Different measures of dietary diversity during infancy and the association with childhood food allergy in a UK birth cohort study

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Conflict of interest:

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Abstract

Background: Diet Diversity (DD) during infancy may prevent food allergies (FA), possibly by exposing the gastrointestinal microbiota to diverse foods and nutrients.

Objective: To investigate the association between four different measures of DD during infancy and development of FA over the first decade of life.

Methods: A birth cohort born between 2001/2002 were followed prospectively, providing information on socio-demographic, environmental and dietary exposures. Information on age of introduction of a range of foods and food allergens were collected during infancy. Children were assessed for food allergy at 1, 2, 3 and 10 years. DD was defined using four measures in the first year of life: the World Health Organisation (WHO) definition of minimum DD at 6 months, as food diversity (FD) and fruit and vegetable diversity (FVD) at 3, 6 and 9 months, and as food allergen diversity (FAD) at 3, 6, 9, 12 months.

Results: 969 pregnant women were recruited at 12 weeks gestation. 900, 858, 891 and 827 offspring were assessed at 1, 2, 3 and 10 years. Univariate analysis showed that WHO DD (p=0.0047), FD (p=0.0009), FAD (p=0.0048) and FVD (p=0.0174) at 6 months and FD (p=0.0392), FAD (p=0.0233), and FVD (0.0163) at 9 months significantly reduced the odds of FA over the first decade of life. DD measures at 3 months were not associated with FA but only 33% of the cohort had solid foods introduced by this age.

Conclusion Increased infant DD, as measured by four different methods, decreased the likelihood of developing FA.

Highlights box:

1. What is already known about this topic?

   Diet Diversity (DD) during infancy may be beneficial for future health.

2. What does this article add to our knowledge? Increased DD measured using four different methods from 6 months onwards, in the first year of life, may decrease the likelihood of FA over the first decade. However, DD at 3 months showed no significant effect on food allergy outcomes.

3. How does this study impact current management guidelines?

   These findings support the recommendation that early oral intake of a variety of foods and food allergens, once the infant is developmentally ready, will reduce incidence of food allergy in the first 10 years of life.

Key words

Dietary variety, dietary diversity, eczema, weaning, complementary feeding, infant feeding, food allergy prevention.

Abbreviations

FAD: Food Allergen diet diversity

CI: Confidence Interval

DBPCFC: Double Blind Placebo Controlled Food Challenge

DD: Dietary diversity

EAACI: European Academy of Asthma, Allergy and Immunology

FD: Food diversity

FA: Food allergy

FVD: Fruit and vegetable dietary diversity

FAIR: Food Allergy Intolerance Research

ISAAC: International Study of Allergy and Asthma in Childhood

OR: Odds ratio

SPT: Skin prick test

WHO DD: World Health Organization diet diversity

Introduction

'Dietary diversity' (DD), is defined as the number of different foods or food groups consumed over a given reference period[1]. DD can also be defined in terms of diversity of foods eaten (FD), number of foods within a food group consumed, e.g., fruit and vegetable diversity (FVD), using the World Health Organisation (WHO) definition of minimum DD, or the number of allergens being consumed, referred to as food allergen diet diversity (FAD).

Recently there has been considerable interest in the effect of infant DD in the prevention of allergic disease. A task force report from the European Academy of Asthma, Allergy and Immunology (EAACI) suggested that increased DD may reduce the risk for allergy development via its effect on the microbiome, increased intake of nutrients related to allergy prevention, and by increased exposure to allergens[2]. The report summarized 14 papers reporting the role of DD on allergy outcomes. However, only one study reported on the association between DD and FA outcomes, suggesting that increased DD in infancy may reduce the risk of food allergy[3].

The aim of this study is to assess the effect of infant DD in the first year of life on food allergy outcomes over the first ten years of life in a population birth cohort.

Materials & methods

The Food Allergy and Intolerance Research (FAIR) birth cohort included children born on the Isle of Wight (UK) (n = 969) between 2001-2002 who were followed up prospectively[4,5]. Demographic and reported allergy data were collected at 12 weeks gestation, at birth and during subsequent follow up studies at set time periods.

Dietary data

Infant feeding data were collected via a standardized questionnaire at ages 3, 6, 9, and 12 months [6]. Specific information was collected regarding breastfeeding duration, introduction of bottle feeding and age of introduction of 21 different foods, categorized into time periods of <3 months (by 3 months), 3-6 months (by 6 months) and 6-9 months (by 9 months). At the 12 month visit, parents were asked questions regarding introduction of eight allergenic foods (dairy, whole egg, wheat, soya, peanut, tree nuts, fish and sesame) during the first year of life[7].

Diet diversity

DD was calculated according to the available information at the different time points and was defined as:

1) Calculated minimum DD according to the WHO classification. This is a population-level indicator designed by the WHO to assess DD as part of infant and young child feeding practices among children aged 6-23 months old. It is calculated by summing the number of food groups included in the child's diet at 6 months (*maximum count of seven).* The seven food groups included are grains/roots/tubers, legumes/nuts, dairy, flesh foods, eggs, vitamin A rich fruit and vegetables, other fruit and vegetables [8].
2) Summing the number of foods introduced at each time point [3,9]; therefore, the *maximum score was 21* at 3, 6 and 9 months, referred to as food diversity (FD).
3) Calculating a sub scale *maximum score of five fruit and vegetable items* (non-citrus fruits, citrus fruits, strawberry, vegetables not including potato and tomato, tomato) was

also computed to give a fruit and vegetable dietary diversity (FVD) score at 3, 6 and 9 months [4].

4) Calculating diversity of main allergen intake calculated at 3, 6, 9 and 12 months, i.e., *score out of 8* (milk, egg, wheat, fish, soy, peanut, tree nuts, sesame) at 4 time points, refered to as food allergen diversity (FAD) [2,10].

DD scores did not take into account frequency of intake or portion size consumed.

