Distribution of peanut protein in the home environment

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Background: To halt the increase in peanut allergy, we must determine how children become sensitized to peanut. High household peanut consumption used as an indirect marker of environmental peanut exposure is associated with the development of peanut allergy.
Objective: We sought to validate a method to quantify environmental peanut exposure, to determine how peanut is transferred into the environment after peanut consumption, and to determine whether environmental peanut persists despite cleaning.
Methods: After initial comparative studies among 3 ELISA kits, we validated and used the Veratox polyclonal peanut ELISA to assess peanut protein concentrations in dust and air and on household surfaces, bedding, furnishings, hand wipes, and saliva.
Results: The Veratox polyclonal peanut ELISA had the best rate of recovery of an independent peanut standard. We demonstrated 100% sensitivity and specificity and a less than 15% coefficient of variation for intra-assay, interassay, and interoperator variability. There was high within-home correlation for peanut protein levels in dust and household surface wipes. Airborne peanut levels were lower than the limit of quantitation for the Veratox polyclonal peanut ELISA in a number of simulated scenarios, except for a brief period directly above peanuts being deshelled. Peanut protein persisted on hands and in saliva 3 hours after peanut consumption. Peanut protein was completely removed from granite tables after cleaning with detergent, and levels were reduced but still present after detergent cleaning of laminate and wooden table surfaces, pillows, and sofa covers.
Conclusions: Peanut spread easily around the home and might be resistant to usual cleaning methods. Peanut protein can be transferred into the environment by means of hand transfer and saliva but is unlikely to be aerosolized. (J Allergy Clin Immunol 2013;132:623-9.)

Key words: *Peanut, sensitization, allergy, environment, dust, aerosolized, airborne, saliva, hand, ELISA, validation*

Peanut allergy is an important public health concern.[1] Ongoing studies on oral tolerance induction to peanut aim to address these issues (www.leapstudy.co.uk).[2] To halt the increase in peanut allergy,[3,4] we must first understand the mechanism of peanut sensitization. Household peanut consumption is 10 times higher in infants with peanut allergy versus high-risk (with egg allergy) control subjects.[5] In this study household peanut consumption was considered an indirect marker of environmental peanut exposure; however, peanut protein levels in the home were not directly quantified.

Few studies have assessed the distribution of peanut in the environment. Surface wipes from desks, cafeteria tables, and water fountains of 6 schools found little evidence of peanut using a monoclonal ELISA against Ara h 1 (INDOOR Biotechnologies, Warminster, United Kingdom).[6] Most cleaning agents (plain water, dishwashing liquid, sanitizing wipes, and bleach cleaner) were able to remove Ara h 1 from tables and hands spiked with 5 mL of peanut butter. Dish soap left residual Ara h 1 on 33% of tables (40-140 ng/mL), and Ara h 1 remained on 25% and 50% of hands after use of water and hand sanitizer, respectively.[6] Previous studies have quantified egg (ovomucoid), milk (β-lactoglobulin), and fish levels in household settled dust.[7,8] More recently, Ara h 2 has been quantified in bedroom dust of 18 (23.4%) of 77 children with asthma.[9] We have shown that peanut levels increase on bed sheets (on which participants have slept) the day after a single peanut-containing meal.[10]

As well as quantifying environmental peanut exposure, it is important to determine how peanut can be transferred into the environment from persons eating peanut. Aircraft often impose restrictions on peanut consumption because of concerns that persons with peanut allergy might inhale airborne peanut from other passengers eating peanuts on board.[11] There are anecdotal reports of allergic reactions after inhalation of peanut; however, when children with severe or reported inhalational reactions to peanut

