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AUTHOR(S)

K Allen, J Koplin, A L Ponsonby, L Gurrin, M Wake, Peter Vuillermin, P Martin, M Matheson, A Lowe, M Robinson, D Tey, N Osborne, T Dang, H T Tan, L Thiele, D Anderson, H Czech, J Sanjeevan, G Zurzolo, T Dwyer, M Tang, D Hill, S Dharmage

PUBLICATION DATE

01-01-2013

HANDLE

10536/DRO/DU:30054674

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Vitamin D insufficiency is associated with challenge-proven food allergy in infants

Katrina J. Allen, MBBS, BMedSc, FRACP, PhD,[a,b,c]* Jennifer J. Koplin, PhD,[a,b]* Anne-Louise Ponsonby, MBBS, PhD,[a,b] Lyle C. Gurrin, PhD,[a,d] Melissa Wake, MD, FRACP,[a,b,e] Peter Vuillermin, MBBS, FRACP, PhD,[a,f] Pamela Martin, PhD,[a] Melanie Matheson, PhD,[d] Adrian Lowe, PhD,[a,d] Marnie Robinson, MBBS, FRACP,[c] Dean Tey, MBBS, FRACP,[c] Nicholas J. Osborne, PhD,[a,b,d,g] Thanh Dang, BSc,[a] Hern-Tze Tina Tan, BSc,[a] Leone Thiele, BA, MNSc,[a] Deborah Anderson, RN,[a] Helen Czech, RN,[a] Jeeva Sanjeevan, MBBS,[a] Giovanni Zurzolo, BSc,[a] Terence Dwyer, PhD,[a] Mimi L. K. Tang, MBBS, FRACP, FRCPA, PhD,[a,b,c] David Hill, MBBD, FRACP,[a] and Shyamali C. Dharmage, MBBS, MSc, MD, PhD[a,d] *Parkville and Geelong, Australia, and Devon, United Kingdom*

Background: Epidemiological evidence has shown that pediatric food allergy is more prevalent in regions further from the equator, suggesting that vitamin D insufficiency may play a role in this disease.

Objective: To investigate the role of vitamin D status in infantile food allergy.

Methods: A population sample of 5276 one-year-old infants underwent skin prick testing to peanut, egg, sesame, and cow's milk or shrimp. All those with a detectable wheal and a random sample of participants with negative skin prick test results attended a hospital-based food challenge clinic. Blood samples were available for 577 infants (344 with challenge-proven food allergy, 74 sensitized but tolerant to food challenge, 159 negative on skin prick test and food challenge). Serum 25-hydroxyvitamin D levels were measured by using liquid chromatography tandem mass spectrometry. Associations between serum 25-hydroxyvitamin D and food allergy were examined by using multiple logistic regression, adjusting for potential risk and confounding factors.

Results: Infants of Australian-born parents, but not of parents born overseas, with vitamin D insufficiency (≤50 nmol/L) were more likely to be peanut (adjusted odds ratio [aOR], 11.51; 95% CI, 2.01-65.79; $P = .006$) and/or egg (aOR, 3.79; 95% CI, 1.19-12.08; $P = .025$) allergic than were those with adequate vitamin D levels independent of eczema status. Among those with Australian-born parents, infants with vitamin D insufficiency were more likely to have multiple food allergies (≥2) rather than a single food allergy (aOR, 10.48; 95% CI, 1.60-68.61 vs aOR, 1.82; 95% CI, 0.38-8.77, respectively).

Conclusions: These results provide the first direct evidence that vitamin D sufficiency may be an important protective factor for food allergy in the first year of life. (J Allergy Clin Immunol 2013;131:1109-16.)

Key words: *Vitamin D, food allergy, peanut allergy, egg allergy, population, oral food challenge, eczema, epigenetic*

Food allergy and food allergy–related anaphylaxis have increased dramatically and inexplicably in the last two decades.[1] Recent hypotheses that low vitamin D levels may increase the risk of food allergy[2] are supported by 2 lines of ecological enquiry.

First, countries further from the equator (and thus with lower ambient ultraviolet radiation [UVR]) have recorded more pediatric admissions to hospitals for food allergy–related events[3] and more prescriptions for adrenaline autoinjectors for the treatment of anaphylaxis in children.[3,4] These findings appear to be independent of longitude, physician density, or socioeconomic status. Second, season of birth may play a role. For example, children attending emergency departments in Boston with a food-related acute allergic reaction were more likely to be born in autumn/winter, when vitamin D levels reach their nadir, than in spring/summer,[5] and similar links of food allergy to birth seasonality were reported in the Southern Hemisphere.[6] However, these indirect associations were not supported by direct serological measurements of vitamin D status nor adjusted for numerous factors that may confound or modify the association between vitamin D status and food allergy.[7] Prominent among these are ethnicity,[8] skin color,[9] and genotype.[10]

From [a]the Murdoch Childrens Research Institute, Parkville, Victoria; [b]the Department of Paediatrics, The University of Melbourne, Parkville, Victoria; [c]the Department of Allergy and Immunology, The Royal Children's Hospital, Parkville, Victoria; [d]the Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, Parkville, Victoria; [e]the Centre for Community Child Health, The Royal Children's Hospital, Parkville, Victoria; [f]the Child Health Research Unit, Barwon Health and Deakin University, Geelong, Victoria, and [g]the European Centre for Environment and Human Health, Peninsula College of Medicine and Dentistry, University of Exeter, Devon.

*These authors contributed equally to this work.

This study was supported by funding from the National Health and Medical Research Council of Australia, the Ilhan Food Allergy Foundation, AnaphylaxiStop, the Charles and Sylvia Viertel Medical Research Foundation, and the Victorian Government's Operational Infrastructure Support Program.

Disclosure of potential conflict of interest: K. J. Allen is a Viertel Senior Medical Research Fellow and has received payment for lectures from Abbott, Wyeth, and Nutricia. J. J. Koplin is partly supported by a postdoctoral fellowship from a National Health and Medical Research Council Capacity Building Grant in Population Health. A.-L. Ponsonby, L. C. Gurrin, M. Wake, M. Matheson, A. Lowe, and S. C. Dharmage are supported by the National Health and Medical Research Council. D. Tey has received payment for lectures from Merck Sharp & Dohme, Abbott Nutrition, and Alphapharm. P. Martin and T. Dang are Australian Postgraduate Award PhD scholars, and H.-T. Tina Tan is a Malaysian Government PhD scholar. N.J. Osborne is supported by the European Regional Development Fund and the European Social Fund Convergence Programme, has received grants and travel support from the National Health and Medical Research Council, and has received grants from the Australian Egg Corporation. M. Tang is on the advisory board for the Nestlé Nutrition Institute and Nutricia and has received speakers fees from Nutricia, Nestlé Nutrition Institute, and Wyeth. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication July 31, 2012; revised December 14, 2012; accepted for publication January 2, 2013.

