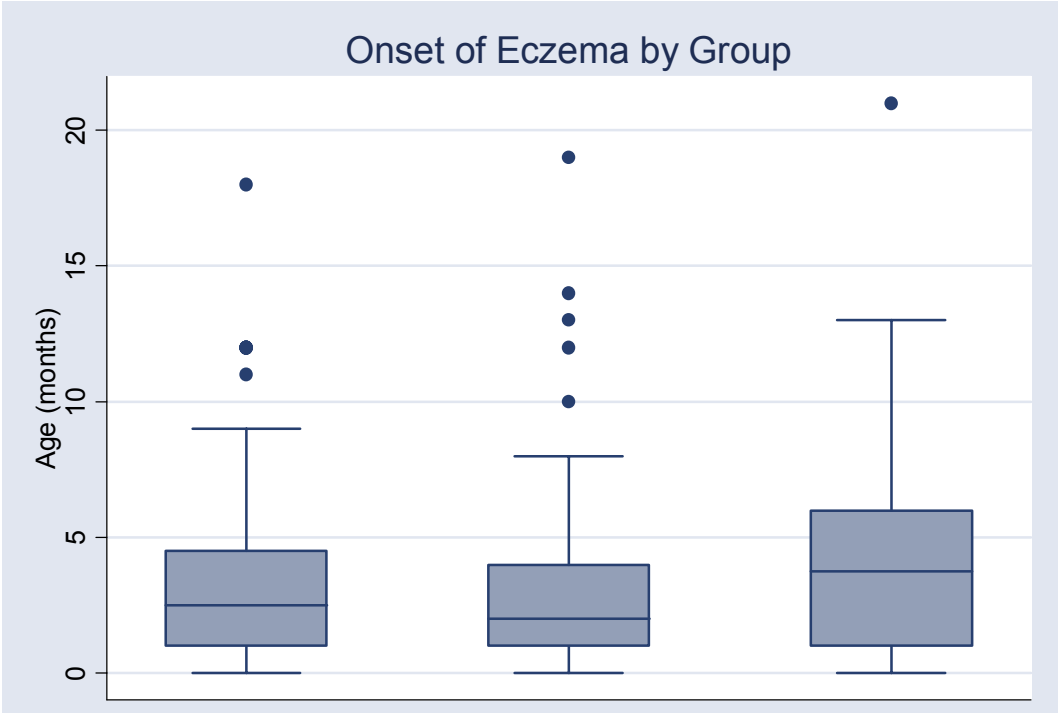

Figure 26: Age of onset of eczema

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

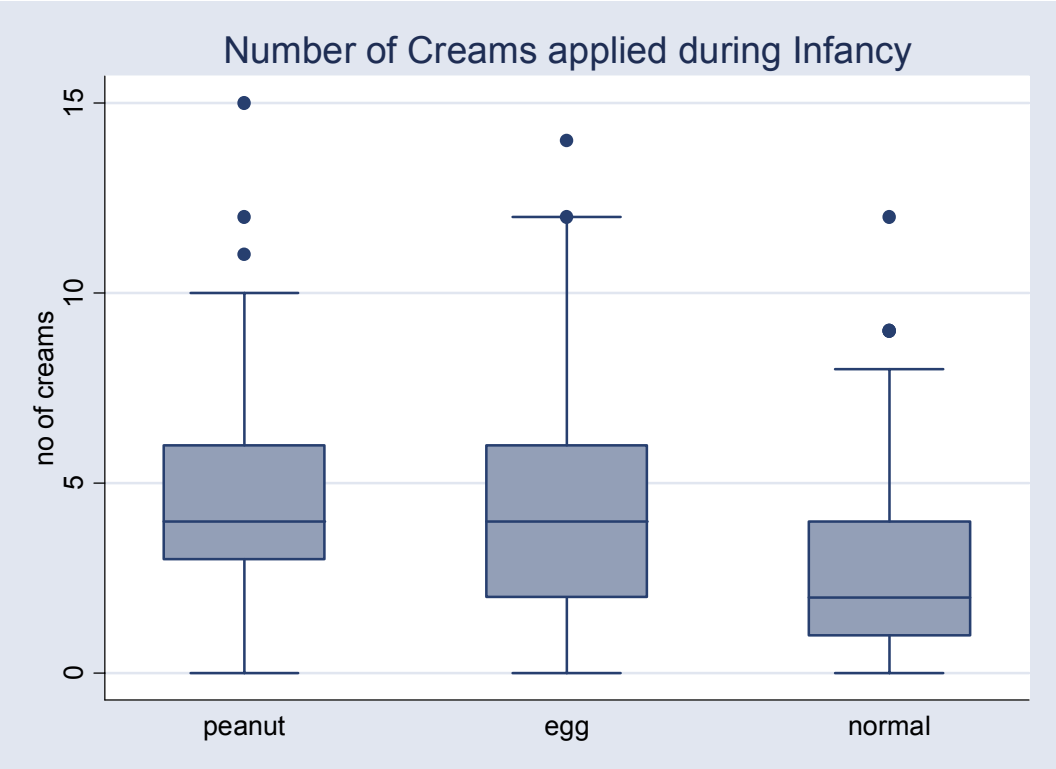

Figure 27: Number of creams applied to infant during first year of life