Food allergy diagnosis

Children were clinically examined and skin prick tests (SPT) were performed to milk, wheat, egg, cod, peanut and sesame at 1, 2, 3 and 10 years as previously described [4,5].

Food allergy was defined as a positive food challenge or a positive SPT and a convincing clinical history, as previously reported. Children were invited for oral food challenges (OFC) according to predefined criteria. Children were invited for a food challenge if they were sensitized to a food which they have never knowingly consumed or a reported adverse reaction to a food irrespective of their sensitization status. OFCs at 1, 2 and 3 years were performed following a previously published algorithm [4,5,7]. All eligible children underwent open OFCs. Those with a history of immediate symptoms from prior ingestion of the food underwent a hospital challenge. For safety reasons, in some instances challenges were not conducted (e.g if there was a clear history of a systemic reaction in addition to sensitisation).Challenges were performed at home for participants with a negative SPT and delayed symptoms. Only those with a positive reaction were invited to participate in a double-blind, placebo-controlled, food challenge (DBPCFC). At 10 years of age the PRACTALL recommendations for food challenge doses were followed [11]. Food allergy outcomes are described at age 1, 2, 3 and 10 years. A new variable was calculated for children diagnosed with any food allergy in the first ten years of life, referred to as “over the first ten years”.

Diagnosis of eczema

Presence of eczema was recorded via parental report using questions from the International Study of Asthma and Allergy in Children (ISAAC study) [12] at ages 3, 6, 9 and 12 months, and 2, 3 and 10 years [4,5,7]. We used the question: Has your child been diagnosed with eczema?

Fillagrin Los of function

DNA was extracted from umbilical cord blood and was genotyped for 4 FLG null variants common among Europeans: R501X, S3247X, R2247X and 2282del4. Genotyping was performed using TaqMan allelic discrimination assays as previously described [13], with PerfeCTa mastermix (VWR International, Radnor, PA, USA) and 5 ng DNA per sample. Control samples of known genotype were included to allow end-point genotype determination. Individuals carrying the minor allele for at least one of the FLG variants were classified as filaggrin haploinsufficient.

Demographic information

Data regarding race/ethnicity, parity, maternal education and socio-economic status were collected by questionnaire. Self-reported history of maternal and family allergies: hay fever, seasonal allergies, or allergic rhinitis and eczema was collected at recruitment using the ISAAC questions [12].

Statistical methods

Data were double entered by different operators on SPSS versions 20 and 21 (SPSS Inc, Chicago, USA). Descriptive statistics with means (standard deviations) or counts (frequencies) were calculated. Univariate analysis was carried out to assess the association of each DD measure and FA outcome.

Logistic regression models were fitted to describe the relationship between the binary food allergy variables, food diversity measures and other related covariates. If independent variables were found to be statistically significant at the p=0.05 level in the univariate analysis the variables were entered into a multivariate model to understand the variables at each time point that are independently associated with food allergies in the first 10 years of life. Spearman correlations were performed to examine relationships between count data. We therefore only performed multivariate analyses if the food count variable was significantly associated with the outcome variable at the p = 0.05 level in the univariate analysis, since we were more interested in the food diversity values than other independent variables. All significance tests were two sided and analyses were performed with SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). We made no adjustments for multiple comparisons in this study because the hypotheses were made *a priori*, and the hypotheses ask the same core question in different ways.

To test whether eczema was a confounder in the relationship between diet diversity and food allergies at the time-points we were investigating, we examined whether food allergies were associated with eczema and whether DD was associated with eczema. If these associations were significant at the $p < 0.05$ level and the estimate for the relationship between DD and food-allergy at each time point changed by more than 10% with eczema included and excluded from the model, eczema was acknowledged as a confounder. We also tested for other possible confounders such as age of introduction of solid foods and DD and eczema. In order to understand the role of FLG-LOF in any of the associations seen, we also tested for Fillagrin Loss of Function outcomes and food allergy and association between Fillagrin Los of Function outcomes and eczema Finally, we tested for an interaction between FD, FLG-LOF and eczema in the logistic regression modelling with food allergy outcomes.

Ethical approval was obtained from the NRES South Central - Southampton B Research Ethics Committee (REF 10/H0504/11). All parents consented and children provided assent.

Results

Study Sample

For the primary analysis, we included N=969 mother-infant dyads with information on diet, eczema and food allergy outcomes. For the secondary analysis, we included N= 296 children with available DNA and information on four filaggrin LOF variants (2282del4, R501X, S3247X and R2247X).The study population consisted of 969 children. Nine hundred (92.9%) children were seen at 1 year, 858 (88.5%) at 2 years, 891 (91.9%) at 3 years and 827/969 (85%) children were seen at 10 years. Over the course of the 10 years, 947/969 (97.7%) children were seen at any time point (19,21,26,29). Demographic, environmental and allergic characteristics of participants are shown in Table 1.

Association of background characteristics with food allergy

Family history or maternal history of allergy was not associated with food allergy outcomes in the child at any time point (Table 2). Maternal history of food allergy increased the odds of having a food allergy at 2 years (OR: 2.588; 95% CI: 1.055 - 6.348, p=0.038) and 10 years (OR: 3.061; 95% CI: 1.442 – 6.497, p = 0.0036), but not at 1, 3 and over the first 10 years of life. Parity did not show an association with food allergy outcomes at any time point other than at 10 years, with an increased number of older siblings reducing the odds of developing a food allergy (OR: 0.499, 95% CI: 0.295 – 0.845; p=0.0096). Breast feeding duration did not affect food allergy outcomes at any time point, but later introduction of solids (continuous variable measured in weeks; table 2) increased the odds of having a food allergy at 1 year (OR: 1.215, 95% CI: 1.087 – 1.359, p=0.0006), and food allergy over the first 10 years of life (OR: 1.157, 95% CI: 1.056- 1.269, p=0.0019. Any eczema in the first year of life (3, 6, 9 or 12 months) was significantly associated with food allergy outcomes at 1 year (OR: 2.731, 95% CI: 1.192- 6.257; p= 0.018), at 2 years (OR: 12.015; 95% CI: 1.605 – 89.959, p = 0.015), 3 years (OR; 3.23.; 95% CI: 1.106 – 9.426, p= 0.032), 10 years (OR: 3.230; 95% CI: 1.107 - 9.426, p = 0.0319) and over the first 10 years of life (OR: 2.823; 95% CI: 1.453 - 5.483, p = 0.0022.)

