

Peanut protein in household dust is related to household peanut consumption and is biologically active

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Background: Peanut allergy is an important public health concern. To understand the pathogenesis of peanut allergy, we need to determine the route by which children become sensitized. A dose-response between household peanut consumption (HPC; used as an indirect marker of environmental peanut exposure) and the development of peanut allergy has been observed; however, environmental peanut exposure was not directly quantified.

Objective: We sought to explore the relationship between reported HPC and peanut protein levels in an infant's home environment and to determine the biological activity of environmental peanut.

Methods: Peanut protein was quantified in wipe and dust samples collected from 45 homes with infants by using a polyclonal peanut ELISA. Environmental peanut protein levels were compared with peanut consumption assessed by using a validated peanut food frequency questionnaire and other

clinical and household factors. Biological activity of peanut protein in dust was assessed with a basophil activation assay. **Results:** There was a positive correlation between peanut protein levels in the infant's bed, crib rail, and play area and reported HPC over 1 and 6 months. On multivariate regression analysis, HPC was the most important variable associated with peanut protein levels in the infant's bed sheet and play area. Dust samples containing high peanut protein levels induced dose-dependent activation of basophils in children with peanut allergy.

Conclusions: We have shown that an infant's environmental exposure to peanut is most likely to be due to HPC. Peanut protein in dust is biologically active and should be assessed as a route of possible early peanut sensitization in infants. (*J Allergy Clin Immunol* 2013;■■■:■■■-■■■.)

Key words: Peanut, sensitization, allergy, environment, dust, ELISA, biological activity, basophil activation test

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Peanut allergy is increasing,^{1,2} is a leading cause of anaphylaxis in food allergy,³ and has a significant effect on quality of life for the child and his or her family.^{4,5} Most children react on first known oral exposure to peanut, thus sensitization must be occurring earlier.⁶⁻⁹ Understanding the way children become sensitized to peanut is therefore imperative to prevent this condition. Observational⁹ and animal¹⁰ work suggest that epicutaneous peanut exposure might play an important role in peanut sensitization; both early-onset severe eczema and the application of *Arachis* (peanut) oil onto eczematous skin are risk factors for the development of peanut allergy.⁹ A dose-response relationship has been demonstrated between household peanut consumption (HPC; used as an indirect marker for environmental peanut exposure) and the risk of peanut allergy in young children.¹¹ Environmental peanut levels were not directly measured in the study by Fox et al¹¹; however, previous studies have demonstrated quantifiable levels of egg, milk, fish, and peanut (*Ara h* 2) in vacuumed household settled dust.¹²⁻¹⁴ In one study egg and milk protein levels in dust were high enough to elicit a positive RAST result with the sera of patients with egg and milk allergy, respectively.¹²

In addition to objectively quantifying environmental peanut protein, we wished to determine whether peanut allergens in dust maintain their biological activity. Functional *in vitro* assays that closely resemble *in vivo* allergic reactions have been used in previous studies to verify the allergenic potential of peanut proteins (ie, their ability to trigger mast cell and basophil activation and degranulation), both pure and in complex mixtures.^{15,16} Levels of mediators, such as β -hexosaminidase and histamine, released by rat basophilic leukemia cells and stripped human basophils passively sensitized with plasma from allergic patients have

Abbreviations used

BAT: Basophil activation test
 FFQ: Food frequency questionnaire
 HPC: Household peanut consumption

been measured after stimulation with allergen.¹⁷ Recently, assays evaluating the expression of activation markers on the surfaces of basophils of allergic patients by using flow cytometry (basophil activation test [BAT]) have gained application in the diagnosis of IgE-mediated food allergy,¹⁸ including peanut allergy.^{19,20} The BAT has also been used to assess the allergenicity of foods²¹ and has been shown to be a sensitive and specific tool to detect trace amounts of peanut in food matrices.²²

Given that household peanut exposure is a risk factor for the development of peanut allergy,¹¹ direct quantification of environmental peanut exposure might be important in future studies to determine threshold levels of peanut exposure associated with peanut sensitization, allergy, or both. Therefore in this study we aimed to relate peanut levels in an infant's home environment to peanut consumption by other household members and other potentially contributing factors. Additionally, we anticipated that the BAT would be useful in verifying the biological activity of peanut proteins in household dust samples.

METHODS

The study was approved by the Brent Medical Research Ethics Committee and South East London Research Ethics Committee 2. Forty-six families with infants were recruited from pediatric allergy clinics. One family did not complete their dietary questionnaire and thus was not included in the study. Three peanut-monoallergic and 3 nonallergic children (not allergic to peanut or to common food or airborne allergens) were recruited for the basophil activation assays. Written informed consent was obtained from parents of all children before any study procedures.

Dust and wipe samples

Families were asked to avoid washing their bed sheets and vacuuming their infant's play areas for 5 days before our home visit. Wipe samples were obtained from the parent's table, infant's highchair table, tap, dishwasher handle, refrigerator handle, and infant's crib rail with 4 × 4 cm Benchkote filter paper (Whatman, Maidstone, United Kingdom) and moistened with PBS. Wipes were weighed before and after sampling to calculate results in micrograms of peanut protein per gram of filter. Dust samples were obtained from the beds of all household members and from the infant's play area. Parents usually slept in the same bed, and thus dust samples were taken from each side of the bed. The infant's play area was the place where the infant spent most of his or her day (eg, Moses basket, play mat/quilt, and living room carpet). A Philips cylinder vacuum FC8262 (1600 Watts) was connected to a Duststream adaptor and collector (INDOOR Biotechnologies, Warminster, United Kingdom) with a nylon collection filter (pore size, 40 μm). The infant's bed sheet was vacuumed for 1 minute within a 0.5-m² surface area. Other bed sheets and the infant's play area were vacuumed for 2 minutes within a 1-m² surface area. Further details of the dust processing and analysis are described in a separate article.²³ We used the Veratox polyclonal peanut ELISA (Neogen, Lansing, Mich) to quantify peanut protein in dust and wipes. This ELISA has been validated for sensitivity, specificity, reliability, and efficacy of extraction for determining peanut protein contamination in food.²⁴⁻²⁶ We have also performed our own validation of this ELISA to measure peanut protein levels in dust and wipe samples.²³ Results were converted from nanograms per milliliter into micrograms of peanut protein per gram of dust. All wipe and dust samples collected were blinded from the researcher performing the ELISA

analysis. The main caregiver was asked to complete a questionnaire detailing factors that could affect environmental peanut levels.

Semiquantitative peanut food frequency questionnaire

The food frequency questionnaire (FFQ) used in this study has been validated for recall against the same FFQ over 2 years.²⁷ It has also been validated against a 7-day food diary over 6 months for maternal and infant peanut consumption.²⁸ The FFQ combined peanut-containing foods and other foods so as to prevent too much emphasis on peanut and potential recall bias. Standard portion sizes were obtained by using the Ministry of Agriculture's food portion sizes.²⁹ Frequency of peanut consumption was assessed over 1 week, 1 month, and 6 months. Moderate to high peanut protein consumption was defined as 10 g/wk or greater, as previously described.²⁸ HPC was the sum of peanut protein consumed by all household members.