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

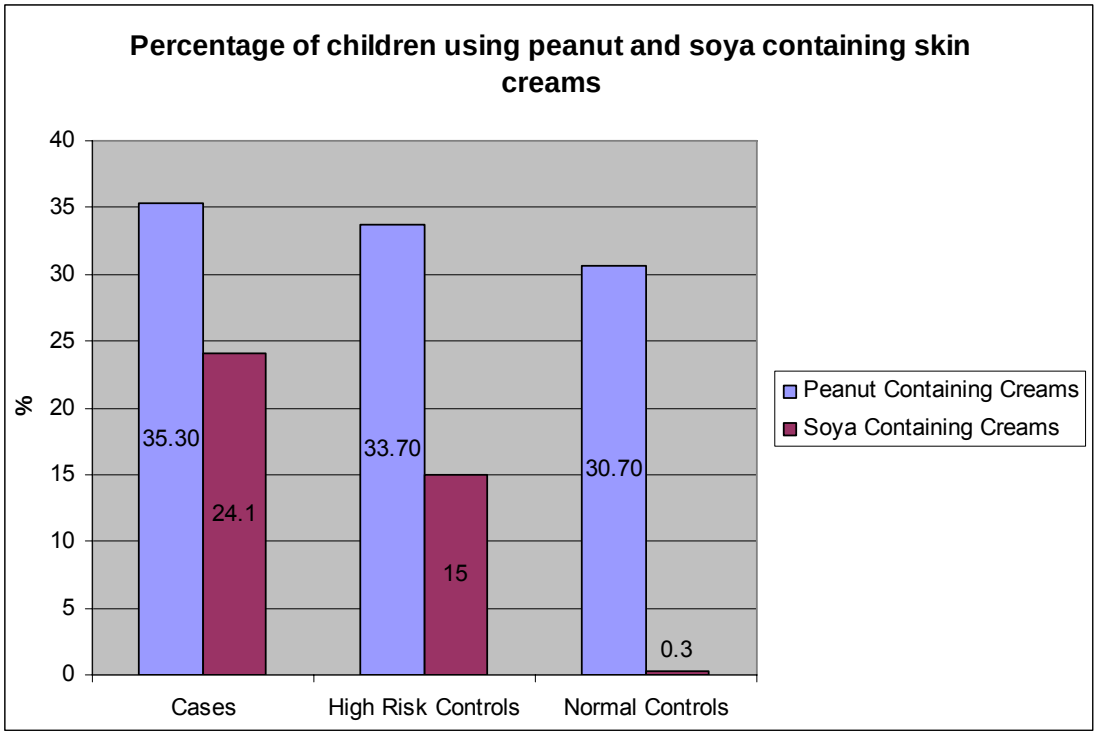

Figure 28: Proportion of children applying creams containing peanut or soy to infant during first year of life