Available online February 27, 2013.

Corresponding author: Katrina J. Allen, MBBS, BMedSc, FRACP, PhD, Murdoch Childrens Research Institute, The Royal Children's Hospital, Flemington Road, Parkville, Victoria 3052, Australia. E-mail: katie.allen@rch.org.au.

0091-6749/$36.00

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http://dx.doi.org/10.1016/j.jaci.2013.01.017

Abbreviations used
25(OH)D: 25-Hydroxyvitamin D
aOR: Adjusted odds ratio
HREC: Human Research Ethics Committee
OFC: Oral food challenge
OR: Odds ratio
sIgE: Specific IgE
SPT: Skin prick test
UVR: Ultraviolet radiation

Melbourne, the most southern major mainland city in Australia, has the highest reported prevalence of documented infantile food allergy in the world, with more than 10% of a population sample of 1-year-old infants having challenge-proven IgE-mediated food allergy.[11] In a separate study population, we have shown that children residing in Australia's southern states have twice the odds (95% CI, 1.2-5.0) of peanut allergy at the age of 4 to 5 years and thrice the odds (95% CI, 1.0-9.0) of egg allergy than do those residing in the northern states.[12] In addition, we found that delaying the introduction of egg, one of breast-fed infants' richest sources of vitamin D in the first year of life, trebled the odds of developing egg allergy by the age of 1 year (95% CI, 1.8-6.5).[13] Finally, a rising prevalence of vitamin D insufficiency over the last 20 years,[14] with up to 30% of Melbourne pregnant women now vitamin D insufficient,[15] has paralleled the rise in food allergy. Australia is one of the few developed countries where routine fortification of the food chain supply with vitamin D does not occur.

By drawing on baseline data from the HealthNuts cohort study, we aimed to examine the association between vitamin D insufficiency and food allergy in infants aged 12 to 18 months.

METHODS

Design, participants, and procedures

HealthNuts is a large-scale, population-based cohort study undertaken to assess the prevalence and risk factors for allergic disease in early childhood.[11,13,16] Briefly, by using a predetermined population-based sampling frame drawn from local government–led immunization clinics in Melbourne, Australia (population 4 million), infants were recruited while attending 1-year-old immunization sessions at 1 of more than 120 locations. Recruitment took place between September 2007 and August 2011. All infants aged between 11 and 15 months (inclusive) and attending council-led immunization sessions were eligible for recruitment (74% response rate). Reasons for nonparticipation included eating and tolerating all foods (24.5%), testing too painful for the child (18.0%), too busy (8.7%), parent did not speak English (5.5%), and existing food allergy diagnosis (0.9%). The sample size of 5000 infants was calculated to provide sufficient power to detect risk factors present in at least 10% of the population, given a prevalence of food sensitization or allergy of 5% to 10%. Power calculations were performed by using simulation studies based on the known prevalence of individual and household risk factors from the 5000-strong infant cohort in the Longitudinal Study of Australian Children based on Wave 1 data collected in 2004 toward the end of the first year of life. Given a risk factor prevalence of 10%, this sample size was calculated to provide 84% power to detect an odds ratio (OR) of 1.75 by assuming a prevalence of food allergy of 5% as well as 98% power to detect an OR of 1.75 by assuming a prevalence of food allergy of 10%.

Parents completed a questionnaire,[16] and infants were skin prick tested to hen's egg, peanut, sesame, and either cow's milk (n = 2715) or shrimp (n = 2405) (ALK-Abelló, Madrid, Spain), with a positive control (histamine 10 mg/mL) and a negative control (saline) using single-tine lancets on the infant's back. All infants were examined for the presence of eczema. All participants with a detectable wheal to 1 or more foods, defined as 1 mm or more bigger than the negative control, were invited to a Royal Children's Hospital–based clinic, where staff administered diagnostic oral food challenges (OFCs), blinded to the infant's skin prick test (SPT) wheal size and history of ingestion (see Table E1 in this article's Online Repository at www.jacionline.org for challenge protocols). We chose any detectable wheal size as our entry criterion to assess the food allergy status of participants to ensure we were not missing any cases of potential food allergy. Repeat SPTs were undertaken at the time of the OFC, and only those infants with both a positive food challenge by objective criteria and an SPT wheal size of 2 mm or more or specific IgE (sIgE) level of 0.35 kU_A/L or more were deemed food allergic. A random sample of infants with negative SPT results was also invited to undergo a food challenge (negative controls for the clinic study sample). SPT was repeated at clinic attendance (6-8 weeks after the initial contact) by using an extended panel of foods: egg, peanut, sesame, cow's milk, shrimp, cashew, hazelnut, almond, wheat, and soy (ALK-Abelló). Blood sample for serum vitamin D and food-specific IgE to egg, peanut, sesame, and cow's milk or shrimp was obtained.

In a substudy, vitamin D supplementation, parents' ethnicity, and skin type were also assessed in a random subsample of infants (N = 350; see Table E4 in this article's Online Repository at www.jacionline.org) by using a graded skin color chart shown to have excellent agreement with melanin density measured with a spectrophotometer.[17]

Measures of outcome: Food allergy definitions

IgE-mediated allergy to egg, peanut, and sesame. Positive OFC, that is, 1 or more than 1 of the following: 3 or more concurrent noncontact urticaria lasting 5 minutes or more; perioral/periorbital angioedema; vomiting; or circulatory or respiratory compromise within 2 hours of ingestion of a challenge dose.[16] Infants underwent OFC irrespective of their history of ingestion or SPT wheal size unless there was a clear history of an immediate reaction to the food in question (as per the HealthNuts challenge criteria) within the past 1 month for egg or 2 months for peanut or sesame. Only infants with a positive food challenge or recent reaction by these objective criteria and an SPT wheal size of 2 mm or more or sIgE level of 0.35 kU_A/L or more were deemed food allergic. Infants with an SPT wheal size of 8 mm or more to one of the other foods (cashew, hazelnut, almond, wheat, soy, cow's milk, or shrimp) on the extended panel (n = 7) at the challenge clinic were presumed food allergic to that food.

Food-sensitized tolerant. SPT wheal size of 2 mm or more and/or sIgE level of 0.35 kU_A/L or more at the clinic to egg, peanut, or sesame and negative OFC. If sensitized to more than 1 food, OFC results had to be negative to all foods to which sensitization was present.