There was also no association between parity and food diversity at 6 months (Spearmans correlation p=0.25).

Association between dietary diversity score and food allergy outcomes

The median number of foods introduced by certain age categories according to the 4 measures of DD (minimum diversity according to the WHO, FD, FAD, and FVD) is shown in Table 3.Table 4 shows the univariate results for the associations between DD and food allergies at years 1, 2, 3, 10 and over 10 years of age.

Diet diversity according to the WHO DD

DD by 6 months, when classified according the WHO definition, reduced the odds of having a food allergy significantly at all time points other than 2 years.

Classifying DD according to number of foods introduced (FD)

By 3 months DD did not show an association with FA at any of the time points studied, though 33% of infants had been introduced to solids by 12 weeks (table 1). By 6 months, increased DD showed a reduced odds of developing FA at 1 year, 3 years, 10 years and over the first 10 years of life but not at 2 years. By 9 months, the number of foods introduced showed a reduced odds for the development of food allergy at 2 years, 3 years, and 10 years of age and over the first 10 years of life but not at 1 year of age.

Classifying DD according to number of allergenic foods introduced (FAD)

As with FD at 3 months, FAD at 3 months did not show any association with food allergy outcomes at any time point studied. FAD at 6 months showed a reduced odds of developing food allergy at 1 year and over the first 10 years of life but not at 2 years, 3 years and 10 years. Similarly, FAD at 9 months showed a reduced odds of developing food allergy by 1 year, 3 years and over the first 10 years of life but not at two years and 10 years. AD at 6 and 9 months was positively correlated with FD at 6 and 9 months (r_s=.69,.64[p<0.0001,p<0.0001), i.e., increased intake of food allergens did not negatively impact on FD. Most interestingly,

FAD at 12 months was significantly associated with a reduced odds of having food allergy at all time points.

Classifying DD according to number fruit and vegetables introduced (FVD)

As with FD and FAD, FVD, at 3 months showed no association with any of the food allergy outcomes studied. However, FVD at 6 and 9 months reduced the odds of food allergy at 1 year, at 10 years and over the first 10 years of life. FVD at 6 months did not reduce the odds of having a food allergy at 2 years or 3 years. FVD at 9 months, did not reduce the odds of having a food allergy at 2 years but did at 3 years.

Using multivariate analysis (Table 5), we showed that after correcting for significant factors, for each additional food introduced by 6 months using the WHO DD, the odds of developing FA was reduced by 21.6%, and for each additional food introduced (FD) by 9 months, the odds of developing food allergy over the first 10 years of life reduced by 9.8%. Similarly for each additional allergenic food (FAD) consumed (of 8) by 6 or 12 months, there was a significant reduction of 24.9% and 33.2%, respectively, in the likelihood of FA over the first 10 years of life. FVD at 6 and 9 months reduced the odds of developing a FA by 10 years by 23% and 16.9%, respectively.

In summary (Table 6 and figure 1), in the multivariate analysis, WHO DD was significantly associated with a reduced odds of FA at all time points, other than at 2 years. FD at 6 months was associated with reduced odds of FA at 1, 3, and over 10 years. FD at 9 months was assocated with reduced FA at 2, 3 and 10 years. FAD at 6 months was associated with less FA at 1 year and over 10 years. FAD at 9 months was only associated with less FA at 3 years. FAD at 12 months was associated with all reduced FA at all time points. FVD at 6 months was associated with less FA at 10 years, and over 10 years and FVD was associated with less FA at 3, 10 and over 10 years.

306 Asesssment of possible confounders

307 *Association between eczema and age of introduction of solid foods*

308 There does not appear to be an association between eczema and age of introduction of solid
309 foods (p=0.57) . Children without eczema started solids on average at 14.93 (2.95) weeks vs
310 those with eczema at 15.04 (2.61) weeks.

311

312 *Association between Diet Diversity and eczema*

313 Exploring the relationship between our four DD measures and eczema showed that only one
314 variable, 'number of allergic foods at 1 yr,' had a negative association with eczema status
315 (p=0.04), but since the estimate for this variable only changed by 3.45%, this variable would
316 not be considered a confounder leading to a reduced DD estimate.

317

318 *Association between Fillagrin Los of Function outcomes and food allergy*

319 We next explored the relationship between filiggrin haploinsufficiency and food allergies at
320 years 1, 2, 3, 10 and over 10 years of age . Children having at least one FLG-LOF mutation
321 were 4.2 times more likely to have food allergies at age 10 years than those children who did
322 not have a filiggrin mutation (OR: 4.224; 95% CI: 1.474 - 12.106, p = 0.007).

323

324 *Association between Fillagrin Los of Function outcomes and eczema*

325 FLG-LOF did not show an association with eczema at age 2, 3, 10 and over 10 years. At 1
326 year the association was: (OR: 2.517, 95% CI: 1.005 – 6.308, p=0.0489). However, there
327 were only 34 children with FLG_LOF and eczema info at 1 year, 15 children at 2 years, 12 at
328 3 years, 35 at age 10 and 35 over the first 10 years of life, affecting the power of our statistical
329 analysis.

330

331 *Interactions between between FA, DD and eczema*

332 We found no statistically significant interaction between FA, DD and eczema (p-values for
333 interaction term between = 0.13 – 0.92).