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Abbreviations used
IOM: Inhalable occupational medicine
LLQ: Lower limit of quantitation
VPPE: Veratox polyclonal peanut ELISA

underwent blind inhalational peanut challenges (peanut butter held 12 inches from the face for 10 minutes), these children had no allergic symptoms or signs.[12] Peanut protein has been detected in the ventilation system filters of commercial airliners after 5000 flight hours by using an inhibition assay with peanut extract[13]; however, the results of this abstract have not been replicated. Peanut protein might be transferred into the environment after peanut consumption through hand transmission[5] or saliva.[14] Ara h 1 has been measured in saliva in levels up to 40 μg/mL (enough to cause an allergic reaction) immediately after peanut consumption; however, it was undetectable in 87% of participants after 1 hour using a monoclonal ELISA against Ara h 1.[14]

This study was designed to validate a method to quantify environmental peanut protein levels in household dust, surfaces, bedding, furnishings, and air to quantify environmental peanut exposure and its potential role in peanut sensitization and allergy. We also wished to assess potential routes of peanut transfer into the environment and the effect of usual detergent cleaning on reducing environmental peanut levels.

METHODS

The study was approved by the Brent Medical Research Ethics Committee. Informed consent was obtained before environmental sampling and from participants who provided saliva and hand-wipe samples before and after peanut consumption.

Validation of method to quantify peanut protein in dust and wipes

The Veratox polyclonal peanut ELISA (VPPE) used in this study was validated according to the International Conference on Harmonization guidelines for validation of analytic procedures.[15] We also assessed aspects of dust processing related to peanut protein. Details of the methods used are included in the Methods section in this article's Online Repository at www.jacionline.org, including the following:

1. Details of samples used
2. Rate of recovery of an independent peanut standard comparing 3 validated commercial ELISA kits:
   A. VPPE (Neogen, Lansing, Mich)
   B. Biokits polyclonal Ara h 1 ELISA (Tepnel Research Products and Services, Flintshire, United Kingdom)
   C. Monoclonal ELISA against Ara h 1 (INDOOR Biotechnologies)
3. Performance characteristics of VPPE:
   A. Sensitivity and specificity
   B. Lower limit of quantitation (LLQ)
   C. Assay precision
4. Dust processing:
   A. Peanut protein in sieved fine dust versus residual fluff
   B. Extraction assays
   C. Effect of freezing and thawing extracted dust samples.

Peanut protein in household dust and surfaces

Forty-five families with infants were recruited from pediatric allergy clinics. Dust samples were obtained from the bed sheets of all household members and from the infant's play area; participants were asked not to wash or vacuum these for 5 days before the home visit. Dust samples were taken from each side of the parent's bed. The infant's play area was the place where the infant spent most of his or her day (eg, play mat/quilt and living room carpet).

A Philips cylinder vacuum FC8262 (1600 W) was connected to a Dustream adaptor and collector with a disposable nylon collection filter (pore size, 40 μm; INDOOR Biotechnologies). Bed sheets and the infant's play area were vacuumed for 2 minutes within a 1-m^2 surface area; the infant's bed sheet was vacuumed for 1 minute within a 0.5-m^2 area. Dust samples were sieved with a 300-μm copper sieve (Endecotts, London, United Kingdom), and fine dust was weighed to express results in micrograms of peanut protein per gram of dust. Dust was extracted in proportional volumes of the VPPE extraction solution and heated for 15 minutes at 60°C (see the Methods section in this article's Online Repository for further details). Dust samples of less than 5 mg were excluded.

Wipe samples made from Benchkote filter paper (Whatman, Maidstone, United Kingdom) cut to 4 × 4 cm and moistened with 0.5 mL of PBS were obtained from the parent's table, infant's highchair table, tap, dishwasher handle, refrigerator handle, and infant's crib rail. Table-surface wipes were collected within A4 paper–sized templates. Wipes were weighed before and after sampling to calculate results in micrograms of peanut protein per gram. Wipe samples were extracted in 2 mL of VPPE extraction solution in a sealed syringe. We used the VPPE to quantify peanut protein levels in dust and wipes. All samples collected were blinded from the researcher performing the ELISAs.