Non–food-sensitized tolerant. SPT wheal size of less than 2 mm and sIgE level of less than 0.35 kU_A/L to all foods at the clinic in conjunction with a negative OFC to either peanut or egg. Infants with a positive OFC despite a negative SPT (n = 11) were excluded from the analysis as their IgE-mediated allergy status was unclear.

The current diagnosis of eczema at age 12 months was defined as either a parent report of diagnosed eczema currently requiring medication or eczema observed by a trained nurse at the time of recruitment.

Measures of exposure: Vitamin D and UVR

Vitamin D status. Vitamin D deficiency: 25-hydroxyvitamin D_3 ($25(OH)D_3$) level of 25 nmol/L or less; vitamin D insufficiency: $25(OH)D_3$ level of 26 to 50 nmol/L; vitamin D sufficient: $25(OH)D_3$ level of more than 50 nmol/L.[18] Serum $25(OH)D_3$ level was measured in 2 batches by using liquid chromatography tandem mass spectrometry at the Royal Melbourne Institute of Technology. Extracts were derivatized with 4-phenyl-1,2,4-triazoline-3,5-dione prior to analysis with liquid chromatography tandem mass spectrometry. This laboratory has previously demonstrated high interbatch agreement for duplicate samples (n = 39 pairs; intraclass correlation = 0.89).[17] We fitted a sinusoidal curve to data on $25(OH)D_3$ levels[19] and the date of blood sampling and took the difference between observed and fitted vitamin D levels (ie, the regression residual) to represent a seasonally adjusted measure of vitamin D.

TABLE I. Characteristics of HealthNuts infants by food allergy outcome

Characteristic	All infants who were nonallergic to food (n = 3794)	Infants included in the current analysis (%)*	
		Infants who were nonallergic and attended clinic (n = 207)	Infants who were allergic to food (n = 274)
Family characteristics			
Family history of allergic disease†	68.9	76.1	75.1
Family history of eczema	29.4	37.1	38.0
Family history of food allergy	13.0	19.3	12.4
Both parents born in Australia	62.5	68.1	47.8
Maternal smoking during pregnancy	4.7	3.8	3.0
Maternal multivitamin use during pregnancy (without additional vitamin D)‡	74.0	78.9	75.5
Maternal vitamin D use during pregnancy	5.3	7.2	4.2
Any siblings	50.6	56.8	47.1
Infant characteristics			
Sex: male	50.0	49.8	59.9
Current eczema (at 12 mo)	21.1	29.2	52.6
History of eczema diagnosis (by age 12 mo)	20.1	31.5	57.8
Ever breast-fed	94.2	96.7	94.9
Ever used infant formula	78.9	72.8	77.4
Ever consumed egg	97.0	95.8	92.0
Vitamin D supplement in infancy	–	14.5	7.5
Vitamin D insufficiency (26-50 nmol/L)	–	16.4	17.9
Vitamin D deficiency (≤25 nmol/L)	–	1.4	6.3

*Restricted to those infants with both vitamin D results available and food allergy status known (see Fig E1) and who also had complete data on all the characteristics captured in this table.

†Asthma, allergic rhinitis, eczema, or food allergy.

‡The most commonly used multivitamin supplements taken during pregnancy in this cohort were Blackmores and Elevit pregnancy supplements containing 6.3 and 12.5 μg vitamin D_3 per capsule, respectively.

UVR at the month of birth. It was defined as the average of ambient daily UVR for the month in which the child was born by using Australian Radiation Protection and Nuclear Safety Agency data for Melbourne's monthly average daily total UVR dose in standard erythemal doses from 2007 to 2012.

Socioeconomic status was assigned on the basis of home postcode by using Socio-Economic Indexes for Areas (SEIFA) measures derived from the 2006 Australian census, which assess relative socioeconomic advantage/disadvantage, economic resources (income, assets, and expenditure), and educational and occupational characteristics.[20]

Statistical analysis

The variation in vitamin D levels attributable to a single risk factor was quantified by calculating the proportional reduction in the residual sum of squares (ie, R^2) from simple linear regression models. We used a multivariable logistic regression model to estimate ORs and thus quantify the association between vitamin D levels and the odds of food allergy. The current use of infant formula (none, formula with continued breast-feeding, and formula alone) and history of egg consumption (none, baked goods containing egg [eg, cakes and biscuits], cooked egg [eg, scrambled or soft boiled egg] on 1 occasion, and cooked egg on multiple occasions) were both selected *a priori* for inclusion in the final model, as both are dietary sources of vitamin D and associated with the odds of infant food allergy.[13,21] Other potential confounders (infant history of eczema, age [in months] at OFC, infant's sex, duration of breast-feeding, infant consumption of fish [yes/no], number of siblings, socioeconomic status, pet ownership, maternal use of vitamin D supplements during pregnancy, maternal smoking during pregnancy, family history of allergy, filaggrin null mutations, season of birth, and UVR at the month of birth) were retained in the regression model only if their inclusion caused a more than 10% change in the magnitude of the association between vitamin D status and the odds of food allergy. Nested models for 2 sample comparisons of proportions (those with or without a binary exposure, those with and without interaction terms, or those with different representations of the same exposure variable) were compared by using the likelihood ratio test.

On the basis of *a priori* decisions, analyses were stratified for parental birthplace (ie, both parents born in Australia) as a proxy for fairer skin. In the substudy (see Table E4), 93% of the infants of Australian-born parents and 51% of the infants of non–Australian-born parents had fair or medium-fair skin. Of infants who were dark or olive-skinned, 92% had a parent born overseas. We also conducted subgroup analyses on the sensitized group only,[22] dichotomizing as food allergic versus tolerant.

To mitigate bias in estimated ORs due to low attendance fraction at the clinic for infants who were nonsensitized, we generated weights as the inverse of the probability of clinic attendance for all infants who were nonsensitized within groups defined by cross-classifying family history of food allergy, infant history of eczema, and parents' country of birth[23] (see this article's Online Repository available at www.jacionline.org). These weights were used in the corresponding logistic regression models, with robust standard errors used to ensure that the precision of estimated ORs reflected the sample size.

Analyses were performed by using Stata (version 11.1, College Station, Tex).

Ethics

Ethical approval was obtained from the Office for Children Human Research Ethics Committee (HREC; ref no CDF/07/492), Department of Human Services HREC (ref no 10/07), and Royal Children's Hospital HREC (ref no 27047).

