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Allergy

Vitamin D insufficiency in the first 6 months of infancy and challenge-proven IgE-mediated food allergy at 1 year of age: a case-cohort study.

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Abstract

Background

Ecological evidence suggests vitamin D insufficiency (VDI) due to lower ambient ultraviolet radiation (UVR) exposure may be a risk factor for IgE-mediated food allergy. However there are no studies relating directly measured VDI during early infancy to subsequent challenge-proven food allergy.

Objective

To prospectively investigate the association between VDI during infancy and challenge-proven food allergy at 1 year.

Methods

In a birth cohort (n=1074), we used a case-cohort design to compare 25-hydroxy-vitamin-D_3 ($25(OH)D_3$) levels among infants with food allergy versus a random subcohort (n=274). The primary exposures were VDI ($25(OH)D_3$<50 nmol/L) at birth and 6 months of age. Ambient UVR and time in the sun were combined to estimate UVR exposure dose. IgE-mediated food allergy status at 1 year was determined by formal challenge. Binomial regression was used to examine associations between VDI, UVR exposure dose and food allergy, and investigate potential confounding.

Results

Within the random subcohort VDI was present in 45% (105/233) of newborns and 24% (55/227) of infants at 6 months. Food allergy prevalence at 1 year was 7.7% (61/786) and 6.5% (53/808) were egg allergic. There was no evidence of an association between VDI at either birth (aRR 1.25, 95% CI 0.70-2.22) or 6 months (aRR 0.93, 95% CI 0.41-2.14) and food allergy at 1 year.

Conclusions

There was no evidence that VDI during the first 6 months of infancy is a risk factor for food allergy at 1 year of age. These findings primarily relate to egg allergy and larger studies are required.

Key words: cohort; eczema; food allergy; paediatrics; vitamin D;

Abbreviations:

UVR: Ultraviolet Radiation

VDI: vitamin D insufficiency

25(OH)D_3: 25-hydroxy-vitamin-D_3

25(OH)D: 25-hydroxy-vitamin-D

BIS: Barwon Infant Study

C3-epi-25(OH)D3: C3-epimeric-25-hydroxyvitamin D3

2D LC-MS/MS: two-dimensional ultra-performance liquid chromatography separation coupled tandem mass spectrometry

SCORAD: Scoring Atopic Dermatitis Scale

RR: Risk Ratio

aRR: adjusted Risk Ratio

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Introduction

A concordant increase in allergic disease prevalence (1-6) and decrease in vitamin D status (7) has been reported in both developed and developing countries. This correlation has led to the hypothesis that low vitamin D status may influence the development of allergic disease including food allergy and eczema (8). Consistent with this, observational studies have found associations between proxy markers of ultraviolet radiation (UVR) exposure, the major source of vitamin D (9), and allergic disease. Latitudes further from the equator appear to be associated with higher rates of food allergy and eczema (10-12); and less consistently, autumn and winter births (periods of low UVR exposure (13)) may be more common among infants and children with food allergy (14-16). There is a substantial literature describing the biological mechanisms by which vitamin D may influence the risk of developing allergic disease (8) including effects on regulatory T cells (17) and bowel epithelial integrity (18). Moreover, ambient UVR has also been shown to affect immune modulation via non-vitamin D pathways (19) and may independently protect against allergic disease development (20).

A previous study reported a cross-sectional association between vitamin D_3 insufficiency (VDI; 25(OH)D_3 <50 nmol/L) and challenge-proven IgE-mediated food allergy among one-year-old infants of Australian-born parents, with evidence of a dose-response relationship (21). However the findings from prospective studies regarding directly measured maternal and infant vitamin D (25(OH)D) (22-26) or 25(OH)D_3 (27, 28) and subsequent allergic outcomes are conflicting, and limited by incomplete measurement of potential confounding factors and suboptimal case definition (22-28). Further, there has been minimal investigation of the potential role of UVR exposure in reducing risk of allergic disease independent of 25(OH)D pathways (29); and the hypothesis that the association between VDI and food allergy may be modified by microbial exposure has not been adequately tested (8).

The primary objective of this study was to utilise a population-derived birth cohort with detailed measurement of relevant covariates to prospectively evaluate the relationship between VDI during the first 6 months of infancy and challenge-proven IgE-mediated food allergy at 1 year of age. In addition, we evaluated the relationship between UVR exposure and food allergy; as well as the relationship between VDI, UVR exposure and eczema.

Methods

Study design

The aims and methodology for the Barwon Infant Study (BIS) have been described previously (30). Briefly, a birth cohort of 1074 mother-infant pairs was assembled in the southeast of Australia using an unselected antenatal sampling frame. The Barwon region population characteristics are similar to those of the Australian population overall, but with a smaller proportion of families from non-English-speaking backgrounds (30). Mother-infant pairs were reviewed at regular intervals during pregnancy and the first year of life. Eczema symptoms and signs were recorded at each review and challenge-proven food allergy status was determined at 1 year. Among the infants who completed the 1 year review, 31% (274/894) were randomly selected to comprise the 'random subcohort'. $25(OH)D_3$ was measured among infants in this subcohort as well as in those who had a positive food skin prick test (SPT) and/or had clinically proven food allergy. To minimise selection bias, analysis of the distribution and determinants of $25(OH)D_3$, and analysis of the association between 25(OH)D3 and eczema, was restricted to infants within the random subcohort. Relationships between UVR exposure dose and food allergy or eczema were investigated in the full BIS cohort.