Interaction between Food diversity and Fillagrin Loss of Function outcomes and food allergy

Finally we tested for an interaction between food diversity and FLG-LOF and food allergy; we found no significant interactions (p-values for interaction term between = 0.41 – 0.90). However, the number of children with food allergies that we had FLG_LOF mutations for was small in each age group. For example, in the 3 year old age group (n=286), there were only 33 who were fillagrin haploinsufficent, and there were only 15 that had food allergies, which resulted in very low power to detect significant differences.

Discussion

In this study we set out to determine if different measures of diet diversity (DD) in the first year of life are associated with food allergy outcomes at 1, 2, 3, 10 years and over the first 10 years of life. We saw a consistent pattern of increased DD measured by the WHO definition, food diversity (FD), food allergen diversity (FAD) and fruit and vegetable diversity (FVD) and allergy outcomes. In particular, we have shown that for each additional food introduced by 6 months (FD), the odds of developing food allergy over the first 10 years of life was reduced by 10.8% even after correcting for other significant factors. Similarly, for each additional allergenic food consumed (FAD) by 1 year, there was a significant reduction of 33.2% in the likelihood of food allergy (FA) over the first 10 years of life. Fillagrin haplo insufficency was associated with food allergy outcomes at 10 years of age and with eczema at 1 year. FAD did not negatively affect FD, which reassures us that an early and diverse intake of foods regarded as allergenic does not negatively impact on overall DD. Our data implies that there were no interactions or confounding seen between FA, DD and eczema. We also tested the association between eczema and age of introduction of solid foods and did not find an association. Finally, we tested for an interaction among food diversity, FLG-LOF and food allergy, and we found none that were statistically significant.

Our findings are in agreement with previous research. Roduit et al. [3,14] reported an inverse association between DD in the first year of life and FA at 4-6 years. Hua et al. [10] showed that increased FAD during the first year of life was associated with reduced sensitization to food and aero-allergens at 12 months, but no study has shown that FAD in early life reduces food allergy during the first decade of life. In accordance with Nwaru et al.[15] and Hesselmar et al.[16], we did not find that having eczema affected age of introduction of solids. Eczema in the first year of life did not have an effect on DD either. Interestingly, we did not find any association between having a fillagrin mutation and any eczema over the first 10 years, in contrast to Flohr et al. [17] and Ziyab et al. [18], though the associations reached significance at 1 year. However, this may represent a lack of power in the subset with genotype data.

Suprisingly, almost a quarter of infants had received solid foods by 3 months of age in our study. The reason for the introduction of solids at this early time point is due to the fact the cohort was born in 2001/2002. At the time in the UK[19], the recommendation was that solid food should be introduced to infants' at ~ 4 months. This guideline was subsequently updated in 2003, when the UK Department of Health adopted the 2001 WHO's recommendations that complementary foods should be introduced at 6 months of age whilst continuing to breastfeed.

A limitation of our study and other previous studies mentioned is the lack of specifying the preparation of foods consumed (whether raw or cooked), or differentiation between homemade and commercially produced foods. Although we asked parents as part of our 6 month questionnaire whether they had introduced any packaged infant foods, we did not collect any further details. This is highly relevant as there is debate whether consumption of commercially produced infant food increases or decreases DD [20,21]. Furthermore, the microbial content is known to vary, with homemade infant meals having a higher aerobic colony count, but lower pesticide count that those made commercially [22], which potentially could influence gut microbiota. Research from our group has recently reported that commercially prepared infant food is consumed 15 times more frequently in young children consuming an exclusion diet for milk allergy [23]. Furthermore, data from a UK birth cohort suggest that a diet high in fruit, vegetables and home prepared foods, with only occasional use of commercially produced infant food, is associated with less FA at age 2 years [24]. Therefore, it is important that future DD research should explore this topic in more depth.

The most recent position statement regarding diet diversity by the European Academy of Allergy and Clinical Immunology recommends that portion size and frequency of consumption should be measured when possible [25]. Typically observational cohort studies use FFQs, a dietary assessment method which do not usually quantify the portion size of food consumed or whether the food was eaten singly or eaten as a minor ingredient in combination with other foods. In terms of our analysis, this does not allow us to differentiate the effect of eating substantial portions of specific foods, versus mere exposure to specific foods. Whilst using a FFQ in our study did not allow us to determine the significance of the portion size

401 consumed, it is a practical method with low participant burden for collecting dietary data in a
402 population at multiple time points. Other limitations are that data collected on maternal atopy
403 and child eczema was reported, rather than diagnosed. As is the case for all observational
404 cohort studies, the associations reported cannot determine causation. Finally, we wanted to
405 test if infant diet diversity modifies the penetrance of ethnically matched filaggrin loss-of-
406 function mutations[13,26,27]. We were however limited by our sample size and were unable to find
407 any significant associations.

408 The unique strengths of this study are FA outcome measures until 10 years; OFC-
409 diagnosed FA, and a broader range of foods considered than previous studies. Additionally,
410 we have demonstrated an excellent retention of participants and have used prospectively
411 collected data, thus limiting the impact of participant attrition and recall bias [28]. We have
412 assessed DD using a variety of different defintions and arrived at the same conclusion,
413 underscoring the robustness of the findings. If only one or two significant associations had
414 been found, these associations could have been discounted as possibly being due to chance
415 alone because of the multiple comparisons undertaken in the present analysis. However, there
416 were many associations found between diet diversity measures and food allergy outcomes
417 that all showed consistent direction. We have accounted for confounding variables using
418 adjusted multivariate regression models based on the methodologies of previous published
419 studies [3,9,29].

420 In conclusion, this study demonstrates that increased DD using 4 different measures
421 in the first year of life is associated with reduced FA over the first 10 years of life, even after
422 correcting for significant factors, particularly eczema. This reinforces the advice that a varied
423 diet should be encouraged, unless otherwise indicated. Future research should ensure a
424 consistent approach is used to quantify DD, consider the method of preparation of
425 complementary foods and investigate the mechanisms involved.