RESULTS

Between September 28, 2007, and August 10, 2011, 7134 eligible parents/guardians were approached and 5276 (average age, 12.7 ± 0.7 months) agreed to participate. Of these, 5120 infants completed an SPT and 1089 (21%) had a positive SPT to 1 or more of the 4 foods tested (see Fig E1 in this article's Online Repository available at www.jacionline.org). Of these, 928 (85%) attended the OFC clinic at the age of 14 to 18 months along with

TABLE II. Association between vitamin D insufficiency (≤50 nmol/L) and food allergy and/or eczema

Outcome and stratification variables	OR (95% CI)	*P* value	aOR (95% CI)*	*P* value
Association between vitamin D and any food allergy				
Among all infants (n = 481)†				
No food allergy (n = 207)	1.0		1.0	
Any food allergy (n = 274)	1.38 (0.88-2.17)	.16	1.29 (0.51-3.25)	.59
Among infants with 1 or both parents born overseas (n = 210)				
No food allergy (n = 67)	1.0		1.0	
Any food allergy (n = 143)	0.73 (0.38-1.41)	.35	0.39 (0.08-1.76)	.22
Among infants with both parents born in Australia (n = 271)				
No food allergy (n = 140)	1.0		1.0	
Any food allergy (n = 131)	2.14 (1.15-4.00)	.017	3.08 (1.10-8.59)	.032
Association between vitamin D and specific food allergies among infants with both parents born in Australia‡				
No food allergy (n = 140)	1.0		1.0	
Single food (n = 96)	2.40 (1.07-5.42)	.035	1.82 (0.38-8.77)	.46
Multiple foods (n = 32)	5.67 (2.26-14.28)	<.001	10.48 (1.60-68.61)	.014
No food allergy (n = 140)	1.0		1.0	
Egg allergy (n = 110)	3.36 (1.56-7.22)	.002	3.79 (1.19-12.08)	.025
No food allergy (n = 140)	1.0		1.0	
Peanut allergy (n = 33)	3.11 (1.17-8.23)	.023	11.51 (2.01-65.79)	.006
Association between vitamin D and eczema§				
Among all infants (n = 481)†				
No eczema (n = 166)	1.0			
History of eczema (n = 103)	1.22 (0.68-2.17)	.50	0.50 (0.09-2.66)	.42
Current eczema (n = 174)	0.98 (0.58-1.64)	.92	1.34 (0.37-4.79)	.66
Among infants with both parents born in Australia (n = 271)				
No eczema (n = 103)	1.0		1.0	
History of eczema (n = 56)	1.29 (0.57-2.91)	.54	1.09 (0.11-10.76)	.94
Current eczema (n = 99)	1.27 (0.63-2.56)	.50	2.19 (0.28-16.92)	.45
Among infants with 1 or both parents born overseas (n = 210)				
No eczema (n = 63)	1.0		1.0	
History of eczema (n = 47)	1.06 (0.46-2.43)	.89	0.21 (0.03-1.45)	.11
Current eczema (n = 75)	0.68 (0.31-1.47)	.33	1.04 (0.18-6.21)	.96

*The adjusted analysis controlled for date of blood draw, current use of infant formula (classified as none, formula with continued breast-feeding, and formula alone), and history of egg consumption (classified as none, baked goods containing egg, cooked egg on 1 occasion, and cooked egg on multiple occasions), and mothers' country of birth (for those born outside Australia). Food allergy outcomes were adjusted for the current eczema status, and eczema outcomes were adjusted for food allergy. Adjustment for socioeconomic status, sex, age at OFC clinic attendance, infant consumption of fish, number of siblings, family history of allergy, maternal use of vitamin D supplements during pregnancy, dog ownership, and maternal smoking—each changed the magnitude of the association between low vitamin D status and food allergy by <10%; thus, these variables were not included in the final model. To mitigate bias in estimated ORs owing to the low attendance fraction at the clinic for infants who were nonsensitized, the final model also included the use of sampling weights defined as the inverse of the probability of clinic attendance for all infants who were nonsensitized within groups defined by cross-classifying family history of food allergy, infant history of eczema, and parents' country of birth.

†This analysis was additionally adjusted for parents' country of birth. Difference in effect for food allergy by parents' country of birth in the adjusted model ($P = .003$). Difference in effect for eczema by parents' country of birth in the adjusted model ($P = .40$ for current eczema; $P = .18$ for history of eczema).

‡Three infants who were allergic to food were excluded from the analysis of single versus multiple food sensitization because of missing food allergy status data for 1 or more foods to which they were sensitized. The number of infants included in the egg- and peanut-specific food allergy analysis does not add up to the total; for example, the egg allergy analysis compared only infants with no food allergies to infants with egg allergy. Infants allergic to peanut but not egg were not included in this analysis.

§Thirty-eight infants had missing data on eczema status and were excluded from this analysis.

197 (20%) of the negative controls. Overall, 708 infants provided a blood sample for vitamin D testing, and food allergy status could be determined for 577 of these (see Fig E1 for inconclusive food allergy classifications). Complete data on confounders were available for 481 (83%) of the 577 infants with known vitamin D and food allergy status. All analyses were restricted to the 481 infants with complete information on vitamin D, food allergy, and confounders. Table I shows characteristics of these 481 infants compared with those of the whole study population. Table E3 in this article's Online Repository at www.jacionline.org compares characteristics of infants with and without blood samples available.

Serum vitamin D (25(OH)D_3) levels in infants at the age of 14 to 18 months

Infants with both parents born in Australia had a significantly higher mean vitamin D level than did those without (mean difference, 7.61 units; 95% CI, 3.19-12.04; $P < .001$; see Fig E2 in this article's Online Repository available at www.jacionline.org). Ambient UVR levels 6 weeks prior to blood draw accounted for only 10% and 9% of the variation in serum vitamin D levels among infants of Australian- and non–Australian-born parents, respectively. Ambient UVR levels at the time of birth explained even less of the variation in serum 25(OH)D levels. Among infants of Australian- and non–Australian-born parents, the use of infant formula accounted for 4.6% and 10.5 % and the consumption of egg 0.8% and 0.7% of the variation in vitamin D levels, respectively.