Whole-blood BAT

Six extracted dust samples containing high (19.3-43.5 μg/mL, n = 3) or low (0.01-0.015 μg/mL, n = 3) levels of peanut protein were used to stimulate basophils of 3 peanut-monoallergic and 3 nonallergic children, resulting in 5 pairs of dust sample-patient experiments. The extracted dust samples with high peanut content were dilution-adjusted to a maximum concentration of 10 μg/mL and then underwent serial dilutions for comparability of dose-response. The low peanut dust samples were prepared by using the same dilution factor to make comparable other potential components of the dust extract that could activate basophils independently of peanut protein. Peanut allergy was diagnosed based on a combination of a recent history of an immediate allergic reaction to peanut and peanut skin prick test responses of 8 mm or greater (Stallergenes, Antony, France) and serum peanut-specific IgE levels of 15 KU/L or greater (ImmunoCAP; ThermoFisher Scientific, Uppsala, Sweden).³⁰ Nonallergic patients were eating peanuts regularly and had negative skin prick test results and negative serum specific IgE levels to peanut and common food and airborne allergens.

Heparinized venous blood was incubated for 30 minutes at 37°C, with equal volumes of extracted dust samples or a peanut protein standard (ALK-Abelló, Hørsholm, Denmark) and diluted in RPMI medium at serial 10-fold dilutions from 10,000 to 0.1 ng of peanut protein per milliliter. Anti-IgE (Sigma-Aldrich, Dorset, United Kingdom) and N-formyl-methionyl-leucyl-phenylalanine (Sigma-Aldrich) were used as positive controls. RPMI alone was used as a negative control. Cells were stained for CD123-fluorescein isothiocyanate, HLA-DR-peridinin-chlorophyll-protein, and CD63-allophycocyanin. Surface expression of these markers was evaluated by using flow cytometry in a FACSCanto II with FACSDiva software (BD Biosciences, San Jose, Calif). Data were analyzed with FlowJo software (version 7.6.1; TreeStar, Ashland, Ore).

Sample size calculation

To determine the sample size for this study, we used data from our previous study of peanut protein in dust.³¹ Peanut levels in dust from beds on which participants had slept before and 18 to 24 hours after eating a peanut butter sandwich (n = 10) were used as an estimate for peanut levels in moderate to high (≥10 g/wk) and low (<10 g/wk) peanut-consuming families.²⁸ The mean difference in peanut levels before and after peanut consumption was 2.9 μg/m², with an SD of 2.9. Using a paired *t* test, we would need 17 moderate to high and 17 low peanut-consuming families (n = 34); however, adding 15% for unknown distribution/nonparametric test, this resulted in a sample size of 40 with a power of 80% and an α value of .05. We accounted for 15% dropout because of withdrawal or dust levels of less than 5 mg and thus recruited 46 families.

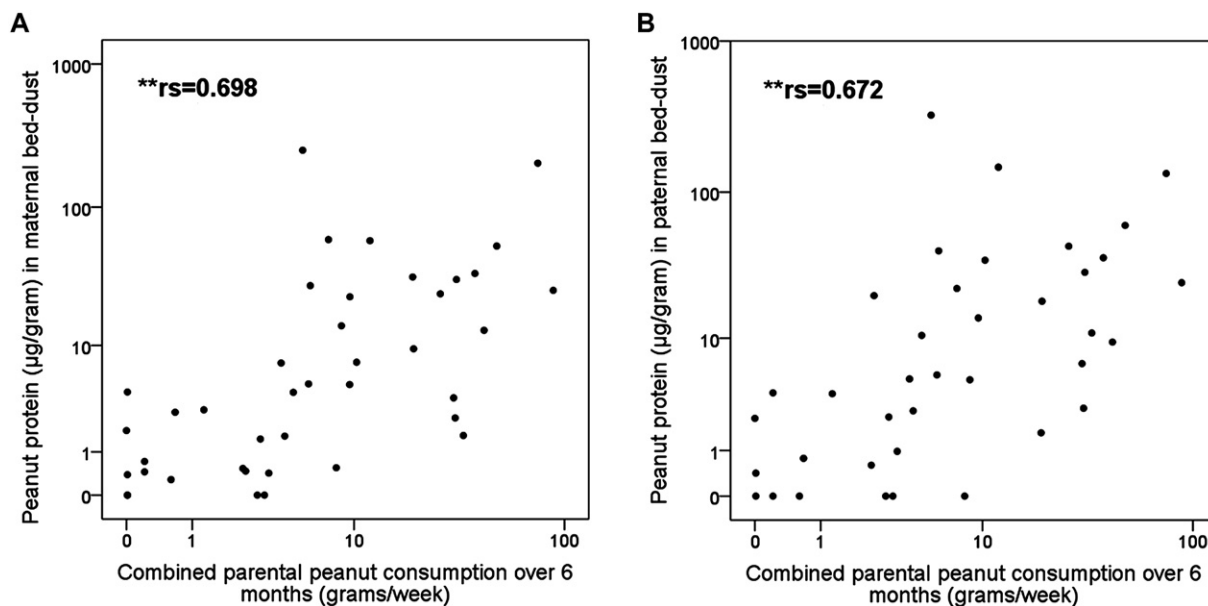
Statistical analysis

Data were entered into an SPSS spreadsheet (SPSS 17.0; SPSS, Chicago, Ill) for the purposes of analysis. Spearman rank correlation was used

TABLE I. Correlation grid (Spearman rank: r_s , 95% CI, and P value) between peanut protein consumption over 6 months (median [IQR] grams per week) and environmental peanut protein levels (median [IQR] $\mu\text{g/g}$) in household dust and surface wipes