Airborne peanut

Airborne peanut was captured with glass-fiber filters (pore size, 0.7 μm) inserted into the inhalable occupational medicine (IOM) sampling head of a personal air-sampling monitor (TUFF; Casella Measurement, Bedford, United Kingdom). The pump was run at 2 L/min, as recommended by the manufacturer, which is equivalent to an infant's minute volume (tidal volume [5 mL/kg] × respiratory rate [40 breaths/min]), using an estimated weight of 10 kg. Glass-fiber filters were processed in the same way as wipes and analyzed with the VPPE. The VPPE LLQ was 100 ng/mL (equivalent to 2.5 μg/m^3). The following experiments were performed to detect airborne peanut:

1. The sampling head was held 1 cm (n = 3) and 1 m (n = 3) above a peanut butter jar/dry-roasted peanut bag for 22 hours and above a simmering pan of satay sauce (10.8 g of peanut; Amoy, Hayes, United Kingdom) for 10 minutes.
2. While eating peanut butter or dry-roasted peanuts, the sampling head was pinned to the researcher's clothes, placed on the dining room table, breathed on for 10 minutes, or placed overnight on the bedside table (n = 3).
3. The IOM was run for 22 hours in homes with high peanut protein levels in dust (n = 5; median peanut protein, 163.8 μg/g; range, 51.2-365.2 μg/g).
4. The sampling head was held 1 cm and 1 m above peanuts being deshelled. New glass-fiber filters were run in the IOM for 10 minutes before, during, immediately after, and 30 minutes and 1 hour after deshelling peanuts (n = 6).

Peanut protein on hands and saliva after peanut consumption

Hand-wipe and saliva samples were taken before and 3 hours after consuming 50 g of salted peanuts (n = 6; KP Nuts, Hayes, United Kingdom). Participants were asked not to eat peanut for 24 hours before and 3 hours after this peanut meal. Hand samples were taken with Benchkote wipes of the right palm (all subjects were right handed) and processed as described above. Saliva samples were collected into Eppendorf tubes and analyzed directly for peanut protein by using the VPPE without extraction.

Persistence of peanut despite cleaning

Table surfaces. Three table surfaces (wood [unpainted], granite, and laminate) were cleaned with water and allowed to air dry. A5 paper templates were sellotaped to the tables (n = 3). Smooth peanut butter (0.5 mL; Sun-Pat; Premier Foods Group, Manchester, United Kingdom) was spread evenly onto

TABLE I. Correlation grid of peanut protein levels (in micrograms per gram) in household dust and surface wipes