Exposure measures

Vitamin D status

Blood samples were collected at four time points: maternal at 28-32 weeks gestation, infant at birth (cord blood), 6 months and 1 year (mean 13.03 months ± S.D. 0.83). The $25(OH)D_3$ metabolites (serum or plasma) and epimeric form, C3-epi-25-hydroxyvitamin D_3 (C3-epi-$25(OH)D_3$) were measured using two-dimensional ultra-performance liquid chromatography separation coupled tandem mass spectrometry detection (2D LC-MS/MS) (31). VDI was defined as $25(OH)D_3$ <50 nmol/L and vitamin D deficiency as $25(OH)D_3$ <25 nmol/L, levels which are based predominantly on markers of bone health (32, 33). An appropriate definition of VDI in relation to immune health remains uncertain.

UVR exposure

Questionnaire data quantifying exposure to direct sunlight daily were recorded during trimesters 1 and 2 of pregnancy, and at 4 weeks, 6 months and 1 year. The ambient UVR was estimated using monthly averages of daily total ambient UVR in standard erythemal doses

(34) for Melbourne from 2010 to 2014, provided by the Australian Radiation Protection and Nuclear Safety Agency. Total UVR exposure dose was calculated as the product of time in direct sun and the average of the daily ambient UVR exposure. A total cumulative postnatal UVR exposure dose over the first year of life was estimated by dividing UVR exposure at 4 weeks, six months and twelve months into tertiles and assigning each tertile a score (0=lowest, 1= moderate and 2=highest). These scores were then summed to generate a cumulative postnatal UVR exposure dose score (lowest=0, highest=6) (35). A categorical score was created describing the parental report of sunscreen use during infant sun exposure. The score was included in a secondary analysis to test for the modifying effect sunscreen may have on actual personal UVR exposure (35).

Outcome measures

Food allergy status

At the 1 year review, infants underwent a SPT to five foods: cow's milk, egg, peanut, cashew and sesame (ALK-Abelló, Madrid, Spain) with a positive (10 mg/ml histamine) and negative (saline) control. Quintip® lancets (Hollister-Stier Laboratories, Spokane, WA) were used to perform SPT's on infant's backs. In clinical practice food sensitisation at 1 year of age is defined as a SPT wheal size 3mm or greater than the negative control in the presence of a positive histamine control (36), however recent studies have used a definition of 2mm or greater than the negative control in infants (21). A food allergen SPT wheal size of at least 2 mm greater than the negative control in the presence of a positive histamine control was defined as food sensitised in this study. All participants with SPT wheals 1 mm or more greater than the negative control were offered an in-hospital open food challenge (4). Those regularly ingesting the sensitised food at the time of SPT were defined as sensitised tolerant without formal challenge (n=12). If, on clinical review, the participant had a clinical history and reaction consistent with a diagnosis of IgE-mediated food allergy within 2 months of the 1 year review and a positive SPT, they were defined as food allergic without proceeding to food challenge (n=3) (4). Open food challenges (including raw egg) were performed under clinical supervision using validated protocols from the HealthNuts study (4). A positive challenge comprised one or more of the following criteria occurring within 2 hours of ingesting a dose of challenge food (4) :

- three or more concurrent non-contact urticaria for five minutes or longer;

- vomiting or diarrhoea;
- angioedema;
- anaphylaxis (circulatory or respiratory compromise).

These criteria were also used to define an IgE-mediated reaction occurring during the subsequent week-long home-based introduction if they had not reacted during the clinic challenge (n=1) (4). A negative challenge was defined by the full ingestion of the highest dose of the challenge food with no reaction and completion of the subsequent home-based introduction without reaction. The refusal of the child to eat the challenge food or complete all doses in the challenge protocol was deemed an inconclusive challenge (n=5).

Eczema status

Data on eczema were collected by questionnaires administered at 1, 3, 6, 9 months and 1 year; and clinical assessments were conducted at 1 month, 6 months and 1 year. Eczema was defined according to the modified UK working party criteria (37, 38). All infants had to have a history of itchy skin plus at least three of the following: a history of dry skin, a family history of allergy, a history of skin rash affecting the flexures or outer surfaces of the limbs or affecting the head or cheeks, or visible dermatitis assessed during a study visit at either 1 month, 6 months or 1 year (39). The Scoring Atopic Dermatitis Scale (SCORAD) was used to quantify eczema severity (40, 41).

Statistical analysis

Our primary hypothesis was that VDI in the first 6 months of infancy would be associated with food allergy at 1 year of age. Infants with an inconclusive hospital food challenge result or indeterminate food allergy status were excluded from the food allergy analysis (n=30). For the food allergy case-cohort analysis we applied inverse probability weighting where the probability of selection for non-cases in the random subcohort was estimated by the fraction of infants included in the subcohort from the total cohort reviewed at 12 months in the BIS study (274/894). The probability of selection for food allergy cases was 1 (42).