Median (IQR)	Maternal peanut consumption, 2.40 g/wk (0.11-5.90 g/wk)	Paternal peanut consumption, 2.97 g/wk (0.00-10.80 g/wk)	Combined parental consumption, 5.40 g/wk (1.03-19.50 g/wk)	Infant peanut consumption 0.00 g/wk (0.0-0.00 g/wk)	HPC 7.38 g/wk (1.84-19.50 g/wk)
Peanut protein in maternal bed dust, 4.19 $\mu\text{g/g}$ (0.54-24.89 $\mu\text{g/g}$)	$r_s = 0.477$ (0.199 to 0.684) [†]	$r_s = 0.588$ (0.335 to 0.762)*	$r_s = 0.698$ (0.498 to 0.827)*	$r_s = 0.364$ (0.064 to 0.604) [†]	$r_s = 0.689$ (0.484 to 0.852)*
Peanut protein in paternal bed dust, 5.11 $\mu\text{g/g}$ (0.92-25.63 $\mu\text{g/g}$)	$r_s = 0.390$ (0.081 to 0.631) [†]	$r_s = 0.591$ (0.331 to 0.807)*	$r_s = 0.672$ (0.449 to 0.816)*	$r_s = 0.346$ (0.030 to 0.599) [†]	$r_s = 0.692$ (0.479 to 0.828)*
Peanut protein in infant bed sheet dust, 4.79 $\mu\text{g/g}$ (0.76-33.27 $\mu\text{g/g}$)	$r_s = 0.417$ (0.108 to 0.652) [†]	$r_s = 0.579$ (0.305 to 0.764)*	$r_s = 0.699$ (0.489 to 0.832)*	$r_s = 0.540$ (0.267 to 0.733)*	$r_s = 0.713$ (0.510 to 0.840)*
Peanut protein in infant play area dust, 6.88 $\mu\text{g/g}$ (3.25-48.95 $\mu\text{g/g}$)	$r_s = 0.370$ (0.058 to 0.616) [†]	$r_s = 0.563$ (0.293 to 0.750)*	$r_s = 0.674$ (0.452 to 0.817)*	$r_s = 0.510$ (0.228 to 0.713)*	$r_s = 0.718$ (0.517 to 0.843)*
Peanut protein on infant crib rail wipe, 0.0 $\mu\text{g/g}$ (0.0-0.0 $\mu\text{g/g}$)	$r_s = 0.150$ (-0.260 to 0.514) [‡]	$r_s = 0.606$ (0.269 to 0.852) [†]	$r_s = 0.612$ (0.287 to 0.811)*	$r_s = 0.782$ (0.560 to 0.899)*	$r_s = 0.585$ (0.247 to 0.796) [†]
Peanut protein on dishwasher wipe, 0.0 $\mu\text{g/g}$ (0.0-1.28 $\mu\text{g/g}$)	$r_s = 0.311$ (-0.078 to 0.617) [‡]	$r_s = 0.454$ (0.090 to 0.711) [†]	$r_s = 0.601$ (0.287 to 0.798)*	$r_s = 0.594$ (0.277 to 0.794)*	$r_s = 0.685$ (0.413 to 0.845)*
Peanut protein tap wipe, 0.0 $\mu\text{g/g}$ (0.0-0.49 $\mu\text{g/g}$)	$r_s = 0.256$ (-0.079 to 0.539) [‡]	$r_s = 0.300$ (-0.042 to 0.579) [‡]	$r_s = 0.431$ (0.120 to 0.665) [†]	$r_s = 0.355$ (0.030 to 0.612) [†]	$r_s = 0.468$ (0.165 to 0.690) [†]
Peanut protein on refrigerator handle wipe, 0.0 $\mu\text{g/g}$ (0.0-1.52 $\mu\text{g/g}$)	$r_s = 0.508$ (0.211 to 0.719) [†]	$r_s = 0.559$ (0.267 to 0.757)*	$r_s = 0.653$ (0.409 to 0.809)*	$r_s = 0.520$ (0.226 to 0.727)*	$r_s = 0.632$ (0.379 to 0.797)*
Peanut protein on parent table wipe, 0.0 $\mu\text{g/g}$ (0.0-0.68 $\mu\text{g/g}$)	$r_s = 0.462$ (0.136 to 0.698) [†]	$r_s = 0.336$ (-0.020 to 0.616) [‡]	$r_s = 0.455$ (0.127 to 0.693) [†]	$r_s = 0.173$ (-0.294 to 0.573) [‡]	$r_s = 0.509$ (0.217 to 0.717) [†]

Statistical significance is shown as follows: * $P \leq .001$, [†] $P = .002-.049$, and [‡] $P \geq .05$.
IQR, Interquartile range.

**FIG 1.** Correlation between combined parental peanut consumption (grams per week) over 6 months and peanut protein levels in the maternal bed (A; $n = 41$; $r_s = 0.698$; 95% CI, 0.460-0.811; $P < .001$) and paternal bed (B; $n = 37$; $r_s = 0.672$; 95% CI, 0.475-0.829; $P < .001$). Axes are displayed in log scale.

to correlate environmental peanut levels and average weekly peanut consumption; 95% CIs were calculated by using Fisher r -to- z transformation. Kendall tau correlation was used to confirm Spearman rank correlations between variables with multiple low values (infant peanut consumption and surface wipes). Peanut protein levels in the infant's environment were compared in moderate to high versus low peanut-consuming households by using the Mann-Whitney U test. Clinical, household, and behavioral factors affecting peanut protein levels in the home were compared by using the Mann-Whitney U test and linear regression

analysis. Peanut protein levels in dust and HPC underwent natural log transformation to meet the assumptions of a regression analysis. Univariate factors associated with peanut protein levels in the infant's bed dust and play area ($P \leq .1$) were included in the multivariate regression model. Basophil activation (as measured by the percentage of CD63⁺ basophils) induced by serial dilutions of high or low peanut extracted dust samples, independent peanut standard, and controls in patients with peanut allergy and nonallergic children was compared by using the Mann-Whitney U test. Statistical significance was assessed at a P value of less than .05.