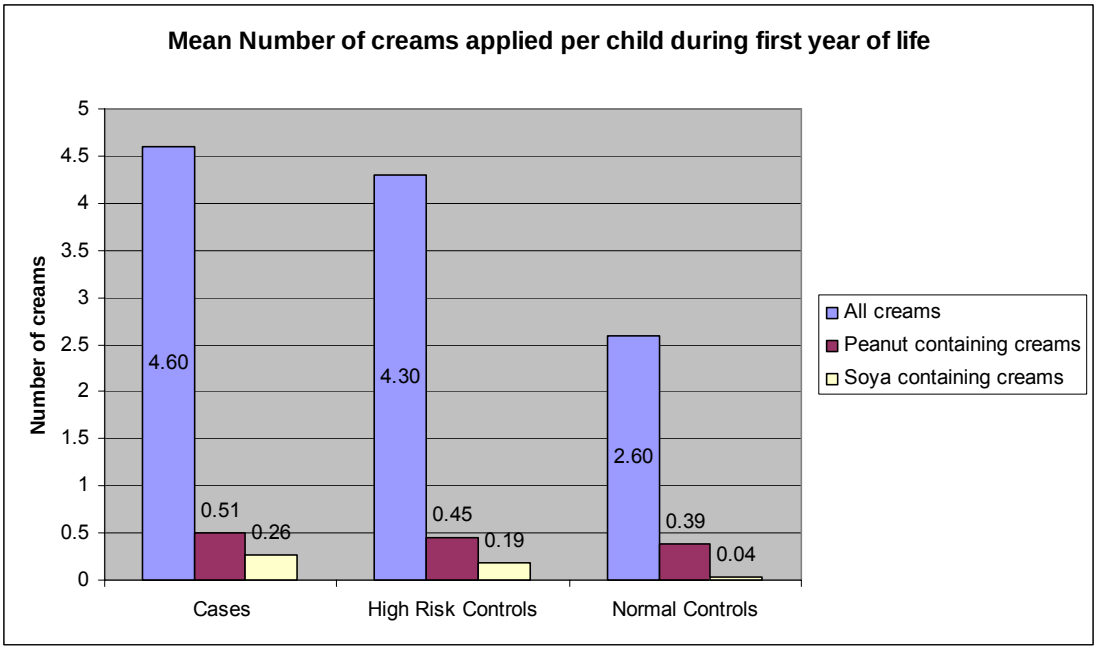

Figure 29: Proportion of children applying creams containing peanut or soy to infant during first year of life