426

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Conflicts of Interest: CV has received honoraria from Mead Johnson Nutrition, Danone, Nestle, Abbott, Lil' Mixins and DBV techonologies. The other authors declare no conflicts of interest.

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Figure legends

Figure 1: Food diversity at 6 months vs. food allergy over 10 years.

Multivariate analysis showed: Food diversity at 6 months (p=0.0111) significantly reduced the odds of food allergy over first 10 years (holding introduction of solids at the mean and having eczema ever = yes).

Dotted line: 95% CI

Solid line: p-value

Figure 2: Food allergen diversity at 12 months vs. food allergy over 10 years.

Multivariate analysis showed: Food Allergen Diversity at 12 months (p=0.0005) significantly reduced the odds of Food Allergy over first 10 years (holding introduction of solids at the mean and having eczema ever = yes).

Dotted line: 95% CI

Solid line: p-value

545 Table I. Participant demographic characteristics

Characteristic	n (%)
Male (n = 969)	492 (50.8)
Number of participants first born in family (n = 969)	401 (41.2)
Type of delivery (n = 969)	755 (77.9% normal 211 (21.8) Caesarean; 0.3% (missing)
Family history of allergy at recruitment; asthma, eczema, rhinitis, food allergy (n = 969)	788 (81.3%)
Maternal history of allergy at recruitment; asthma, eczema, rhinitis, food allergy (n = 969)	558 (57.6)
Maternal FA at recruitment (n = 969)	189 (19.5)
Maternal education at recruitment (n = 969) No education Secondary school education (up to 16 years of age) Post secondary school education (between 16-18 years of age) Third level education (18 years of age)	 12 (1.2%) 363 (37.5%) 437 (45.1) 152 (15.7%)
Median breastfeeding duration in days (IQR)	35 (1 ,154)
Any breastfeeding; even just 1 feed (n = 969)	743 (76.7)
Median age of introduction of solid foods in weeks (IQR)	16 (13,16)
Number of infants introducing solids by 3 months (n = 925)	207 (22.38)
Median age of introduction of infant formula in days (IQR)	14 (0, 56)
Eczema at 3 months (n = 927)	200 (21.6%)
Eczema at 6 months (n = 918)	424(42.8%)
Eczema at 12 months (n = 932)	535 (57.4%)
Diagnosed FA at 1 year* (n = 969)	39 (4.0%)
Any reported allergy at 1 year (asthma, eczema, rhinitis, food allergy)	496 (51.2)
Diagnosed FA at 2 years* (n = 858)	21 (2.5%)
Any reported allergy at 2 years (asthma, eczema, rhinitis, food allergy) (n=858)	498 (55.9%)

Diagnosed FA at 3 years (n = 891)	27 (3.0%)
Any reported allergy at 3 years (asthma, eczema, rhinitis, food allergy) (n=891)	409 (45.9%)
Diagnosed FA at 10 years* (n = 827)	30 (3.6%)
Any reported allergy at 10 years (asthma, eczema, rhinitis, food allergy) (n=827)	434 (52.5%)
Any food allergy over the first 10 years of life (n=947)	64 (6.8%)
Any reported allergy over the first 10 years of life (n=947)	809 (86.4%)
Any filaggrin mutation (n=296)	35 (11.8%)

546 FA:Food Allergy *Includes both IgE and non IgE FA

547 Table 2: Association between food allergy outcomes, family history of allergic disease, maternal history
548 of allergic disease, maternal history of food allergy, parity and eczema in the first year of life

	Food Allergy	*OR*(95% CI)*	*p-value*
Family history of allergic disease	1 year	1.582 (0.609 - 4.107)	0.346
	2 years	4.541(0.605 - 34.091)	0.141
	3 years	1.849 (0.550 – 6.214)	0.320
	10 years	1.567 (0.539 - 4.555)	0.4095
	Over first 10 years	1.928 (0.863 - 4.303)	0.1092
Maternal history of allergic disease	1 year	1.330 (0.682 - 2.594)	0.402
	2 years	2.373 (0.862 - 6.539)	0.094
	3 years	1.240 (0.561 - 2.740)	0.595
	10 years	1.804 (0.816 - 3.989)	0.1448
	Over first 10 years	1.422 (0.834 - 2.423)	0.1956
Maternal history of FA	1 year	1.883 (0.934 - 3.795)	0.077
	2 years	2.588 (1.055 - 6.348)	*0.038*
	3 years	1.402 (0.583 - 3.369)	0.450
	10 years	3.061 (1.442 - 6.497)	*0.0036*
	Over first 10 years	1.692 (0.955 - 2.995)	0.0713
Parity	1 year	0.762 (0.538 - 1.080)	0.127
	2 years	0.770 (0.481 - 1.233)	0.277
	3 years	0.837 (0.563 - 1.245)	0.380
	10 years	0.499 (0.295 - 0.845)	*0.0096*
	Over first 10 years	0.769 (0.584 - 1.011)	0.0597
Breast feeding duration (days)	FA year 1	1.001 (1.000 - 1.003)	0.1594
	FA year 2	1.001 (0.999 - 1.004)	0.1686
	FA year 3	1.001 (0.999 - 1.003)	0.4497
	FA year 10	1.001 (1.000 - 1.003)	0.1139
	FA over 10	1.001 (1.000 - 1.002)	0.0832
Age of introduction	FA year 1	1.215 (1.087 - 1.359)	*0.0006*

of solid foods (weeks)	FA year 2	1.154 (0.989 - 1.346)	0.0690
	FA year 3	1.082 (0.939 - 1.247)	0.2775
	FA year 10	1.088 (0.952 - 1.243)	0.2147
	FA over 10	1.157 (1.056 - 1.269)	*0.0019*
Any eczema in first year of life	1 year	2.731 (1.192 - 6.257)	*0.018*
	2 years	12.015 (1.605. - 89.959)	*0.015*
	3 years	3.230 (1.107 - 9.426)	*0.032*
	10 years	2.776 (1.051 - 7.334)	*0.0319*
	Over first 10 years	2.823 (1.453 - 5.483)	*0.0022*

549 FA: Food Allergy

Table 3: Dietary diversity score at each time point.