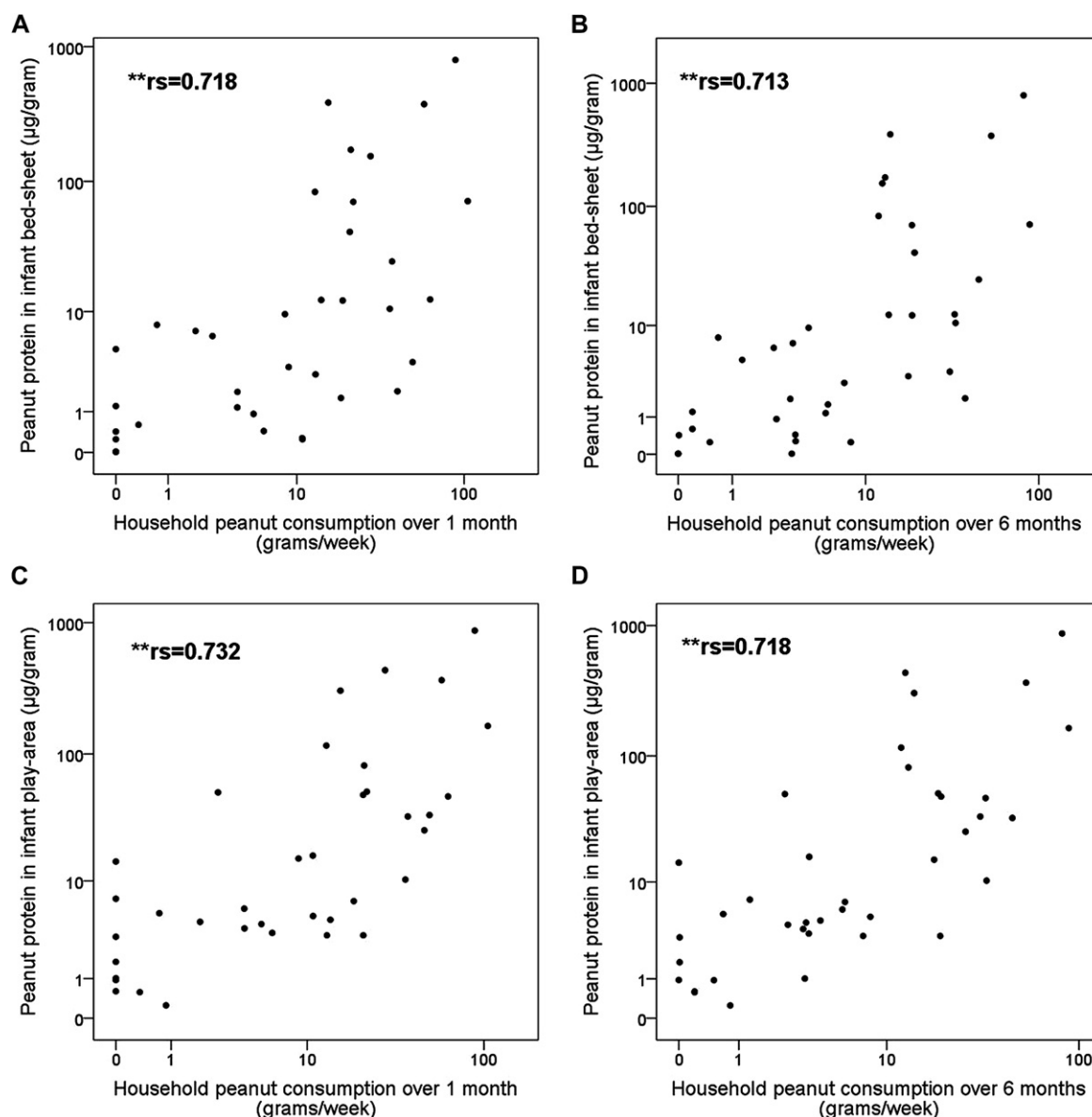


FIG 2. Correlation between peanut protein (micrograms per gram of dust) in the infant's bed sheet and HPC (grams per week) over 1 month (**A**; $n = 38$; $r_s = 0.718$; 95% CI, 0.517-0.843; $P < .001$) and 6 months (**B**; $n = 38$; $r_s = 0.713$; 95% CI, 0.510-0.840; $P < .001$). Correlation between peanut protein in the infant's play area and HPC over 1 month (**C**; $n = 38$; $r_s = 0.732$; 95% CI, 0.539-0.852; $P < .001$) and 6 months (**D**; $n = 38$; $r_s = 0.718$; 95% CI, 0.517-0.843; $P < .001$). Axes are displayed in log scale.

RESULTS

Peanut consumption is positively correlated with peanut protein levels in the environment

Results and correlations between environmental peanut levels in dust and surface wipes and peanut consumption in the home are displayed in Table I. Individual paternal and maternal peanut consumption was associated with peanut in the respective person's bed dust. However, combined parental peanut consumption showed higher correlation coefficients for peanut in the maternal and paternal sides of the bed (Fig 1). Infant peanut consumption was related to peanut levels in the infant's home environment (bed, crib rail, and play area). All 6 infants consuming peanut had high (≥ 10 $\mu\text{g/g}$ dust) peanut protein levels in their play areas and bed dust.

We then went on to test whether parental peanut consumption and HPC affected peanut protein levels in the infant's environment. Paternal peanut consumption was more highly correlated with peanut levels in the infant's bed and play area than maternal peanut consumption. HPC was most highly correlated with peanut protein in the infant's bed and play area dust, with an r_s value of greater than 0.700 ($P < .001$) both at 1 and 6 months (Fig 2). There was also a positive correlation between HPC and peanut protein levels on the infant's crib rail and kitchen surfaces, particularly dishwasher and fridge handles. Spearman rank correlations were similar to Kendall tau correlations between surface wipes and infant peanut consumption (data not shown). Infant highchair table wipes were not included because only 3 wipes had detectable peanut levels. Peanut protein levels were

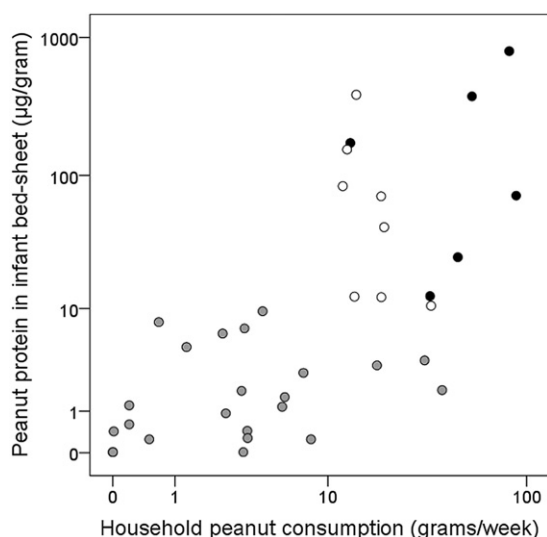


FIG 3. Average HPC over 6 months (grams per week) versus peanut protein levels in the infant's bed dust. High ($>10 \mu\text{g/g}$) peanut protein levels in the infant's bed dust are present when both the infant and parents eat peanut (black dots). If the infant is not eating peanut, high peanut protein levels ($>10 \mu\text{g/g}$) in their bed dust is associated with moderate to high parental peanut consumption ($\geq 10 \text{ g/week}$, white dots). Axes are displayed in log scale.

significantly higher ($P < .001$) in the infant's bed and play area in moderate to high ($\geq 10 \text{ g/wk}$) versus low ($< 10 \text{ g/wk}$) peanut-consuming households.

High peanut protein levels in the infant's bed dust were found if both infants and parents were eating peanuts (Fig 3, black dots). If the infant was not eating peanut, high peanut protein levels ($>10 \mu\text{g/g}$) in their bed dust was associated with moderate to high parental peanut consumption ($\geq 10 \text{ g/wk}$; Fig 3, white dots). Importantly, when we excluded families with infants consuming peanut from the analysis (thereby excluding high peanut-consuming parents and households), there was still a significant positive correlation between HPC and peanut protein in the infant's bed dust ($r_s = 0.620$; 95% CI, 0.341-0.798; $P < .001$) and play area ($r_s = 0.629$; 95% CI, 0.360-0.801; $P < .001$).