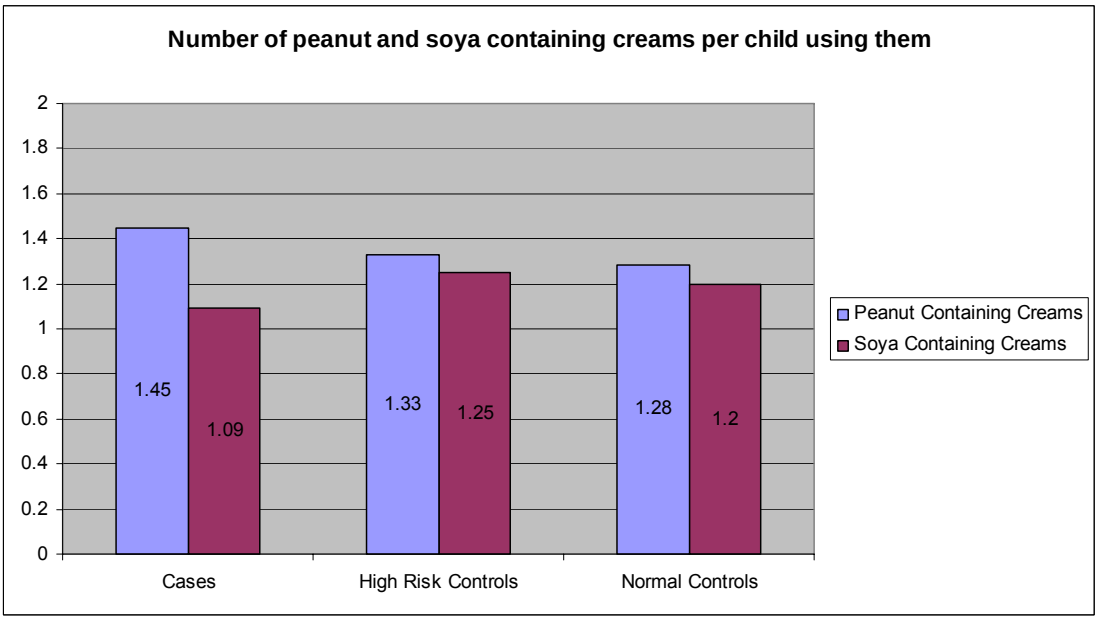

Figure 30: Number of creams containing peanut or soy applied to each infant using them during first year of life

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

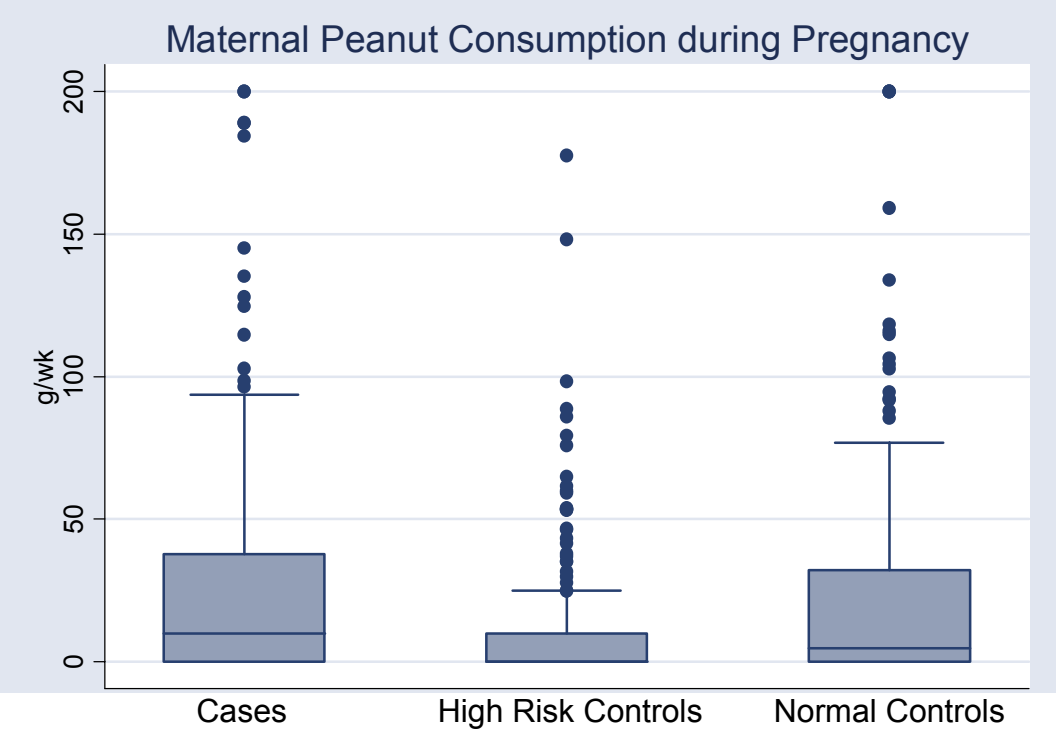

Figure 31: Average Weekly Maternal Peanut Consumption During Pregnancy. Note there are an additional two outliers with peanut-consumption values >200 in the Cases, and three such outliers in the Normal Controls.

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