Association between serum vitamin D levels and food allergy and/or eczema

Parents' country of birth was the only variable investigated that modified the association between vitamin D and food allergy

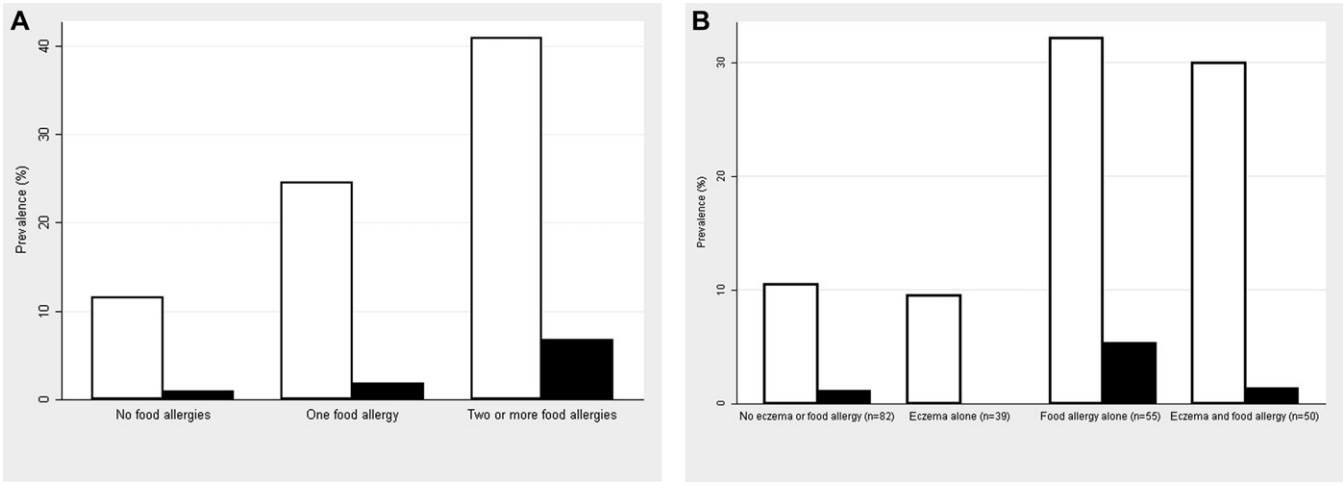


FIG 1. Prevalence of vitamin D insufficiency (*open bar*, ≤50 nmol/L) and deficiency (*solid bar*, ≤25 nmol/L) among various food allergy phenotypes **(A)** and food allergy with and without eczema among infants with both parents born in Australia **(B)**. Compared with infants with neither eczema nor food allergy, vitamin D insufficiency was significantly more common in infants with food allergy with or without eczema ($P < .005$ for both comparisons) but not in infants with eczema alone ($P = .87$). Vitamin D insufficiency was also significantly more common among infants with 2 or more food allergies compared with infants with 1 food allergy ($P = .045$).

(P for interaction = .003). Among those with Australian-born parents, those with vitamin D insufficiency were more likely to be food allergic. This association was not evident for infants of parents born outside of Australia (Table II). The prevalence of both vitamin D deficiency and insufficiency was higher in all infants with any type of food allergy but not in those with eczema alone (Fig 1). We found no evidence that vitamin D levels in quintiles compared with a binary variable for vitamin D insufficiency (cut point 50 nmol/L) provided a better model for the prevalence of food allergy ($P = .24$ and .28 for infants of parents born in Australia and outside Australia, respectively), and so the latter representation was retained.

Adjusting for date of blood draw strengthened the magnitude of the association between vitamin D and food allergy among Australian-born infants (adjusted odds ratio [aOR], 3.21; 95% CI, 1.21-7.88) as did adjustment for infant diet (Table II). There was a dose-response association between the number of food allergies recorded and the frequency of vitamin D insufficiency (more than 2 food allergies vs no food allergy: aOR, 10.48; 95% CI, 1.60-68.61). Among infants with both parents born in Australia, vitamin D insufficiency was independently associated with food allergy after adjusting for eczema, but not with eczema after adjusting for food allergy (Table II).

We found no strong evidence of an association between food allergy in the first year of life and any of the following: ambient UVR levels at birth, season of birth, ambient UVR levels 6 weeks prior to blood draw, and maternal use of vitamin D supplements during pregnancy (Table III). However, there was some suggestion of a threshold effect of low ambient UVR at birth, with infants born in the lowest quintile having a small increase in odds of food allergy compared with those in other quintiles (OR, 1.33; 95% CI, 0.99-1.80; $P = .062$).

Among infants who were food sensitized, vitamin D insufficiency was associated with increased odds of food allergy (Table IV). This association was also evident in the substudy by using skin color rather than country of birth stratification (data not shown).

To investigate the impact of missing data on food allergy status, we conducted a sensitivity analysis that reclassified as "sensitized and tolerant" those infants (n = 44) with vitamin D data available who had at least 1 negative challenge to a food to which they were sensitized but had been excluded from the primary analysis as they had not undergone challenge to all foods to which they were sensitized (see Fig E1 for details). This did not substantially alter any of the associations reported (data not shown).

DISCUSSION

Infants of Australian-born parents with vitamin D insufficiency were 3 times more likely to have egg allergy and 11 times more likely to have peanut allergy, the odds increasing 10-fold among those with 2 or more food allergies. Furthermore, among infants who were food sensitized, those with vitamin D insufficiency were 6 times more likely to be food allergic than tolerant. This is the largest study to ascertain objectively food sensitization status in an entire population-based sample,[16] to use the gold standard measure of food allergy status of all infants who were sensitized, and to explore directly the association between serum vitamin D_3 levels and challenge-proven food allergy status adjusting for a wide range of potential confounders.

A key finding was the interaction between vitamin D and parents' country of birth. The differential effect of vitamin D on food allergy depending on the parents' country of birth may be related to skin color or other unmeasured genetic, epigenetic, or environmental variables.[24] A recent study found a differential effect by genotype of the risk of vitamin D deficiency at birth and subsequent food sensitization including an effect modification by *CYP24A1*, the gene regulating the degradation of the active form of vitamin D_3.[10] Alternatively, migratory (eg, gastrointestinal microbial changes)[25] or cultural (eg, variation in lifestyle factors such as diet or vitamin D supplementation in infancy at critical developmental windows) influences may be important. In our substudy, infants of parents born overseas were more likely to have received vitamin D supplementation in early infancy (15.9% vs 8.4%). It is possible that

TABLE III. Association between season of birth, UVR, maternal vitamin D supplementation, and food allergy among infants with both parents born in Australia