Categorical variables were summarised using proportions. A propensity weighting approach was used to adjust the eczema prevalence to account for loss to follow-up over the first year of the study and provide an estimated eczema prevalence over the first year for the inception cohort (43). T-tests were used to compare the mean $25(OH)D_3$ levels between food allergic infants and the random subcohort, and between infants within the random subcohort with and without eczema (Supplemental Tables EI and EII). Binomial regression models were fitted to estimate risk ratios (RR) for associations between exposures (VDI and UVR exposure dose) and food allergy at 1 year of age or eczema. A further analysis examined the associations between VDI and food sensitisation. $25(OH)D_3$ was also divided into quintiles to assess potential non-linear associations and thresholds other than 50 nmol/L (Supplemental Figures E3 & E4).

Ethnicity, family history of allergy, number of siblings, formula feeding and pet ownership were selected *a priori* to be included as covariates in the model as they have each been related to both $25(OH)D_3$ and allergic disease. Smoking, livestock ownership, socioeconomic status (SES), birth weight, gender, season of birth, egg avoidance and time of solid introduction in infancy, maternal and infant vitamin D supplementation, C3-epi-$25(OH)D_3$ (31) concentration and mode of delivery at birth were retained in the model if they made a greater than 10% change to the risk ratio point estimate.

The relationship between change in $25(OH)D_3$ status over the first 6 months of life and food allergy at 1 year of age was investigated by fitting a multivariable binomial regression model, using $25(OH)D_3$ at birth as a baseline variable, and change in $25(OH)D_3$ status from birth to 6 months of age as an explanatory variable (44). An interaction term was specified to investigate effect modification by pet ownership (8).

To adjust for seasonal variation in $25(OH)D_3$ levels, we fitted a sinusoidal curve with a period of 12 months to $25(OH)D_3$ data using linear regression, and added the residuals from this model to the population average $25(OH)D_3$ levels (45). For the C3-epi-$25(OH)D_3$ sensitivity analysis, C3-epi-$25(OH)D_3$ and $25(OH)D_3$ were summed as a new variable representing total vitamin D exposure for the binary regression.

Analyses were performed using Stata (version 14.1, College Station, Texas).

Ethics

The study was approved by Barwon Health Human Research and Ethics Committee (HREC 10/24). Parents or guardians provided written informed consent for this study.

Results

Study population

The majority of participants were full term, Caucasian infants with Australian born parents (Table 1). The majority of mothers took vitamin D supplements during pregnancy and up to one third of infants were exposed to formula feeding (Table 1).

Vitamin D levels within the random subcohort

Within the random subcohort vitamin D_3 levels were measured among 233 infants at birth and 227 infants at six months. (Supplemental Figure E1). Forty five percent (105/233) of infants had VDI at birth and 24% (55/227) at 6 months. More than 82% of mothers reported taking a supplement containing vitamin D during pregnancy and maternal VDI was uncommon (9%), (Table I and Figure 1). The average ratio of birth 25(OH)D_3 to maternal 25(OH)D_3 was 0.61. The mean change in 25(OH)D_3 status from birth to 6 months was +15.0 nmol/L ± S.D. 34.0 nmol/L.

Determinants of vitamin D status

At each time point, ambient UVR 6 weeks prior to blood draw was associated with 25(OH)D_3 status (Supplemental Table EVI). VDI in the first 6 months of life was less common among infants who were formula fed and who had no siblings (Supplemental Table EVI).

Food allergy and eczema prevalence

Of the 1074 eligible infants in the inception cohort, 83.2% (894) completed the 1 year review. 91.6% (819/894) of these had a valid skin prick test; and 82% (92/114) infants with a skin prick reaction to a food at least 1 mm larger than the negative control subsequently attended a

formal in-hospital food challenge. A total of 58 had positive food challenges and 3 had previous clinical reactions meeting the criteria for clinically proven IgE-mediated food allergy. The prevalence of challenge-proven food allergy among infants completing the 1 year review with valid outcome measures was 7.7% (95%CI, 6.0-9.8), with egg allergy the most common, 6.5% (95%CI, 5.0-8.4) (Table II).

Eczema point prevalence rates in the cohort were: 0% at 1 month; 1.2% (12/973) at 3 months; 8.5% (78/923) at 6 months; 13.6% (120/880) at 9 months; and 8% (71/884) at 1 year. The estimated eczema prevalence over the first year of life for the inception cohort was 24.2% (95% CI 21.2%-27.3%). The average SCORAD in infants with eczema at 6 months was 9.9 (S.D. 11.4) with 69% (54/78) having a score consistent with mild disease (41) At 1 year the average SCORAD was 6.6 (S.D. 8.4) with 86% (61/71) having a score consistent with mild disease.

Vitamin D status and IgE-mediated food allergy

There was no evidence of an association between VDI at either birth (aRR 1.25, 95% CI 0.70-2.22) or 6 months (aRR 0.93, 95% CI 0.41-2.14) and food allergy at 1 year of age (Figure 2). Analyses using deseasonalised $25(OH)D_3$ (Supplemental Figure E2 & Table EI) showed similar patterns of associations to the primary analysis. The mean $25(OH)D_3$ level was similar at birth (Mean Difference 2.10 nmol/L, 95%CI -3.70-7.91) and 6 months (Mean Difference -2.16 nmol/L, 95%CI -10.2-5.9) among infants with and without food allergy at 1 year of age (Supplemental Table EI). There was no evidence of an association between $25(OH)D_3 < 25$ nmol/L at birth and food allergy at 1 year of age (p=0.94). At each timepoint, there was some non-linearity between $25(OH)D_3$ levels and food allergy risk (Supplemental Figures E3 & E4) but there was no evidence to support the use of a threshold other than 50 nmol/L.