Linear regression analysis of factors affecting peanut protein levels in the environment

Associations between clinical, environmental, and behavioral characteristics and environmental peanut protein levels in the infant's bed dust are displayed in Table II. There was a positive linear relationship between HPC ($\ln$ grams per week over 6 months) and peanut protein in the infant's bed dust ($\ln$ micrograms per gram; Coefficient (B) = 0.733; 95% CI, 0.399-1.068; $P < .001$) and play area (B = 0.602; 95% CI, 0.346-0.859; $P < .001$). The unstandardized coefficient (B) shows that for a natural log unit of peanut consumption (2.72 g/wk), peanut protein in the infant's bed dust increases by $\ln$ 0.733 units (2.08 $\mu\text{g/g}$). Maternal, paternal, combined parental, and infant peanut consumption were also associated with peanut protein in the infant's bed and play area dust but were not included in the multivariate analysis because HPC comprises each of these variables.

A reported family member with peanut allergy was associated with lower environmental peanut protein levels in the infant's bed and play area. On subgroup analysis, peanut protein levels were

highest in the infant's bed in homes with no peanut allergy, followed by households with infants with peanut allergy, followed by households with "other" members with peanut allergy (excluding those with infants with peanut allergy). This pattern was mirrored by HPC (Fig 4). Age at assessment, sex, siblings, peanut allergy in the infant, size of the home, antiallergenic covers in the infant's crib, and age of the infant's mattress were not associated with peanut levels in the infant's bed (Table II).

In the multivariate regression model HPC was the only variable that remained significantly associated with peanut protein levels in the infant's bed dust (B = 0.492; 95% CI, 0.084-0.900; $P = .020$), adjusting for a household member with peanut allergy, ethnicity, where the infant slept, and dust weight. HPC was also the determining factor for peanut protein levels in the infant's play area (B = 0.539; 95% CI, 0.275-0.804; $P < .001$), adjusting for a household member with peanut allergy. Other clinical and household factors were not associated with peanut protein levels in the infant's play area. "Old" mattresses (>10 years) were associated with higher environmental peanut protein levels in the paternal (B = 1.945; 95% CI, 0.455-3.434; $P = .012$) and maternal (B = 1.434; 95% CI, -0.031-2.899; $P = .055$ [trend]) sides of the bed.

Biological activity of peanut protein in house dust

Three children with peanut allergy and 3 nonallergic children were recruited for the basophil activation assay on dust samples. Two patients with peanut allergy were sensitized to the 3 major peanut allergens (Ara h 1, Ara h 2, and Ara h 3), and 1 patient was sensitized to Ara h 1 and Ara h 2. None of these patients were sensitized to any other food or airborne allergens. Extracted dust samples with high or low peanut protein content were tested by using blood from children with peanut allergy and nonallergic children, forming 5 pairs of dust sample-patient experiments. Dust samples containing peanut protein levels of less than 0.375 ng/mL did not induce significant basophil activation either in children with peanut allergy (Fig 5, A) or in nonallergic children (Fig 5, C). However, basophils of children with peanut allergy showed clear dose-dependent upregulation of the activation marker CD63 with serial dilutions of peanut protein levels in dust (Fig 5, B), as opposed to basophils from nonallergic children, which did not respond to peanut protein in dust (Fig 5, D). The proportion of CD63⁺ basophils was significantly higher in children with peanut allergy at peanut concentrations ranging between 1 and 10,000 ng/mL ($P < .01$). The threshold for basophil activation was 1 ng/mL using basophils from the 3 selected donors with peanut allergy (Fig 5, B [with peanut allergy], vs 5, D [nonallergic]). Basophils of allergic and nonallergic patients showed comparable spontaneous basophil activation and CD63 expression induced by anti-IgE and N-formyl-methionyl-leucyl-phenylalanine. In children with peanut allergy, the dose response of basophil activation by dust samples was comparable with the dose response to similar concentrations of the independent peanut standard. Basophils of nonallergic patients were not activated by the peanut standard up to 10 $\mu\text{g/mL}$ peanut protein.

DISCUSSION

This is the first study to demonstrate that peanut protein levels in an infant's home environment are positively correlated with HPC and that peanut protein in household dust is biologically active. Previous work showed that HPC was a risk factor for the development of peanut allergy.¹¹ One criticism of this study was

TABLE II. Clinical, behavioral, and household factors associated with peanut protein levels in the infant's bed dust (*ln* micrograms per gram)

Clinical, environmental, and behavioral factors	Median (IQR) or frequency (%)	Univariate analysis for peanut protein in the infant's bed dust (<i>ln</i> µg/g)			Multivariate analysis for peanut protein in the infant's bed dust (<i>ln</i> µg/g)		
		Coefficient	95% CI	P value	Coefficient	95% CI	P value
Age at assessment	9 mo (6-10.5 mo)	-0.150	-0.396 to 0.095	.222		NA	
Female sex	Male: 29/45 (64.4%) Female: 16/45 (35.6%)	1.243	-0.344 to 2.830	.121		NA	
White ethnicity	Nonwhite: 14/45 (31.1%) White: 31/45 (68.9%)	1.748	0.180 to 3.316	.030*	0.533	-0.969 to 2.034	.474
Household siblings	Yes: 30/45 (66.7%) No: 15/45 (33.3%)	0.598	-1.004 to 2.200	.454		NA	
Questionnaire: infant with peanut allergy	No: 37/45 (82.2%) Yes: 8/45 (17.7%)	-0.967	-2.950 to 1.015	.329		NA	
Questionnaire: family member with peanut allergy	No: 32/45 (71.1%) Yes: 13/45 (28.9%)	-2.179	-3.684 to 0.674	.006*	-0.794	-2.428 to 0.840	.328
Size of home	≤1 bed: 3/45 (6.7%) 2-3 beds: 28/45 (62.2%) ≥4 beds: 14/45 (31.1%)	0.124	-1.299 to 1.548	.860		NA	
Age of infant's mattress	<5 y: 31/37 (83.8%) ≥5 y: 6/37 (16.2%)	-0.714	-2.275 to 2.004	.785		NA	
Infant anti-allergenic covers	Yes: 9/45 (20%) No: 36/45 (80%)	-0.768	-2.582 to 1.045	.396		NA	
Infant's sleep location	Parent bed: 8/45 (17.8%) Crib: 37/45 (82.2%)	1.706	-0.351 to 3.763	.101*	0.534	-1.503 to 2.572	.596
Infant's bed dust weight (<i>ln</i> mg)	19.00 mg (7.25-76.80 mg [not <i>ln</i> transformed])	0.828	0.060 to 1.596	.035*	0.602	-0.061 to 1.266	.074
Maternal peanut consumption (<i>ln</i> g/wk)	2.40 g/wk (0.11-5.90 g/wk [not <i>ln</i> transformed])	0.329	0.044 to 0.614	.025*		NA	
Paternal peanut consumption (<i>ln</i> g/wk)	2.97 g/wk (0.0-10.80 g/wk [not <i>ln</i> transformed])	0.301	0.420 to 0.561	.025*		NA	
Combined parental peanut consumption (<i>ln</i> g/wk)	5.40 g/wk (1.03-19.50 g/wk [not <i>ln</i> transformed])	0.610	0.333 to 0.887	<.001*		NA	
Infant's peanut consumption (<i>ln</i> g/wk)	0.00 g/wk (0.0-0.00 g/wk [not <i>ln</i> transformed])	0.636	0.293 to 0.980	.001*		NA	
HPC (<i>ln</i> g/wk)	7.38 g/wk [1.84-19.50 g/wk [not <i>ln</i> transformed])	0.733	0.399 to 1.068	<.001*	0.492	0.084 to 0.900	.020*

Initial univariate linear regression analysis was performed, followed by multivariate regression analysis.