Age range	Median WHO DD score (IQR, minimum – maximum)	Median Food DD score (IQR, minimum – maximum	Median AllD score (IQR, minimum – maximum	Median FVD score (IQR, minimum-maximum
By 3 months*	NA	0 (0, 0-15)	0 (0, 0-3)	0 (0, 0-4)
By 6 months*	5 (3-4; 0-5)	11 (9-13, 0-21)	2 (2-3; 0 -6)	3 (3-4, 0-5)
By 9 months*	NA	16 (14 – 17; 5-21)	4 (1-8; 3-4)	5 (4-5, 1-5)
By 12 months**	NA	NA	5 (4-6; 0-8)	NA

WHO: World Health Organization Diet Diversity

*21 foods included in questionnaire at 3, 6 and 9 months. N/A: not applicable (not calculated at 3 and 9 months)

** only allergen intake reported

557 Table 4: Measures of diet diversity vs. food allergy outcomes over the first 10 years of life using univariate
558 analysis

		OR (95% CI)	*p-value*
WHO DD at 6 months	1 year	0.766 (0.638 - 0.920)	*0.004*
	2 years	0.782 (0.611 - 1.001)	0.051
	3 years	0.707 (0.573 - 0.873)	*0.001*
	10 years	0.752 (0.605 - 0.934)	*0.0099*
	Over first 10 years	0.801 (0.687 - 0.934)	*0.0047*
Number of foods at 3 months	1 year	0.731 (0.428 - 1.250)	0.252
	2 years	0.799 (0.419 - 1.524)	0.495
	3 years	1.048 (0.774 - 1.418)	0.762
	10 years	0.976 (0.689 - 1.384)	0.8925
	Over first 10 years	0.835 (0.593 - 1.176)	0.3016
Number of foods by 6 months	1 year	0.833 (0.752 - 0.921)	*0.0004*
	2 years	0.883 (0.770 - 1.012)	0.073
	3 years	0.845 (0.747 - 0.955)	*0.007*
	10 years	0.877 (0.780 - 0.986)	*0.0279*
	Over first 10 years	0.871 (0.803 - 0.945)	*0.0009*
Number of foods by 9 months	1 year	0.893 (0.773 - 1.032)	0.125
	2 years	0.806 (0.676 - 0.961)	*0.016*
	3 years	0.801 (0.683 - 0.940)	*0.007*
	10 years	0.812 (0.697 - 0.946)	*0.0074*
	Over first 10 years	0.886 (0.789 - 0.994)	*0.0392*
Allergenic foods at 3 months	1 year*	NA	NA
	2 years*	NA	NA
	3 years	0.810 (0.139 - 4.706)	0.814
	10 years	0.751 (0.133 - 4.249)	0.7457
	Over first 10 years	0.336 (0.050 - 2.247)	0.2606
Allergenic foods by 6 months	1 year	0.619 (0.454 - 0.843)	*0.002*

	2 years	0.844 (0.562 - 1.268)	0.414
	3 years	0.691 (0.476 - 1.002)	*0.051*
	10 years	0.721 (0.505 - 1.031)	0.0729
	Over first 10 years	0.703 (0.551 - 0.898)	*0.0048*
Allergenic foods by 9 months	1 year	0.810 (0.670 - 0.979)	*0.029*
	2 years	0.804 (0.626 - 1.033)	0.088
	3 years	0.785 (0.626 - 0.985)	*0.037*
	10 years	0.825 (0.667 - 1.022)	0.0779
	Over first 10 years	0.842 (0.726 - 0.977)	*0.0233*
Allergenic foods by 12 months	1 year	0.683 (0.525 - 0.888)	*0.0045*
	2 years	0.632 (0.442 - 0.904)	*0.0119*
	3 years	0.628 (0.451 - 0.875)	*0.0059*
	10 years	0.648 (0.470 - 0.894)	*0.0081*
	Over first 10 years	0.677 (0.545 - 0.841)	*0.0004*
Number of fruit and vegetables introduced by 3 months	1 year	0.979 (0.463 - 2.071)	0.956
	2 years	0.942 (0.331 - 2.684)	0.911
	3 years	1.373 (0.719 - 2.624)	0.337
	10 years	1.253 (0.652 - 2.410)	0.498
	Over first 10 years	1.000(.0.561-1.781)	1.000
Number of fruit and vegetables introduced by 6 months	1 year	0.737 (0.549 - 0.990)	0.*043*
	2 years	0.884 (0.587 - 1.333)	0.556
	3 years	0.703 (0.491 - 1.007)	0.055
	10 years	0.697 (0.495 - 0.982)	*0.0388*
	Over first 10 years	0.748 (0.588 - 0.950)	*0.0174*
Number of fruit and vegetables introduced by 9 months	1 year	0.822 (0.682 - 0.990)	*0.039*
	2 years	0.881 (0.683 - 1.135)	0.326
	3 years	0.786 (0.633 - 0.976)	*0.029*
	10 years	0.799 (0.651 - 0.982)	*0.0332*
	Over first 10 years	0.831 (0.714 - 0.966)	*0.0163*

559 WHO: World Health Organization * Data not shown as numbers did not converge.

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565 Table 5: Measures of diet diversity vs. food allergy outcomes over the first 10 years of life using
566 multivariate analysis, including only factors that have shown significance in the univariate analysis.