There was no evidence that change in $25(OH)D_3$ status from birth to 6 months of age was associated with food allergy at 1 year of age (aRR 0.99, 95%CI 0.96-1.02). Adjusting for C3-

epi-25(OH)D_3 in maternal samples, at birth or 6 months by adding to the 25(OH)D_3 level did not change the lack of association between 25(OH)D_3 and food allergy (Supplemental Table EIII).

There was no evidence that the lack of association between VDI during the first 6 months of infancy and food allergy at 1 year of age was modified by pet ownership status as a marker of microbial experience (p=0.96).

There was also no association evident between maternal VDI and offspring food allergy at 1 year of age (aRR 0.45 95%CI 0.06-3.47) (Supplemental Table EI), and only a weak indication of a cross-sectional association between VDI and food allergy at 1 year of age (aRR, 1.89 95%CI 0.86-4.13) (Figure 2).

Vitamin D status and eczema

Within the random subcohort there was no evidence of an association between VDI at either birth (aRR 0.88, 95%CI 0.54-1.43) or 6 months (aRR 0.84, 95%CI 0.33-2.14) and subsequent eczema (Figure 3). Adjustment for potential confounders made <10% difference to the estimated risk ratios. Analysis using deseasonalised 25(OH)D_3 also indicated no association between VDI and eczema (Supplemental Table EII & Figure E5).

Ambient UVR exposure dose and IgE-mediated food allergy

In the full cohort analysis there was no evidence of a prospective association between UVR exposure dose at individual time points, and food allergy at 1 year of age (Figure 4 & Supplemental Table EIV). Similarly, there was no evidence of a prospective association between maternal or infant UVR exposure dose and eczema in the first year of life (Supplemental Figure E6 & Table EV). There was no evidence that the relationship between UVR exposure and food allergy was modified by any level of sunscreen use (p=0.32 for moderate UVR exposure, p=0.92 for high UVR exposure).

Vitamin D status and food sensitisation

348 *Food sensitisation at year 1 defined as a SPT wheal size 3mm or greater than the negative*
349 *control:* There was no evidence that VDI at any time point was associated with food
350 sensitisation (Supplemental Table EVII). *Food sensitisation at year 1 defined as a SPT wheal*
351 *size 2mm or greater than the negative control:* There was no evidence of an association
352 between VDI during pregnancy or at birth and food sensitisation. There was however weak
353 evidence of associations between VDI at six (p=0.04) and twelve months (p=0.04) and food
354 sensitisation defined using this more inclusive threshold (≥2mm) (Supplemental Table
355 EVIII).

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357

358 **Discussion**

359 This is the first study to investigate 25(OH)D_3 measured during the first 6 months of infancy
360 in relation to challenge-proven food allergy at 1 year of age. In a predominantly Caucasian
361 cohort, among whom raw egg was the most common food allergy, there was no evidence of
362 an association between infant VDI during the first 6 months of infancy and IgE-mediated
363 food allergy at 1 year of age or eczema. There was also no evidence of an association
364 between estimated UVR exposure dose and either food allergy or eczema.

365 VDI was present in almost half of the infants at birth and a quarter of infants at 6 months of
366 age. Egg avoidance, the absence of formula feeding and a greater number of siblings were
367 each associated with increased risk of VDI during infancy. However, a large proportion of the
368 variation in vitamin D status remained unexplained and unmeasured genetic factors are likely
369 to be important (35). Food allergy prevalence, whilst less common than observed in the
370 Australian HealthNuts study (4), was still substantially higher than recent reports from
371 Europe with similar outcomes (46, 47).

372

373 Over the last decade there has been intense interest in the potential relationship between VDI
374 and food allergy, given a range of ecological findings and a wealth of mechanistic studies
375 regarding the influence of 25(OH)D3 on gut epithelial integrity (18, 48, 49) and immune
376 function (17). However the data from studies relating directly measured 25(OH)D or
377 25(OH)D_3 during early infancy to various markers of allergic disease are conflicting. Early
378 papers reported positive associations between low cord blood 25(OH)D and subsequent

allergic sensitisation (50, 51) and eczema (52). Another study using cord blood 25(OH)D_3 also reported similar associations with eczema (27). More recent studies have been unable to replicate these positive associations using either 25(OH)D (22, 53) or 25(OH)D_3 (28) as the exposure. The current paper extends the body of evidence against a prospective association between VDI and allergy by measuring 25(OH)D_3 at two time-points during early infancy and including the more robust and clinically relevant measure of challenge-proven food allergy status.

Birth 25(OH)D_3 was generally around sixty percent of the maternal level, consistent with previous studies (54). Thus relatively lower 25(OH)D_3 levels in cord blood may be physiological, and it is uncertain whether a cut-off of 50 nmol/L to define VDI in cord blood is appropriate, particularly as this threshold is based predominantly on markers of bone health in adults (32) and an appropriate immune related level remains uncertain. Accordingly, we explored a range of threshold levels including 25(OH)D_3 < 25 nmol/L as well as 25(OH)D_3 quintiles, but remained unable to identify a relationship between cord 25(OH)D_3 and food allergy at 1 year of age.