Maternal bed dust (n = 41)	Paternal bed dust (n = 38)	Infant's bed dust (n = 38)	Infant's play area dust (n = 38)	Sibling's bed dust (n = 17)	Parent's table wipe (n = 27)	Dishwasher wipe (n = 27)	Refrigerator handle wipe (n = 35)	Tap wipe (n = 36)	Infant's crib rail (n = 24)
4.19 (0.54 to 24.89)	*5.11 (0.92 to 25.63)*	*4.79 (0.76 to 33.27)*	*6.88 (3.25 to 48.95)*	*2.83 (0.39 to 50.4)*	*0 (0 to 0.68)*	*0 (0 to 1.28)*	*0 (0 to 1.52)*	*0 (0 to 0.49)*	*0 (0 to 0)*
Maternal bed dust	0.864 (0.753 to 0.927)*	0.844 (0.708 to 0.919)*	0.635 (0.383 to 0.799)*	0.700 (0.313 to 0.887)†	0.540 (0.224 to 0.753)†	0.619 (0.288 to 0.818)†	0.790 (0.610 to 0.892)*	0.516 (0.210 to 0.729)†	0.595 (0.253-0.805)†
	Paternal bed dust	0.760 (0.564-0.875)*	0.720 (0.501 to 0.852)*	0.609 (0.163 to 0.848)†	0.656 (0.382 to 0.824)*	0.773 (0.530 to 0.898)*	0.833 (0.676 to 0.917)*	0.668 (0.411 to 0.826)*	0.512 (0.137 to 0.758)†
		Infant's bed dust	0.862 (0.734 to 0.930)*	0.757 (0.241 to 0.914)*	0.645 (0.344 to 0.825)*	0.691 (0.381 to 0.861)*	0.728 (0.488 to 0.865)*	0.490 (0.151 to 0.726)†	0.692 (0.360 to 0.868)*
			Infant's play area dust	0.725 (0.339 to 0.902)†	0.639 (0.335 to 0.822)*	0.731 (0.473 to 0.873)*	0.694 (0.440 to 0.845)*	0.419 (0.07 to 0.677)†	0.693 (0.362 to 0.896)*
				Sibling's bed dust	0.532 (−0.026 to 0.837)‡	0.131 (−0.543 to 0.702)‡	0.595 (0.033 to 0.871)†	0.501 (−0.069 to 0.824)‡	0.247 (−0554 to 0.810)‡
					Parent's table wipe	0.571 (0.228 to 0.788)†	0.728 (0.499 to 0.862)*	0.759 (0.554 to 0.877)*	0.574 (0.212 to 0.797)†
						Dishwasher wipe	0.743 (0.500 to 0.877)*	0.770 (0.546 to 0.891)*	0.570 (0.141 to 0.818)†
							Refrigerator handle wipe	0.756 (0.566 to 0.870)*	0.633 (0.299 to 0.828)*
								Tap wipe	0.559 (0.201 to 0.785)†
									Infant's crib rail

Median peanut protein levels and interquartile ranges are displayed in italics. Spearman rho correlation coefficients (r_s) and 95% CIs are displayed for each combination. Statistical significance is shown as follows: *$P \leq .001$, †$P = .002$-$.049$, and ‡$P \geq .05$.

an index card, placed onto the template area with the peanut side down, held for 5 seconds, and removed. Three table surfaces were also exposed to plain butter as negative controls (n = 3). Benchkote wipes were used to obtain samples before the peanut spike, immediately after, after the water wipe, and after detergent cleaning (n = 3). The water-wipe technique was a single wipe with a clean paper kitchen towel (Bounty; Proctor & Gamble, Weybridge, United Kingdom) moistened with 1 mL of water. In the detergent clean the template was lifted, and the area was vigorously cleaned with 6 circular motions with a kitchen towel and washing up liquid (Fairy Liquid, Proctor & Gamble) and 1 mL of water followed by a water wipe with a new kitchen towel. New templates were placed over the original area, and wipe samples were taken again.

Pillows and sofa covers. Dust samples were vacuumed from 5 sofa covers (70 cm × 70 cm removed from sofa cushions) and 5 standard pillows (65 cm × 45 cm with pillow cases removed) to determine the effect of a detergent wash on household bedding and furnishings. The sofa covers and pillows were washed separately in a Hotpoint washer/dryer (model BHWD129; GE Appliances, Fairfield, Conn) on a 60°C cotton cycle with a 40-minute tumble dry using 1 cap of washing detergent (Lenor, Procter & Gamble) and fabric softener (Fairy). Peanut butter was consumed while sitting on the sofa cover (n = 5), and the pillow was slept on overnight (n = 5). Repeat dust samples were collected from the sofa covers and pillows; these were then washed separately, and when completely dry, further dust samples were collected (n = 5). In between each detergent wash, the washing machine was cleaned by using a 90°C cotton cycle and 1 cap of soda crystals.

Statistical analysis

Data were entered into an SPSS spreadsheet (SPSS 17.0; SPSS, Chicago, Ill) for the purposes of analysis. Spearman rank correlation coefficients (r_s) were used for all correlations, and 95% CIs were calculated by using Fisher r-to-z transformation. Paired differences between peanut protein levels in dust versus fluff, and in hand wipes and saliva before and after peanut consumption were assessed by using the Wilcoxon signed-rank test. Paired analysis between peanut protein levels in extracted dust processed immediately or frozen and then thawed over 2 or 24 hours was performed with the Friedman test. Statistical significance was assessed at a P value of less than .05.