Variable	*Food allergy*	*OR (95% CI)*	*p-value*
WHO DD 6 months[$$]	1 year	0.683 (0.533 - 0.874)	*0.0025*
WHO DD 6 months*	3 years	0.658 (0.524 - 0.825)	*0.0003*
WHO DD[$]	10 years	0.689 (0.544 - 0.873)	*0.0021*
WHO DD[$$]	Over 10 years	0.784 (0.638 - 0.964)	*0.0207*
Number of foods by 6 months[$$]	1 year	0.861 (0.771 - 0.962)	*0.0082*
Number of foods by 6 months*	3 years	0.837 (0.737 - 0.951)	*0.0062*
Number of foods by 6 months[$]	10 years	0.869 (0.767 - 0.984)	*0.0264*
Number of foods by 6 months[$$]	Over 10 years	0.892 (0.817 - 0.974)	*0.0111*
Number of foods by 9 months[*&]	2 years	0.785 (0.653 - 0.943)	*0.0097*
Number of foods by 9 months[$]	10 years	0.766 (0.649 - 0.905)	*0.0017*
Number of foods by 9 months	3 years	0.972 (0.672 - 0.933)	*0.0053*
Number of foods by 9 months[$$]	Over 10 years	0.912 (0.807 - 1.032)	0.1442
Number of allergic foods by 6 months[$$]	1 year	0.683 (0.501 - 0.931)	*0.0159*
Number of allergic foods by 6 months[$$]	Over 10 years	0.751 (0.587 - 0.961)	*0.0229*
Number of allergic foods by 9 months[$$]	1 year	0.850 (0.704 - 1.026)	0.0899
Allergenic foods by 9 months*	3 years	0.785 (0.624 - 0.986)	*0.0373*
Number of allergic foods by 9 months[$$]	Over 10 years	0.869 (0.749 - 1.010)	0.0664
Number of allergenic foods by 12 months	1 year[$$]	0.679 (0.518 - 0.889)	*0.0049*
	2 years[*&]	0.643 (0.447 - 0.926)	*0.0177*

	3 years*	0.640 (0.458 - 0.895)	*0.0090*
	10 years$	0.622 (0.441 - 0.879)	*0.0070*
	Over 10 years$$	0.668 (0.532 - 0.838)	*0.0005*
Number of fruit and vegetables by 6 months$$	1 year	0.771 (0.563 - 1.056)	0.1049
Number of fruit and vegetables by 6 months$	10 year	0.679 (0.474 - 0.972)	*0.0346*
Number of fruit and vegetables by 6 months$$	Over 10 years	0.770 (0.598 - 0.991)	*0.0426*
Number of fruit and vegetables by 9 months$$	1 year	0.826 (0.674 - 1.012)	0.0654
Number of fruit and vegetables by 9 months*	3 years	0.787 (0.633 - 0.978)	0.0308
Number of fruit and vegetables by 9 months$	10 years	0.771 (0.620 - 0.960)	0.0201
Number of fruit and vegetables by 9 months$$	Over 10 years	0.831(0.708 - 0.976)	0.0243

567 WHO DD: World Health Organization Diet Diversity

568 * corrected for eczema

569 *& corrected for eczema and maternal history of food allergy

570 $corrected for eczema, maternal food allergy and parity

571 $$ eczema and age of introduction of solid foods

572 *FG-LOF at 10 years was associated with FA outcomes but we did not include this in the multivariate*

573 *model as the numbers were too small.*

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575 Table 6: Summary of statistically significant association between diversity and food allergy outcomes

	At 1 year	At 2 years	At 3 years	At 10 years	Over 10 years
WHO Diet Diversity 6 months	x		x	x	x
Food diversity 3 months					
Food diversity 6 months	x		x		x
Food diversity 9 months		x	x	x	
Allergen diversity 3 months					
Allergen diversity 6 months	x				x
Allergen diversity 9 months			x		
Allergen diversity 12 months	x	x	x	x	x
Fruit and vegetable diversity 3 months					
Fruit and vegetable diversity 6 months				x	x
Fruit and vegetable diversity 9 months			x	x	x