It has been proposed that 25(OH)D_3 status may be particularly important during the period of introduction of dietary solids (8, 55). A small study has previously suggested 25(OH)D_3 may promote immune tolerance during initial exposure to solids (56), but our study is the first to relate 25(OH)D_3 around the time of weaning to the clinically relevant outcome of challenge-proven food allergy. There was no evidence of an association between VDI at 6 months and food allergy at 1 year of age.

Whilst there is consistent evidence of an association between latitudes further from the equator and various proxy markers of food allergy prevalence (10, 11, 14, 57), we were unable to demonstrate an association between UVR exposure measured at the individual level and allergic outcomes. Our measure, which combined ambient UVR and parent-reported time in the sun (35) was associated with 25(OH)D_3 at each time point, and is likely to provide a more accurate estimate than latitude alone, which may be a proxy for a wide range of factors including ethnicity, genetics and microbial environment. Our findings therefore suggest that the relationship between latitude and allergic disease may relate to factors other than 25(OH)D_3 status.

The strengths of the study include the use of a population-derived cohort with adequate retention rates; measurement of infant 25(OH)D_3 at both birth and during the introduction of solids; delineation of food allergy status by formal food challenge; and detailed measurement of relevant covariates, including UVR exposure at the individual level. We were also able to conduct relevant sensitivity analyses and tests of effect modification, and show that these did not substantially alter the findings. The predominantly Caucasian cohort limits the generalisability of the findings, and this may be important in the context of growing evidence that the relationship between either 25(OH)D (58) or 25(OH)D_3 (45) and food allergy may be modified by genetic factors. On the other hand, the relative homogeneity of the BIS cohort assists internal validity. We did not measure free 25(OH)D and vitamin D binding protein which limited our ability to investigate the role of bioavailable 25(OH)D. The statistical power was limited by the number of food allergy cases and it is important to recognise that the 95% confidence intervals around our point estimates did not exclude potential effects of a relative risk up to about 2-fold. It is also noteworthy that there were only 12 cases of peanut allergy; and the aRR regarding a cross-sectional association between VDI and food allergy at 1 year is not inconsistent with the positive association observed among infants of Australian parents in the HealthNuts study (21). We were unable to conduct a meaningful analysis of the relationship between 25(OH)D_3 status and severe eczema as there were very few children with severe eczema in the current study.

In conclusion, in a predominantly Caucasian cohort, in which egg was the commonest food allergy, there was no evidence of a longitudinal association between either VDI during the first 6 months of infancy, or UVR exposure, and challenge-proven food allergy at 1 year of age or eczema. Our findings require replication in larger observational studies and/or clinical trials, including a sufficient number of children with peanut allergy, and relating directly measured 25(OH)D_3 to robust measures of clinically relevant food allergy status. Until such studies are completed, the balance of current evidence does not support the widespread implementation of routine vitamin D supplementation during early infancy for the purpose of preventing allergic outcomes.

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Tables and Figures:

Table I: Participant baseline characteristics

Characteristic	Inception birth cohort (n =1074)	Random subcohort (n=274)	Food allergic (n=61)
Sex of child			
– Male	557 (51.9%)	153 (55.8%)	34 (55.7%)
– Female	517 (48.1%)	121 (44.2%)	27 (44.3%)
Maternal country of birth			
– Australia	946 (88%)	235 (85.8%)	56 (91.8%)
– Other	107 (10%)	32 (11.7%)	5 (8.2%)
– unknown	21 (2%)	7 (2.5%)	0 (0.0%)
Paternal country of birth			
– Australia	923 (85.9%)	233 (85.0%)	49 (80.3%)
– other	109 (10.2%)	25 (9.1%)	8 (13.1%)
– unknown	42 (3.9%)	16 (5.8%)	4 (6.6%)
Participant Caucasian ethnicity			
– yes	772 (71.9%)	206 (75.2%)	43 (70.5%).
– no	299 (27.8%)	67 (24.4%)	17 (27.9%)
– unknown	3 (0.3%)	1 (0.4%)	1 (1.6%)
Number of siblings			
– 0	453 (42.2%)	98 (35.8%)	22 (36.07%)
– 1	383 (35.7%)	112 (40.9%)	28 (45.9%)
– 2	183 (17.0%)	52 (18.9%)	10 (16.3%)
– 3 or more	55 (5.1%)	12 (4.4%)	1 (1.6%)
Pet ownership			
– yes	815 (75.9%)	215 (78.5%)	35 (55.7%)
– no	197 (18.3%)	55 (20.0%)	25 (44.3%)
– unknown	62 (5.8%)	4 (1.5%)	1 (0.0%)
Livestock ownership			
– yes	73 (6.8%)	16 (5.9%)	1 (1.6%)
– no	985 (91.7%)	253 (92.3%)	59 (96.7%)
– unknown	16 (1.5%)	5 (1.8%)	1 (1.6%)

Family history in a first degree relative of			
– asthma	542 (50.5%)	144 (52.5%)	43 (70.5%)
– hay fever	674 (62.8%)	183 (66.8%)	47 (77.1%)
– eczema	480 (44.7%)	135 (49.3%)	40 (65.6%)
– food allergy	265 (24.7%)	65 (23.7%)	16 (26.2%)
Delivery via caesarean section	332 (30.9%)	93 (33.9%)	20 (32.8%)