RESULTS

Validation of method to quantify peanut protein in dust and wipes

Full details are described in the Results section in this article's Online Repository at www.jacionline.org. To summarize, the VPPE kit showed the best rate of recovery (66.3% to 119.8%) of the ALK-Abelló (Hørsholm, Denmark) independent peanut standard compared with the polyclonal and monoclonal Ara h 1 ELISA kits. Sensitivity and specificity were 100% with receiver operating characteristic curve analysis comparing 20 wooden tables spiked with peanut (and cleaned with detergent) versus tables that had not been spiked with peanut. There was no cross-reactivity with potentially cross-reactive proteins, including soya milk; crushed almond, cashew, or pistachio nuts; house dust mite; and human skin cells (given dust composite). We determined an LLQ of 62.5 ng/mL using the signal-to-noise approach (see Fig E1 in this article's Online Repository at www.jacionline.org). The coefficient of variation was less than 15% for intra-assay, interassay, and interoperator variability (see Fig E2 in this article's Online Repository at www.jacionline.org) for the VPPE standard curve, independent peanut standard, and peanut in dust. There was no significant difference in peanut protein levels in sieved dust versus residual fluff (see Fig E3 in this article's Online Repository at www.jacionline.org). The efficacy of extraction was greater than 99% (see Fig E4 in this article's Online Repository at www.jacionline.org). No peanut remained on the disposable nylon collection filter (see Fig E5 in this article's