	Nonallergic (n = 2518)	Food allergic (n = 240*)	OR† (95% CI)	*P* value
Season of birth				
Summer	91.5%	8.5%	1.0	
Spring	92.0%	8.0%	0.94 (0.64-1.38)	.74
Autumn	90.2%	9.8%	1.18 (0.80-1.72)	.41
Winter	91.4%	8.6%	1.01 (0.69-1.48)	.94
UVR at birth (quintiles)‡				
<8.7	89.4%	10.6%	1.0	
8.7-16.3	92.4%	7.6%	0.69 (0.46-1.05)	.084
16.4-29.1	90.8%	9.2%	0.85 (0.59-1.23)	.39
29.2-40.8	92.8%	7.2%	0.66 (0.43-1.01)	.055
>40.8	91.6%	8.4%	0.77 (0.51-1.17)	.22
UVR at recruitment/SPT (quintiles)‡				
<7.9	89.7%	10.3%	1.0	
7.9-15.7	92.7%	7.4%	0.69 (0.46-1.04)	.077
15.8-28.5	90.2%	9.8%	0.95 (0.65-1.38)	.79
28.6-40.0	91.9%	8.1%	0.76 (0.49-1.18)	.22
>40.0	92.5%	7.5%	0.71 (0.47-1.07)	.10
Maternal vitamin D supplements during pregnancy				
None	92.0%	8.0%	1.0	
Multivitamin	91.1%	8.9%	1.12 (0.77-1.62)	.55
Additional vitamin D	88.4%	11.6%	1.50 (0.78-2.88)	.22

*Defined as the average of ambient daily UVR for the month in which the child was born by using Australian Radiation Protection and Nuclear Safety Agency data for Melbourne's monthly average daily total UVR dose in standard erythemal doses from 2007 to 2012.
†Adjustments for date of blood draw, current use of infant formula (classified as none, formula with continued breast-feeding, and formula alone), and history of egg consumption (classified as none, baked goods containing egg, cooked egg on 1 occasion, and cooked egg on multiple occasions), age at OFC clinic attendance, sex, mothers' country of birth (for those born outside Australia), socioeconomic status, number of siblings, family history of allergy, maternal use of vitamin D supplements during pregnancy, dog ownership, and maternal smoking did not change the estimates of the association of UVR at recruitment and food allergy and therefore the unadjusted estimate is provided.
‡This analysis includes 109 infants who were allergic to food not included in the serum vitamin D analysis, as they did not have blood samples tested for vitamin D levels.

TABLE IV. Association between vitamin D levels and food allergy among infants who were sensitized (positive SPT results) stratified by parents' country of birth

		Food allergic, n (%)		Odds of food allergy among infants who were sensitized			
				Unadjusted		Adjusted*	
Parent's country of birth	Vitamin D (nmol/L)	No	Yes	OR (95% CI)	*P* value	OR (95% CI)	*P* value
All infants† (n = 361)	>50	59 (20.8)	225 (79.2)	1.0		1.0	
	≤50	6 (7.8)	71 (92.2)	3.10 (1.29-7.49)	.012	3.40 (1.26-9.21)	.016
Both born in Australia (n = 186)	>50	39 (26.9)	106 (73.1)	1.0		1.0	
	≤50	3 (7.3)	38 (92.7)	4.66 (1.36-15.97)	.014	5.98 (1.44-24.79)	.014
One or both born outside Australia (n = 175)	>50	20 (14.4)	119 (85.6)	1.0		1.0	
	≤50	3 (8.3)	33 (91.7)	1.85 (0.52-6.60)	.34	1.99 (0.44-8.89)	.37

*The adjusted analysis controlled for date of blood draw, current use of infant formula (classified as none, formula with continued breast-feeding, and formula alone), history of egg consumption (classified as none, baked goods containing egg, cooked egg on 1 occasion, and cooked egg on multiple occasions), age at OFC clinic attendance, sex, and mothers' country of birth (for those born outside Australia).
†Difference in effect by parents' country of birth in the adjusted model ($P = .29$).

among this group, a course of vitamin D supplementation[26] may have protected them from food allergy earlier in the first year of life, even though their measured vitamin D levels by age 12 months were low. This could lead to a spurious association between low vitamin D levels at age 12 months and protection against food allergy in infants of non–Australian-born parents.

A similar interaction between vitamin D and ethnicity has been reported by Keet et al,[27] who found that birth in autumn was associated with food allergy only among white children, although they did not directly examine individual vitamin D levels. However, unlike Keet et al we found no evidence of an interaction between eczema and vitamin D levels, possibly reflecting the differences in age groups studied (all participants in HealthNuts were aged 1 year, while individuals who were allergic to food in the study by Keet et al were aged up to 21 years). It is also possible that the interaction between season of birth and eczema on food allergy could be related to factors other than vitamin D, such as skin care practices (with regards to eczema management and sun avoidance), in different countries.

Plausible mechanisms for the association between vitamin D and food allergy include the lack of vitamin D induction of innate epithelial defenses (such as cathelicidins) or dysregulation of tight junction proteins,[28] resulting in compromised intestinal barrier function or vitamin D–mediated alteration of gastrointestinal microbiota composition.[2] The potential role of vitamin D in the promotion of food tolerance among individuals who were sensitized in our cohort may be explained by vitamin D's capacity to induce expression of IL-10-secreting regulatory T cells.[29,30]

Season of birth, ambient UVR level, or dietary determinants explained relatively little of the variation in serum vitamin D levels compared with data from adult studies.[31,32] Unsurprisingly, therefore, these factors did not strongly predict the development of food allergy in infants in the first year of life at the population level, although there was modest evidence of a threshold effect of low ambient UVR at birth. Although of borderline significance, this is consistent with the findings of previous studies that have used season and latitude as proxy measures for ambient UVR and have suggested a link between food allergy and winter birth and latitude.[3-6] Personal UVR measures are required to minimize the nondifferential misclassification of the exposure assessment and evaluate this more thoroughly. Our failure to replicate previous studies of season of birth and increased risk of food allergy may also be complicated by the presence of transient infantile food allergy. Against this is the fact that vitamin D is more strongly associated with both peanut allergy and multiple food allergies (having more than 1 of the following: peanut, egg, or sesame allergy) in our cohort—factors more closely associated with persistence.[33]

It remains possible that atopy-related changes in behavior led to a low vitamin D status, rather than the reverse. However, the fact that most parents were as yet unaware of the food allergy status of the infant at the time of the blood draw argues against this. Furthermore, adjustment for other factors that would have led to any behavioral changes such as family history did not confound the association. Finally, the association between low vitamin D levels and food allergy remained evident among the parents who did not perceive their child to have a food allergy prior to challenge (data not shown). Although the low uptake of OFC among infants who were nonsensitized is a limitation, the rich baseline data enabled backweighting to better reflect the nonsensitized population. This backweighting increases the external validity of our findings. Parents may also have altered their child's diet because of other factors such as family history of food allergy, colic, or reflux or because of apparent intolerance or dislike of a food, which may be associated with food allergy. However, we have measured infant dietary factors important for vitamin D (consumption of egg, fish, and formula) and have adjusted for these directly; thus, this is unlikely to explain our findings. In Australia, although infant formula is fortified with vitamin D, cow's milk is not. Although there was a proportion of eligible participants who did not participate in the study, who may have been less likely to have food allergy, it is unlikely that there was also differential participation based on vitamin D levels as this was not primarily a study of vitamin D and the hypothesis that vitamin D may be associated with food allergy was not widely known at the time the study was conducted. In addition, those who participate in research studies tend to be more likely to comply with health guidelines (such as those recommending sun protection) and thus may have had lower vitamin D levels, leading to an underestimate of the magnitude of the association between vitamin D and food allergy.