Gestational age at birth			
– 32 to 36 completed weeks	47 (4.4%)	11 (4.0%)	0 (0.0%)
– 37 to 42 completed weeks	1,027 (95.6%)	263 (96.0%)	61 (100%)
– > 42 completed weeks	0 (0.0%)	0 (0.0%)	0 (0.0%)
Birth weight (kg), mean (SD)	3.53 (0.525)	3.56 (0.556)	3.51 (0.459)
Smoking			
– yes	165 (15.4%)	30 (11.0%)	9 (14.8%)
– no	891 (83.0%)	242 (88.3%)	52 (85.2%)
– unknown	18 (1.6%)	2 (0.7%)	0 (0.0%)
[#]SEIFA			
– low	268 (25.0%)	66 (24.1%)	15 (24.6%)
– middle	204 (19%)	59 (21.5%)	9 (14.8%)
– high	582 (54.2%)	146 (53.3%)	35 (57.4%)
– unknown	20 (1.9%)	3 (1.1%)	2 (3.2%)
Season of birth			
– autumn	274 (25.5%)	66 (24.1%)	10 (16.4%)
– winter	275 (25.6%)	77 (28.1%)	19 (31.2%)
– spring	309 (28.8%)	52 (19.0%)	17 (27.9%)
– summer	216 (20.1%)	79 (28.8%)	15 (24.6%)
Infant feeding (at six months)			
– breastfed exclusively	429 (39.9%)	113 (41.2%)	29 (47.5%)
– formulafed exclusively	320 (29.8%)	104 (38.0%)	15 (24.6%)
– mixed breast/formula	171 (15.9%)	46 (16.8%)	14 (22.9%)
– unknown	154 (14.4%)	11 (4.0%)	3 (5.0%)
infant feeding (at twelve months)			
– breastfed exclusively	271 (25.2%)	83 (30.3%)	18 (29.5%)
– formulafed exclusively	354 (33.0%)	111 (40.5%)	23 (37.7%)
– mixed breast/formula	260 (24.2%)	89 (28.1%)	18 (29.5%)
– unknown	189 (17.6%)	3 (1.1%)	2 (3.3%)
Introduction of solids in infancy by six months of age			
– yes	896 (83.4%)	256 (93.4%)	55 (90.2%)

– no	24 (2.2%)	7 (2.6%)	3 (4.9%)
– unknown	154 (14.4%)	11 (4.0%)	3 (4.9%)
Maternal antenatal vitamin D supplementation			
– yes	917 (85.4%)	226 (82.5%)	54 (88.5%)
– no	10 (0.9%)	1 (0.4%)	1 (1.6%)
– unknown	147 (13.7%)	47 (17.1%)	6 (9.8%)

[#]SEIFA, Socio-Economic Indexes for Areas (tertiles)

Table II: Food Allergy prevalence

Food tested	[+]Sensitised (n) ≥1mm	[#]Sensitised (n) ≥2mm	Prevalence sensitised≥2mm % 95% CI	Challenge proven food allergy (n)	Food allergy prevalence % 95% CI
All Foods	114/805	93/804	11.6 (9.5-13.9)	61/786	7.7 (6.0-9.8)
Raw Egg	83/818	70/818	8.6 (6.8-10.6)	53/808	6.5 (5.0-8.4)
Peanut	41/817	28/817	3.4 (2.3-4.9)	12/812	1.5 (0.8-2.6)
Cashew	21/818	15/818	1.8 (1.1-3.0)	4/816	0.4 (0.1-1.3)
Cow's Milk	11/818	10/818	1.2 (0.6-2.2)	4/815	0.4 (0.1 -1.3)
Sesame	3/802	2/802	0.2 (0.0-0.9)	1/801	0.1 (0.0-0.8)

[+]All foods 1 mm sensitised: Infants with a SPT 1 mm or more to one or more of the five foods were compared to infants with negative SPT's to all five foods.

[#]All foods 2 mm sensitised: Infants with a SPT 2 mm or more to one of more of the five foods were compared to infants with negative SPT to all five foods. (A single infant had a 1 mm SPT to a single food but had missing data on other SPT's so had an incomplete SPT and was excluded from the 2 mm sensitisation analysis).

Figure 1: Prevalence of 25(OH)D_3 insufficiency and sufficiency in participants in the random subsample.