IQR, Interquartile range; NA, not applicable.

*Factors with *P* values of .1 or less on univariate analysis were included in the multivariate model. Maternal, paternal, combined parental and infant peanut consumption were not entered into the multivariate model as HPC comprises each of these variables.

that peanut levels in the environment were not directly measured. Our study has now confirmed the relationship between HPC and peanut protein levels in the infant's home environment. A univariate followed by multivariate regression analysis was carried out to determine the factor that best explained peanut levels in the infant's home environment. HPC was the most important factor for peanut protein in the infant's bed and play area dust. A household member with peanut allergy was associated with lower peanut protein levels in the infant's bed and play area; this was a result of lower HPC because in the multivariate model only HPC remained significant. Interestingly, lower peanut consumption was most pronounced when a household member other than the infant was allergic to peanut; thus it might take time for households to change their peanut eating habits after a new household member (the infant) is given a diagnosis of peanut allergy. Alternatively, cessation of peanut consumption might not be reflected in peanut dust levels for several months.

The presence of detectable biologically active peanut protein in dust lends credence to the notion that high environmental peanut exposure can lead to peanut allergic sensitization. To give more weight to the notion that peanut in dust can sensitize young

children, we wanted to confirm that we were measuring peanut levels that were biologically significant in patients with peanut allergy. Therefore we demonstrated the ability of peanut protein in dust to cause allergen-specific activation of basophils from children with peanut allergy. Peanut protein in household dust induced a dose-dependent activation of basophils from children with peanut allergy that was comparable with the dose-response to similar concentrations of an independent peanut standard. These observations are highly suggestive of a peanut-specific basophil response. Given that these children were solely allergic to peanut and neither sensitized nor allergic to any other food or airborne allergen, basophil activation is very unlikely to have been caused by allergens other than peanut present in the dust samples (eg, house dust mite or grass and tree pollen allergens). The allergen-specific response is further confirmed by the fact that activation was not observed when basophils from nonallergic children were stimulated with the high peanut level-containing dust samples. Furthermore, the inability of dust samples with negligible levels of peanut to cause basophil activation in both children with peanut allergy and nonallergic children excludes non-allergen-specific basophil activation. Taken together, these findings confirm that

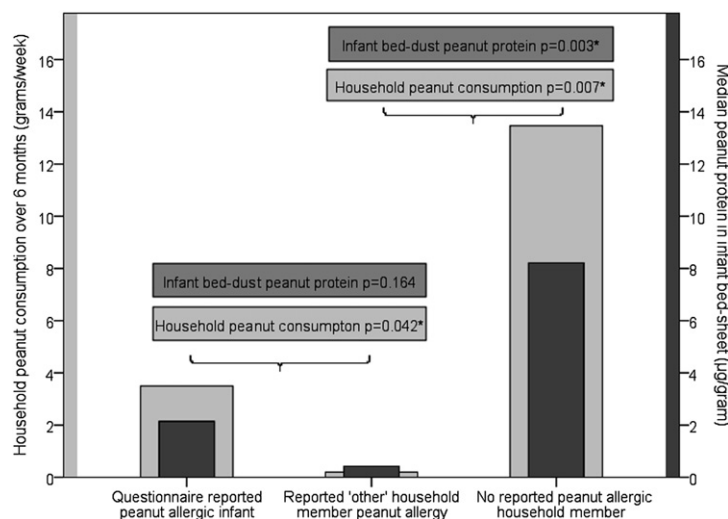


FIG 4. Peanut protein (micrograms per gram of dust) in the infant's bed (dark gray) and HPC (grams per week over 6 months, light gray) comparing households with reported peanut allergy in the infant (n = 8) versus reported peanut allergy in another household member (n = 5) versus no reported household member with peanut allergy (n = 32). *P < .05.

peanut allergens in house dust are able to interact with immune cells, such as basophils, and we hypothesize that, being biologically active, peanut allergens in house dust are not only able to activate mast cells and basophils but also to be captured by dendritic cells and presented to T cells in the context of a T_H2 -driven immune response leading to allergic sensitization.

Allergen exposure threshold levels for sensitization have not been described for food allergens but have been described for inhalant allergens, such as house dust mite (*Dermatophagoides pteronyssinus*, >2 µg/g),³² cat (Fel d 1, 1-8 µg/g),^{33,34} and cockroach (Bla g 1, >8 µg/g).^{35,36} Aeroallergens might behave differently to food allergens with respect to their mechanism of sensitization, and thus these allergen threshold levels might not be directly comparable. We found higher peanut protein concentrations in dust (median, 4.95 µg/g; interquartile range, 1.12-29.34; range undetectable to 870 µg/g) than previously reported for peanut (Ara h 2; median, 1.13 µg/g; range, 0.40-9.79 µg/g),¹⁴ which is likely to be because the Veratox polyclonal peanut ELISA is directed against whole peanut protein. Additionally, peanut proteins are very stable allergens and thus might build up in reservoirs, such as mattresses; this is supported by peanut protein levels being higher in older mattresses. We do not believe environmental peanut protein levels are likely to cause clinical reactions to peanut but might be sufficient to induce sensitization. This is particularly pertinent in the context of infantile eczema in which skin is inflamed and abraded and immune cells are potentially directly exposed to peanut proteins in dust.

Peanut protein levels in bed dust were also related to individual peanut consumption; combined parental peanut consumption was positively correlated with peanut levels in the maternal and paternal sides of the bed, and infant peanut consumption was positively correlated with peanut protein in the infant's bed (Table I). These findings suggest that peanut protein in bed dust can be used as an objective marker of peanut consumption. This might be useful in intervention studies in which peanut consumption versus avoidance needs to be assessed, such as in peanut allergy prevention or peanut oral immunotherapy randomized controlled trials. Although 39 (86.7%) of 45 of the infants consumed no

peanut, there were high (>10 µg/g dust) peanut protein levels in the infant's bed dust of all 6 infants consuming peanut. This supports the hypothesis that if an infant eats peanut, then there will be peanut protein in the infant's bed dust; however, larger studies with infants eating peanut are required to confirm this.