Vitamin D insufficiency at age 12 months is associated with an increased odds of food allergy among infants with Australian-born parents, particularly among infants demonstrating allergic sensitization. Randomized controlled trials stratified by genetic, racial, or migratory status are required to determine whether the correction of vitamin D status either prevents infantile food allergy or promotes the development of tolerance in infants who were allergic to food.

We thank the children and parents who participated in the HealthNuts study as well as the staff of Melbourne's Local Government Areas for providing access to community immunization clinics. We thank Nadine Bertalli for formatting and proofing the document; ALK-Abelló SA, Madrid, Spain, for supplying the skin prick testing reagents; and the HealthNuts safety committee comprising Associate Professor Noel Cranswick (Australian Paediatric Pharmacology Research Unit, Murdoch Childrens Research Institute, Parkville, Victoria, Australia), Dr Jo Smart (Department of Allergy and Immunology, Royal Children's Hospital, Melbourne, Victoria, Australia), and Associate Professor Jo Douglass (Director, Department of Allergy and Immunology, Royal Melbourne Hospital, Melbourne, Victoria, Australia).

Clinical implications: Vitamin D sufficiency may be an important protective factor for food allergy in the first year of life.

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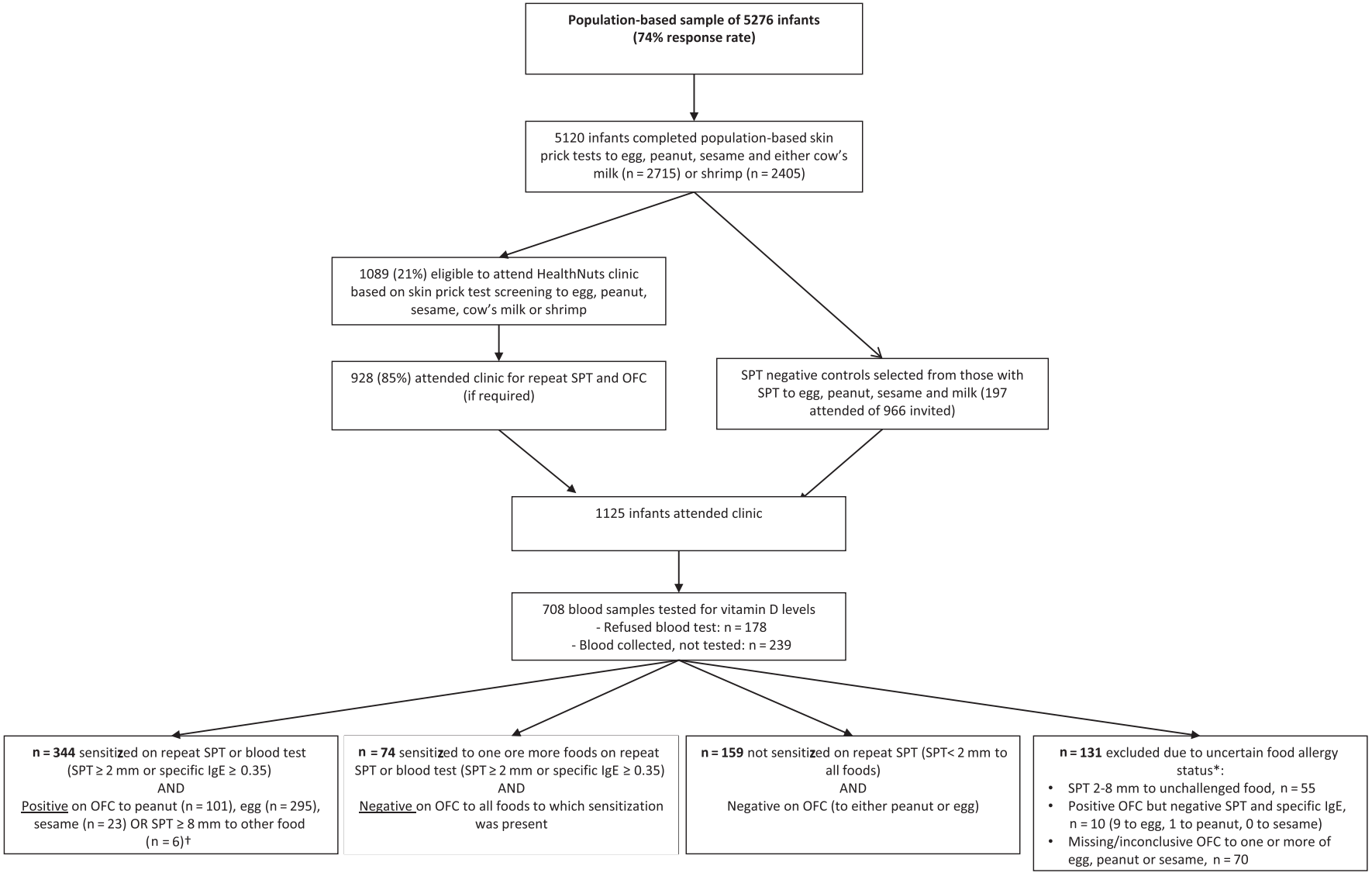


FIG E1. HealthNuts study participation and classification of food allergy status. *Note that some infants fell into more than 1 of these categories. Of these infants, 44 had at least 1 negative challenge to a food to which they were sensitized; however, their full status was unknown as they did not undergo a challenge to all foods to which they were sensitized. A sensitivity analysis was undertaken with these infants classified as "sensitized and tolerant" to examine the effect on the observed association between vitamin D levels and food allergy. †Some infants allergic to more than 1 food.