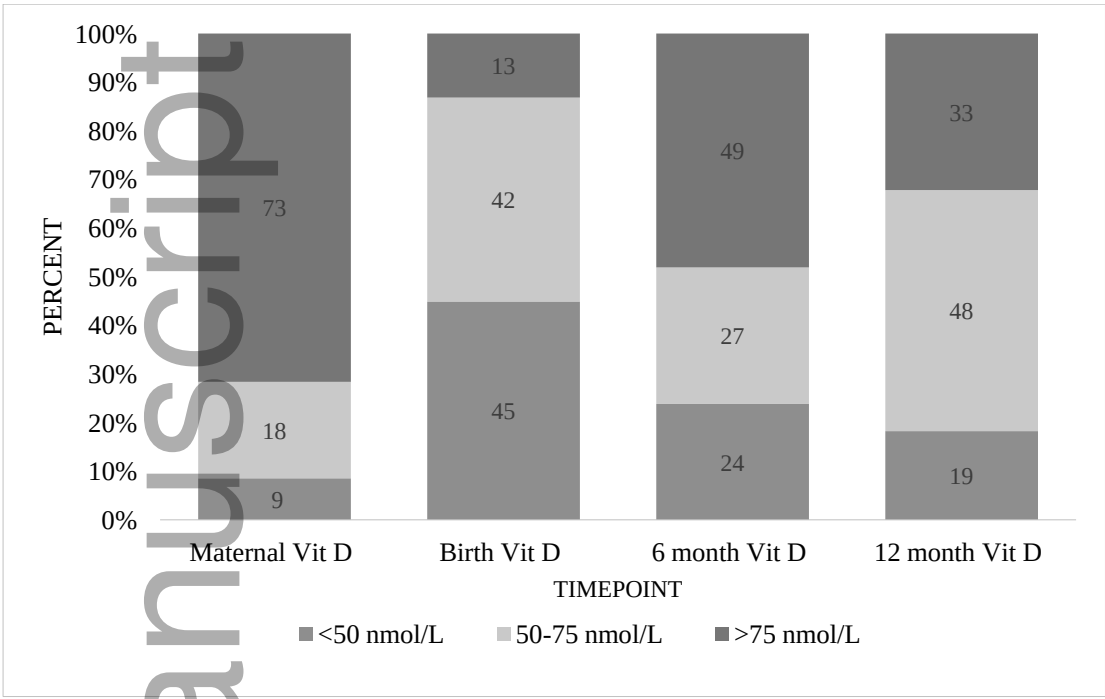
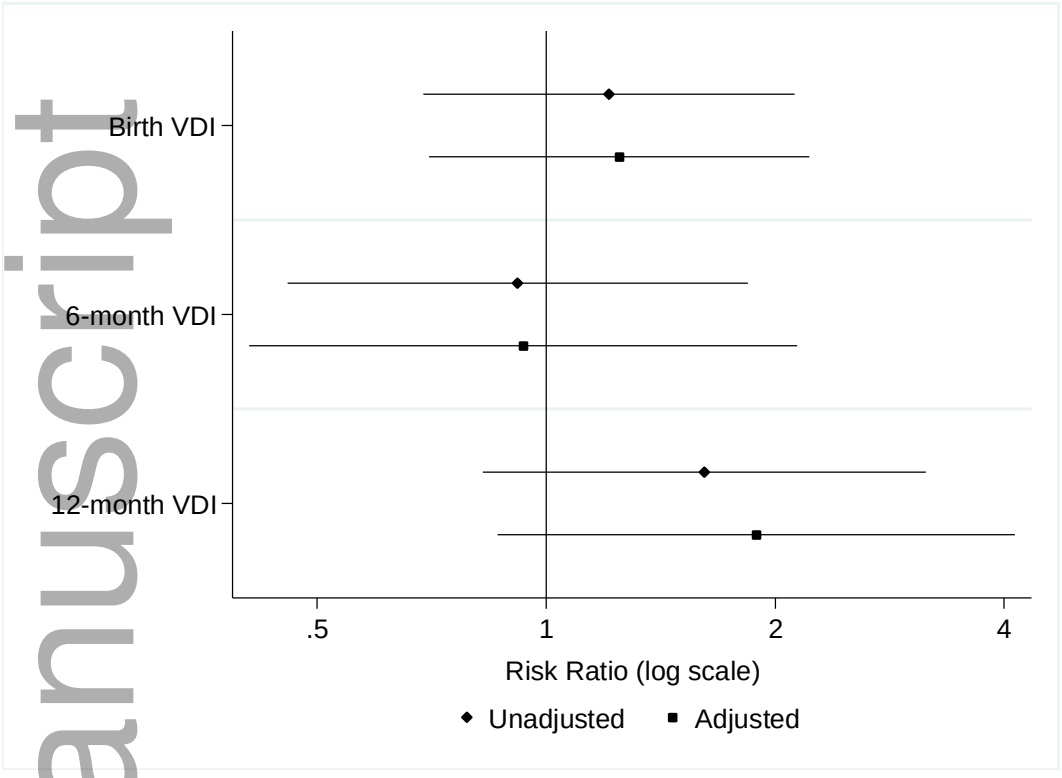
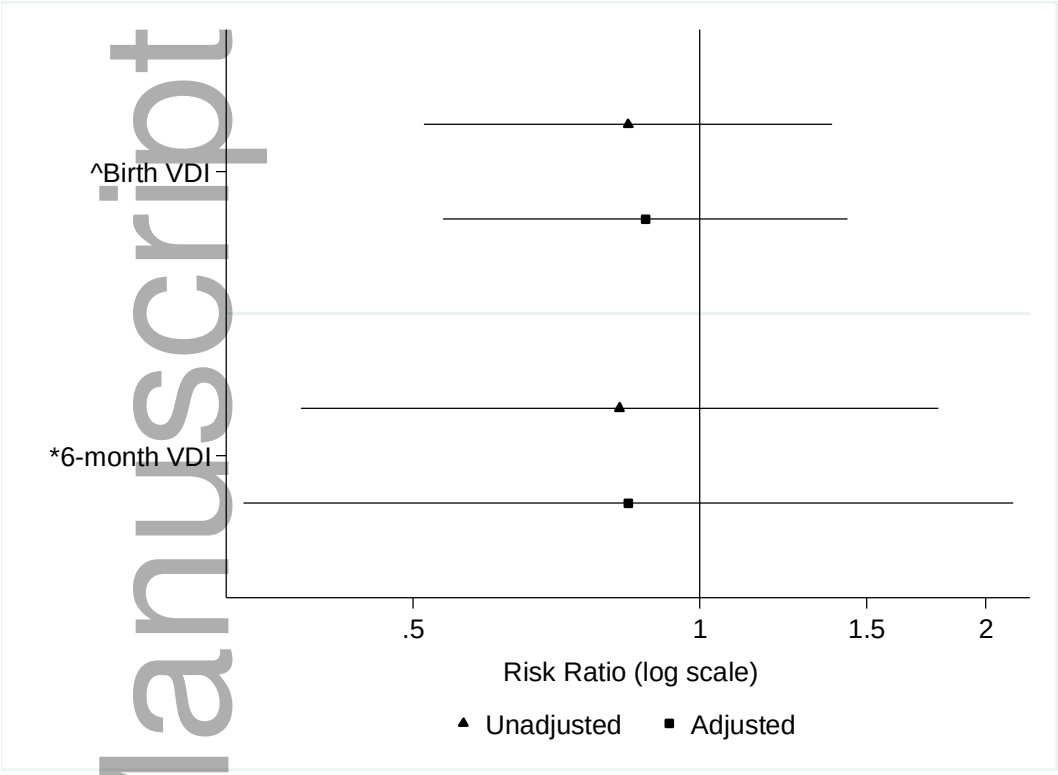