Environmental peanut exposure is only one consideration in the development of peanut sensitization and allergy. There are many other factors to consider, such as genetic factors,³⁷ the hygiene hypothesis,³⁸ gut flora,³⁹ and numerous other factors.⁴⁰ There is ongoing work on the induction of oral tolerance through high-dose early peanut consumption (www.leapstudy.co.uk)⁴¹ on the basis of previous observational work showing a protective effect of early peanut consumption in infants.⁴² In the study by Fox et al,¹¹ children who had consumed peanut before 1 year of age were less affected by high HPC. In animal studies high-dose repeated peanut consumption leads to oral tolerance induction; however, this might be prevented by preceding epicutaneous sensitization.⁴³ Recent *in vitro* work has shown differential proliferation of peanut-specific skin-homing versus gut-homing memory T cells in the presence of peanut in children with peanut allergy versus peanut-tolerant children, respectively. In children with peanut allergy, skin-homing T cells (cutaneous lymphocyte antigen positive) proliferated and produced a predominantly T_H2 cytokine profile. In peanut-tolerant children gut-homing T cells ($\alpha 4\beta 7$ positive) proliferated and produced a predominantly T_H1 cytokine profile. This suggests that in children with peanut allergy, allergic sensitization occurs through the skin, whereas in peanut-tolerant children they become tolerant through the gut.⁴⁴ Thus it might be that environmental peanut exposure poses a risk for the development of peanut sensitization through epicutaneous exposure only in the absence of the protective effect of oral peanut consumption (the dual-allergen exposure hypothesis).⁴⁰

In conclusion, this study confirms the relationship between HPC and environmental peanut exposure in young children and demonstrates that peanut measured in dust is biologically active. Further prospective environmental population studies are needed to address whether there is a threshold level of environmental peanut exposure that can lead to peanut sensitization.

Serial dilutions of dust samples with:

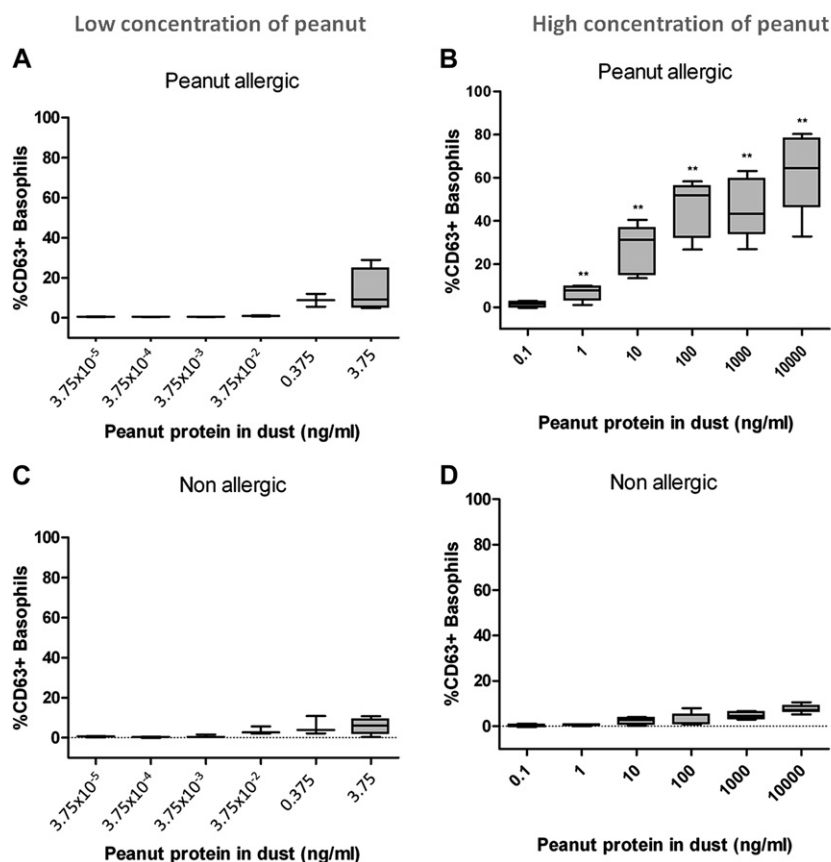


FIG 5. Basophil activation by serial dilutions of dust samples containing low (**A** and **C**) or high (**B** and **D**) peanut protein in peanut-monoallergic (Fig 5, **A** and **B**) or nonallergic (Fig 5, **C** and **D**) patients ($n = 5$ pairs of sample-patient experiments). Medians, 25th and 75th percentiles, and minimum and maximum percentages of CD63⁺ basophils are displayed. High peanut dust extracts were dilution-adjusted to the maximum same concentration of peanut protein (10 $\mu\text{g/mL}$) and then underwent serial dilutions for comparability of dose-response, and low peanut dust samples were prepared by using the same dilution factor. Basophil activation for each concentration of peanut was compared between allergic and nonallergic children (ie, Fig 5, **A**, vs Fig 5, **C**, and Fig 5, **B**, vs Fig 5, **D**). ** $P < .01$.

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Clinical implications: Peanut dust levels in the infant's environment correlate with household consumption of peanut. The peanut in dust is biologically active. Environmental peanut exposure should be studied as a possible risk factor for peanut sensitization.

REFERENCES

- Hourihane JO, Aiken R, Briggs R, Gudgeon LA, Grimshaw KEC, DunnGalvin A, et al. The impact of government advice to pregnant mothers regarding peanut avoidance on the prevalence of peanut allergy in United Kingdom children at school entry. *J Allergy Clin Immunol* 2007;312:1197-202.
- Grundy J, Matthews S, Bateman B, Dean T, Arshad SH. Rising prevalence of allergy to peanut in children: data from 2 sequential cohorts. *J Allergy Clin Immunol* 2002;110:784-9.
- Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001;107:191-3.
- King RM, Knibb RC, Hourihane JO. Impact of peanut allergy on quality of life, stress and anxiety in the family. *Allergy* 2009;64:461-8.
- Roy KM, Roberts MC. Peanut allergy in children: relationships to health-related quality of life, anxiety, and parental stress. *Clin Pediatr* 2011;50:1045-51.
- Hourihane JO, Kilburn SA, Dean P, Warner JO. Clinical characteristics of peanut allergy. *Clin Exp Allergy* 1997;27:634-9.
- Sicherer SH, Burks AW, Sampson HA. Clinical features of acute allergic reactions to peanut and tree nuts in children. *Pediatrics* 1998;102:e6.
- Ewan PW. Clinical study of peanut and nut allergy in 62 consecutive patients: new features and associations. *BMJ* 1996;312:1074-8.
- Lack G, Fox D, Northstone K, Golding J. Avon Longitudinal Study of Parents and Children Study Team. Factors associated with the development of peanut allergy in childhood. *N Engl J Med* 2003;348:977-85.
- Strid J, Hourihane J, Kimber I, Callard R, Strobel S. Disruption of the stratum corneum allows potent epicutaneous immunization with protein antigens resulting in a dominant systemic Th2 response. *Eur J Immunol* 2004;34:2100-9.
- Fox AT, Sasieni P, Du Toit G, Syed H, Lack G. Household peanut consumption as a risk factor for the development of peanut allergy. *J Allergy Clin Immunol* 2009;123:417-23.
- Witteman AM, van Leeuwen J, van der Zee J, Aalberse RC. Food allergens in house dust. *Int Arch Allergy Immunol* 1995;107:566-8.
- Dybendal T, Elsayed S. Dust from carpeted and smooth floors. VI. Allergens in homes compared with those in schools in Norway. *Allergy* 1994;49:210-6.