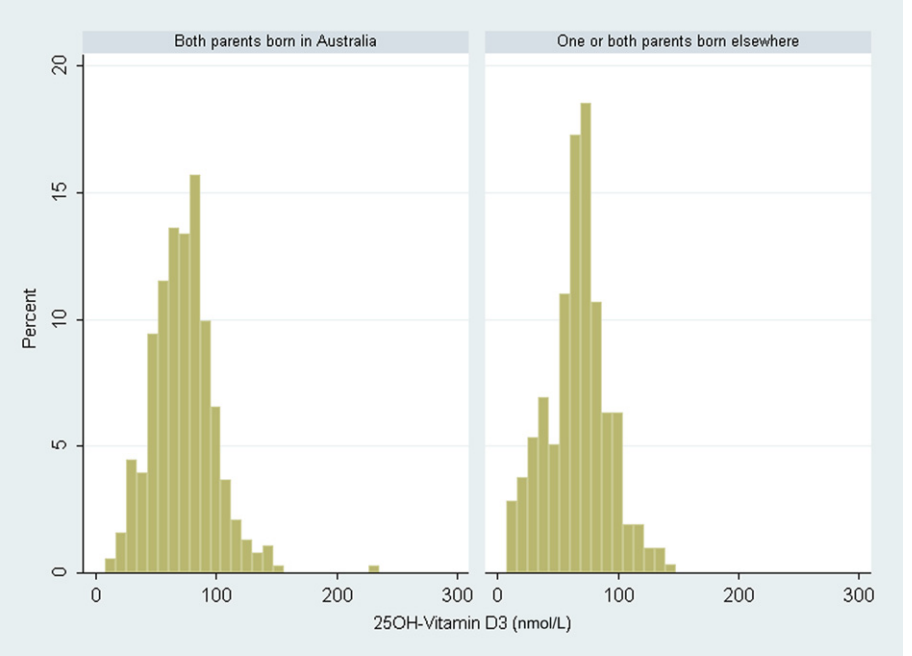


FIG E2. Distribution of vitamin D levels in HealthNuts serum samples by parents' country of birth (n = 708).

TABLE E1. Description of peanut, egg, and sesame oral food challenges

	Food challenge type		
	Peanut	**Sesame**	**Egg**
Food used for challenge	Smooth peanut butter (Kraft, Port Melbourne, Victoria, Australia)	Unhulled tahini (Mayver's Altona North, Victoria, Australia)	Raw free range egg (Coles, Glen Iris, Victoria, Australia)
Time between doses	20 min	20 min	15 min
Total dose	1.94 teaspoons (11.3 g), contains ~2.6 g protein	9.7 mL (11.3 g), contains ~2.9 g protein	Whole egg white from 1 large egg (~58 g), contains ~4.5 g protein
Amount given in each dose	1. Smear inside lip*	1. Drop inside lip*	1. Drop inside lip*
	2. 1/16 tsp	2. 0.31 mL	2. 0.5 mL
	3. 1/8 tsp	3. 0.62 mL	3. 1 mL
	4. 1/4 tsp	4. 1.25 mL	4. 2 mL
	5. 1/2 tsp	5. 2.5 mL	5. 5 mL
	6. 1 tsp	6. 5 mL	6. 10 mL
	7. –	7. –	7. Remainder of egg white (usually 10-13 mL)

tsp, Tablespoon.

*Initial smear or drop inside the lip was administered without allowing contact with the outside of the lip. The initial dose was administered without mixing with other foods. All subsequent doses were mixed with an age-appropriate previously tolerated food based on parental preference.

TABLE E2. Sampling weights used in logistic regression models

Group	Family history of food allergy	Infant history of eczema	Parents' country of birth	Weight (inverse probability of clinic attendance)
1	No	No	Australia	21
2	No	Yes	Australia	15
3	No	No	Other	43
4	No	Yes	Other	26
5	Yes	No	Australia	12
6	Yes	No	Other	24
7	Yes	Yes	Australia	5
8	Yes	Yes	Other	2

TABLE E3. Differences between infants with and without blood samples available

Characteristic	Infants without blood samples (n = 417)	Infants with blood samples (n = 708)	*P* value for difference (χ^2 test)
Family characteristics			
Family history of eczema	38.5%	37.3%	.71
Family history of food allergy	17.6%	15.8%	.46
Both parents born in Australia	54.4%	54.6%	.96
Maternal smoking during pregnancy	2.8%	3.1%	.75
Maternal vitamin use during pregnancy			
None	12.8%	16.9%	
Multivitamin only	78.7%	77.2%	
Additional vitamin D	8.5%	5.9%	.074
Any siblings	42.8%	48.7%	.054
Infant characteristics			
Sex: male	49.6%	45.5%	.18
Current eczema (at 12 mo)	41.7%	44.3%	.46
History of eczema diagnosis (by age 12 mo)	44.4%	48.7%	.18
Ever breast-fed	97.3%	95.0%	.075
Ever used infant formula	75.1%	78.0%	.29
Ever consumed egg	91.7%	93.0%	.44
Food allergic	54.2%	59.6%	.11

TABLE E4. Association between parent's country of birth, skin color, and ethnicity in a substudy of 350 consecutive infants attending the OFC clinic

	Both parents born in Australia (n = 192)	Both parents born in Asia (n = 32)	Mother born in Australia, father born elsewhere (n = 49)	Father born in Australia, mother born elsewhere (n = 37)	Neither parent born in Australia or Asia (n = 37)
Maternal skin tone (%)					
Dark	0.5	0	0	2.7	29.7
Olive	5.7	26.7	12.5	43.2	21.6
Olive/medium	18.2	33.3	25.0	13.5	10.8
Medium/fair	31.8	33.3	29.2	21.6	27.0
Fair	43.8	6.7	33.3	18.9	10.8
Infant skin tone (%)					
Dark	0	3.1	0	0	21.6
Olive	1.6	25.0	6.3	16.2	18.9
Olive/medium	10.6	37.5	33.3	24.3	16.2
Medium/fair	40.8	28.1	35.4	37.8	27.0
Fair	47.1	6.3	25.0	21.6	16.2
Maternal ethnicity (%)					
Caucasian	90.1	3.1	79.6	40.5	32.4
Asian	2.6	96.9	18.4	37.8	27.0
African	0	0	0	2.7	13.5
Aboriginal or Torres Strait Islander	0	0	0	0	0
Middle Eastern	0.5	0	2.0	2.7	10.8
Other	6.8	0	0	16.2	16.2
Paternal ethnicity (%)					
Caucasian	95.7	3.1	61.7	81.1	45.9
Asian	1.6	96.9	25.5	10.8	29.7
African	0	0	0	0	10.8
Aboriginal or Torres Strait Islander	0.5	0	0	0	0
Middle Eastern	0.5	0	2.1	0	5.4
Other	1.5	0	10.6	18.1	8.1