Figure 2: Association between vitamin D insufficiency and challenge proven food allergy at 1 year



Adjusted for family history of allergy, ethnicity, number of siblings, domestic pets, formula feeding at six and twelve months. Whiskers represent 95% CI for RR estimates.

Figure 3: Association between vitamin D insufficiency and eczema

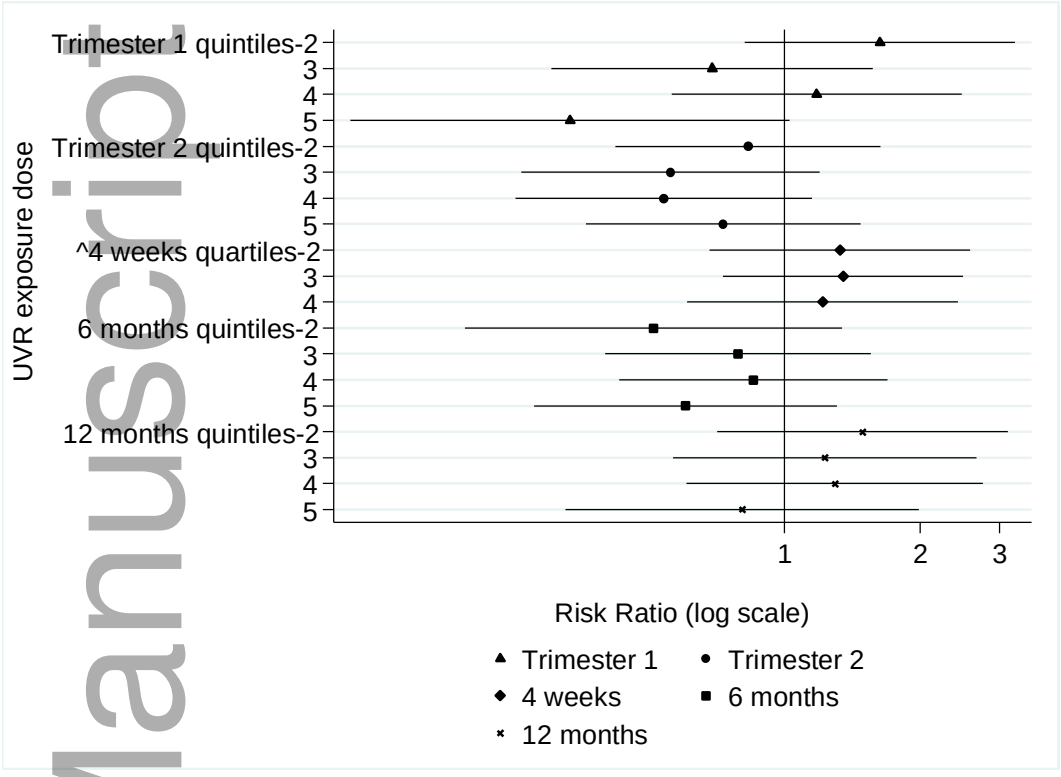


^Eczema identified during the first year of life in infants compared to infants without eczema in the random subcohort (n=274).

* Eczema identified between six and twelve months compared with infants who did not develop eczema within the random subcohort (n=274). Infants with eczema identified at six months of age or earlier were excluded (n=19).

Adjusted for family history of allergy, ethnicity, number of siblings, domestic pets and formula feeding at six months. Whiskers represent 95% CI for RR estimates.

Figure 4: Adjusted association between UVR exposure dose and challenge proven food allergy at 1 year in the whole cohort



Y axis: UVR exposure divided into quintiles at Trimester 1, Trimester 2, 6 and 12 months and lowest quintile used as baseline comparison group.

^At 4 weeks 40% of infants were reported to have minimal or no exposure to direct sunlight so UVR exposure dose at 4 weeks is divided into quartiles and lowest quartile used as baseline comparison group.

Adjusted for family history of allergy, ethnicity, number of siblings and domestic pets. Whiskers represent 95% CI for RR estimates.