14. Sheehan WJ, Hoffman EB, Freidlander DR, Gold DR, Phipatanakul W. Peanut allergen (Ara h 2) in settled dust samples of inner-city schools and homes of children with asthma [abstract]. *J Allergy Clin Immunol* 2012;129:AB236.
15. Palmer GW, Dibbern DA Jr, Burks AW, Bannon GA, Bock SA, Porterfield HS, et al. Comparative potency of Ara h 1 and Ara h 2 in immunochemical and functional assays of allergenicity. *Clin Immunol* 2005;115:302-12.
16. Koppelman SJ, Wensing M, Ertmann M, Knulst AC, Knol EF. Relevance of Ara h1, Ara h2 and Ara h3 in peanut-allergic patients, as determined by immunoglobulin E Western blotting, basophil-histamine release and intracutaneous testing: Ara h2 is the most important peanut allergen. *Clin Exp Allergy* 2004;34:583-90.
17. McDermott RA, Porterfield HS, El Mezayen R, Burks AW, Pons L, Schlichting DG, et al. Contribution of Ara h 2 to peanut-specific, immunoglobulin E-mediated, cell activation. *Clin Exp Allergy* 2007;37:752-63.
18. Ebo DG, Bridts CH, Hagendorens MM, Aerts NE, De Clerck LS, Stevens WJ. Basophil activation test by flow cytometry: present and future applications in allergology. *Cytometry B Clin Cytom* 2008;74:201-10.
19. Glaumann S, Nopp A, Johansson SG, Rudengren M, Borres MP, Nilsson C. Basophil allergen threshold sensitivity, CD-sens, IgE-sensitization and DBPCFC in peanut-sensitized children. *Allergy* 2012;67:242-7.
20. Ocmant A, Mulier S, Hanssens L, Goldman M, Casimir G, Mascart F, et al. Basophil activation tests for the diagnosis of food allergy in children. *Clin Exp Allergy* 2009;39:1234-45.
21. Dolle S, Lehmann K, Schwarz D, Weckwert W, Scheler C, George E, et al. Allergenic activity of different tomato cultivars in tomato allergic subjects. *Clin Exp Allergy* 2011;41:1643-52.
22. Sabato V, van Hengel AJ, De Knop KJ, Verweij MM, Hagendorens MM, Bridts CH, et al. Human basophils: a unique biological instrument to detect the allergenicity of food. *J Invest Allergol Clin Immunol* 2011;21:179-84.
23. Brough HA, Makinson K, Penagos M, Maleki SJ, Cheng H, Stephens AC, et al. Distribution of peanut protein in the home environment. *J Allergy Clin Immunol* 2013 [in press].
24. Park LP, Coates S, Brewer VA, Garber AE, Abouzied M, Johnson K, et al. Performance tested method multiple laboratory validation study of ELISA-based assays for the detection of peanuts in food. *J AOAC Int* 2005;88:156-60.
25. Poms RE, Agazzi ME, Bau A, Brohee M, Capelletti C, Norgaard JV, et al. Inter-laboratory validation study of five commercial ELISA test kits for the determination of peanut proteins in biscuits and dark chocolate. *Food Addit Contam* 2005;22:104-12.
26. Trucksess MW, Whitaker TB, Slate AB, Williams KM, Brewer VA, Whittaker P, et al. Variation of analytical results for peanuts in energy bars and milk chocolate. *J AOAC Int* 2004;87:943-9.
27. Fox AT, Du Toit G, Lack G, Meyer R, Syed H, Sasieni P. Two-year recall of maternal peanut consumption using a food-frequency questionnaire. *S Afr J Clin Nutr* 2006;19:154-60.
28. Sofianou K, Fox AT, Du Toit G, Lack G. Assessing peanut consumption in a population of mothers and their children in the UK: a validation study of a food frequency questionnaire. *WAO J* 2011;4:38-44.
29. Crawley H. Food portion sizes, fisheries & food Great Britain. 2nd ed. London: Ministry of Agriculture: The Stationery Office Books; 1994.
30. Roberts G, Lack G. Diagnosing peanut allergy with skin prick and specific IgE testing. *J Allergy Clin Immunol* 2005;115:1291-6.
31. Brough H, Stephens A, Turcanu V, Lack G. Distribution of peanut in the home. *Allergy* 2009;64(suppl 90):543.
32. Platts-Mills TA, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor allergens and asthma: report of the Third International Workshop. *J Allergy Clin Immunol* 1997;100:S2-24.
33. Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 2001;357:752-6.
34. Custovic A, Hallam CL, Simpson BM, Craven M, Simpson A, Woodcock A. Decreased prevalence of sensitization to cats with high exposure to cat allergen. *J Allergy Clin Immunol* 2001;108:537-9.
35. Gelber LE, Seltzer LH, Bouzoukis JK, Pollart SM, Chapman MD, Platts-Mills TA. Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. *Am Rev Respir Dis* 1993;147:573-8.
36. Call RS, Smith TF, Morris E, Chapman MD, Platts-Mills TA. Risk factors for asthma in inner city children. *J Pediatr* 1992;121:862-6.
37. Sicherer SH, Furlong TJ, Maes HH, Desnick RJ, Sampson HA, Gelb BD. Genetics of peanut allergy: a twin study. *J Allergy Clin Immunol* 2000;106:53-6.
38. Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;299:1259-60.
39. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997;159:1739-45.
40. Lack G. Update on risk factors for food allergy. *J Allergy Clin Immunol* 2012;129:1187-97.
41. Du Toit G, Roberts G, Sayre PH, Plaut M, Bahnson HT, Mitchell H, et al. Identifying infants at high risk of peanut allergy—the LEAP Screening Study. *J Allergy Clin Immunol* 2013;131:135-43, e1-12.
42. Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J Allergy Clin Immunol* 2008;122:984-91.
43. Strid J, Hourihane J, Kimber I, Callard R, Strobel S. Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization. *Clin Exp Allergy* 2005;35:757-66.
44. Chan SM, Turcanu V, Stephens AC, Fox AT, Grieve AP, Lack G. Cutaneous lymphocyte antigen and alpha4beta7 T-lymphocyte responses are associated with peanut allergy and tolerance in children. *Allergy* 2012;67:336-42.