576 WHO: World Health Organization

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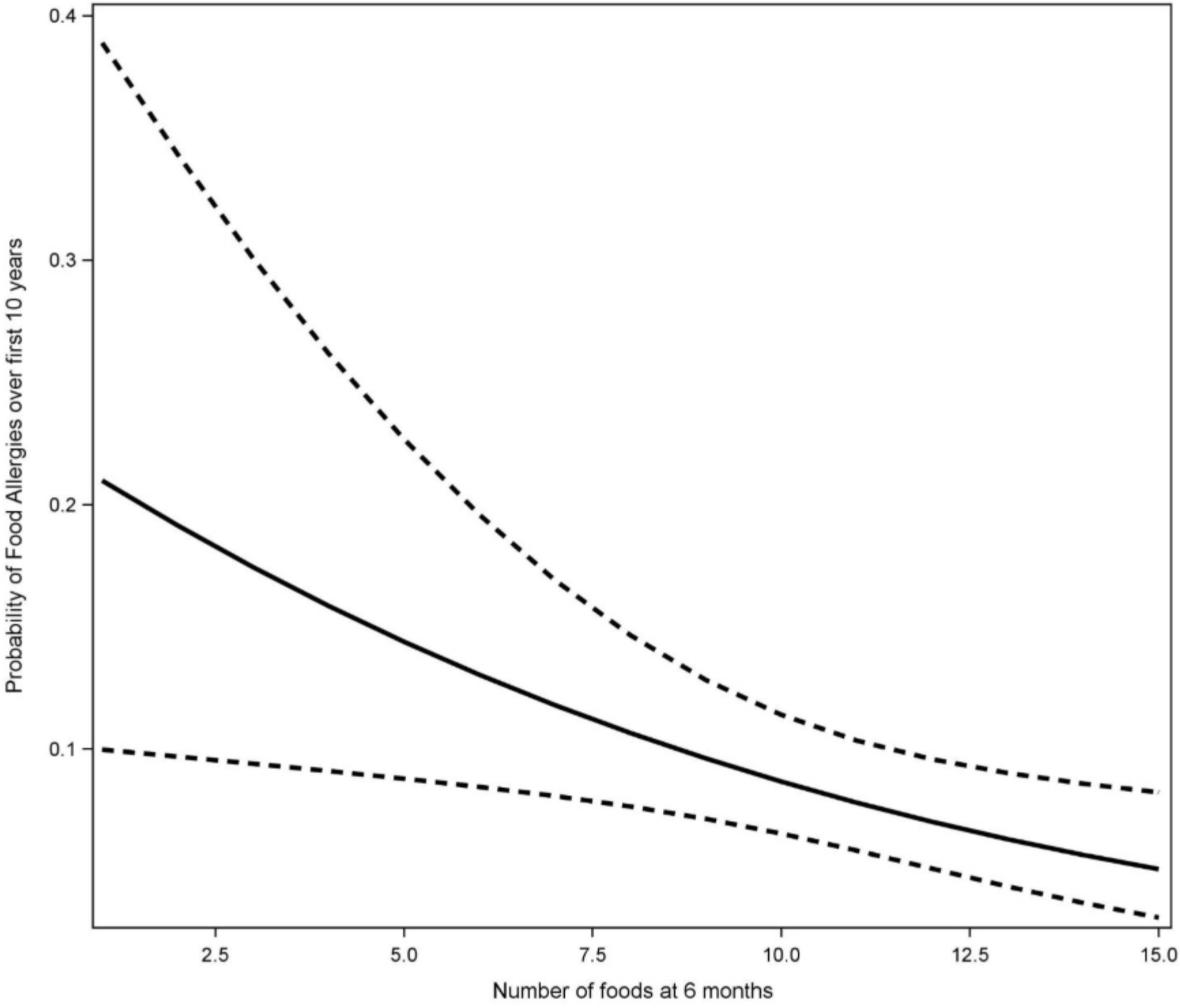
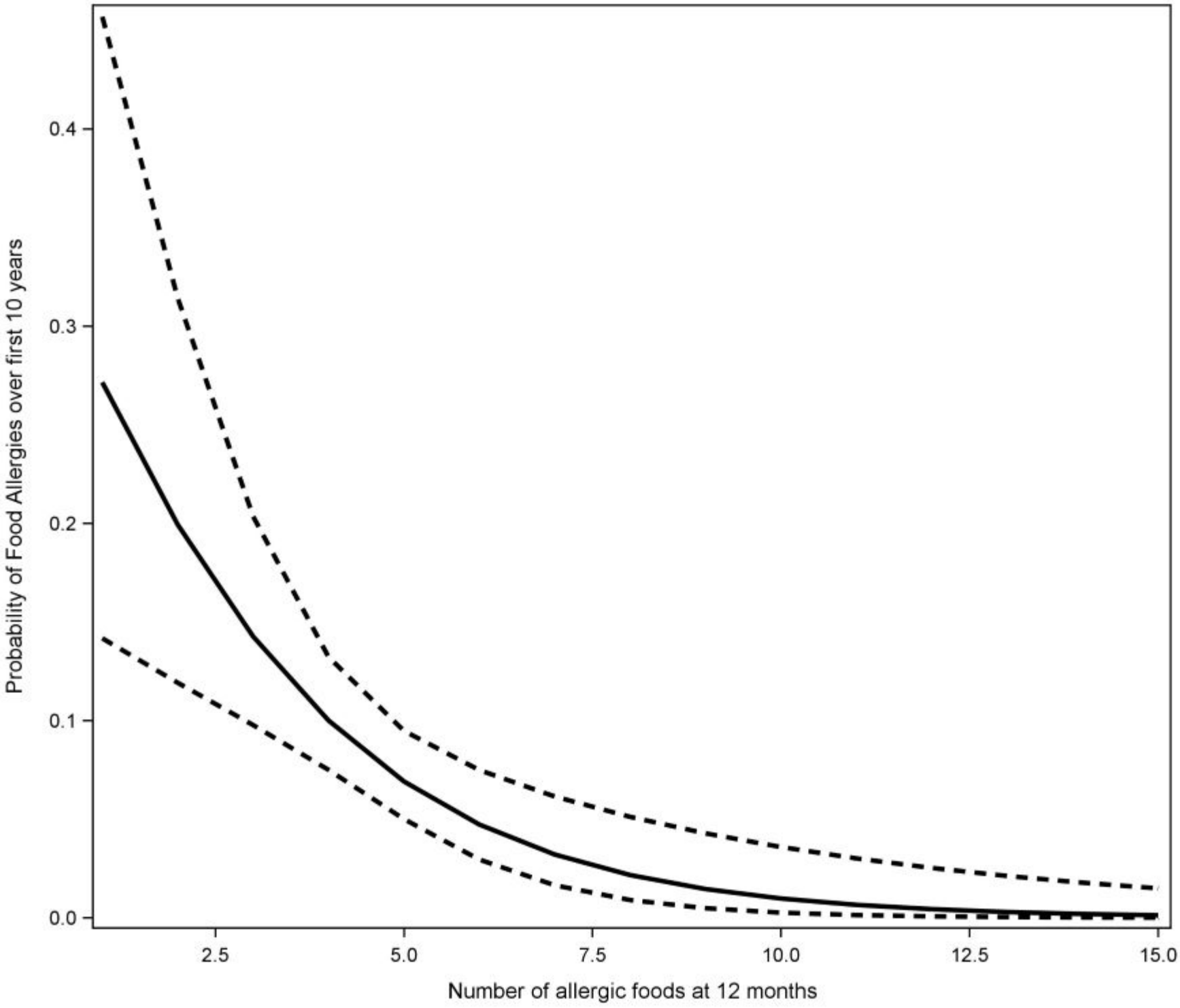

0.4
0.3
0.2
0.1
Probability of Food Allergies over first 10 years
2.5
5.0
7.5
10.0
12.5
15.0
Number of foods at 6 months


Probability of Food Allergies over first 10 years
0.0
0.1
0.2
0.3
0.4
2.5
5.0
7.5
10.0
12.5
15.0
Number of allergic foods at 12 months