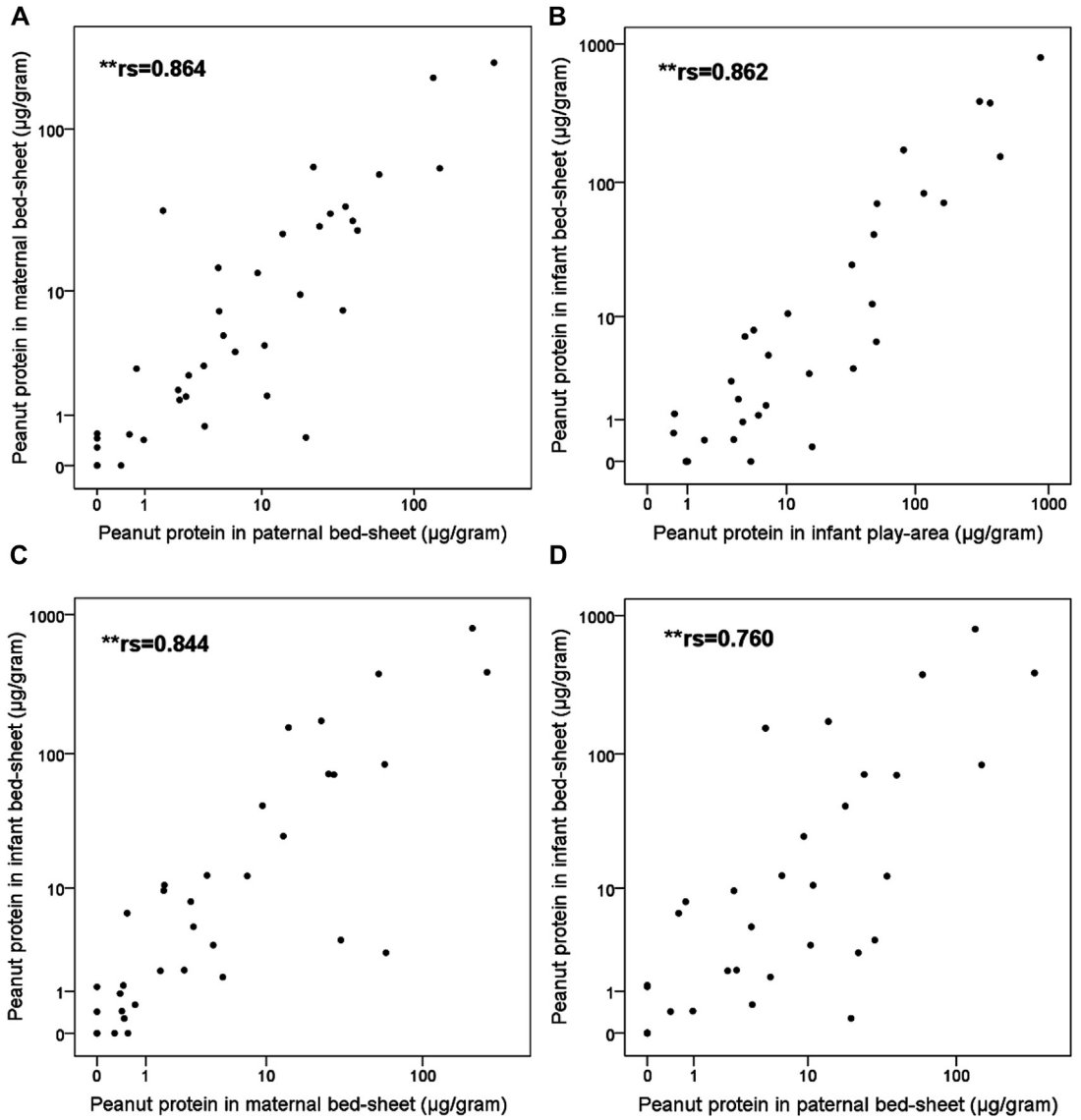

FIG 1. Within-home environmental peanut protein correlation. Correlations between peanut protein levels (in micrograms per gram) in maternal and paternal bed sheets (**A**; n = 38, r_s = 0.864; 95% CI, 0.753-0.927; P < .001), infant's bed sheets and play areas (**B**; n = 32; r_s = 0.862; 95% CI, 0.734-0.930; P < .001), maternal and infant's bed sheets (**C**; n = 34; r_s = 0.844; 95% CI, 0.708-0.919; P < .001), and paternal and infant's bed sheets (**D**; n = 33; r_s = 0.760; 95% CI, 0.564-0.875; P < .001) are shown. Axes are displayed in log scale.

Online Repository at www.jacionline.org). Freezing and thawing extracted dust samples did not affect peanut protein levels (see Fig E6 in this article's Online Repository at www.jacionline.org).

Peanut protein in household dust and wipes

Details of peanut protein levels in dust and wipes, as well as correlations between peanut protein levels in dust and wipes, are shown in Table I. Dust from the infant's play area had the highest peanut protein concentration, followed by dust from the paternal bed, infant's bed, maternal bed, and then sibling's bed. Peanut protein levels were lower in wipe samples than dust samples. Median results for peanut protein were less than the LLQ in wipe samples; however, the 75th percentile was highest for the refrigerator handle, followed by the dishwasher handle, parent's table, tap, and then infant's crib rail and table. There was high within-home correlation between peanut protein levels in dust, particularly between the maternal and paternal bed, the infant's bed and play area, and the maternal and infant's bed (all r_s > 0.840, P < .001, Fig 1). Peanut protein levels also correlated between dust samples and surface wipes (Table I). There were only 3 infant's table-surface wipe samples with peanut levels greater than the LLQ, and thus we did not include this in the correlation analysis.

Peanut protein levels measured with air-sampling monitors

Median peanut protein levels were less than the LLQ (2.5 µg/m^3) at all time points, except while peanuts were being deshelled. The median peanut protein level extracted from glass-fiber filters 1 cm above peanuts being deshelled (n = 6) was 330.90 µg/m^3 (range, 292.64-692.08 µg/m^3) and was still detectable 1 m above peanut deshelling (median, 4.76 µg/m^3;

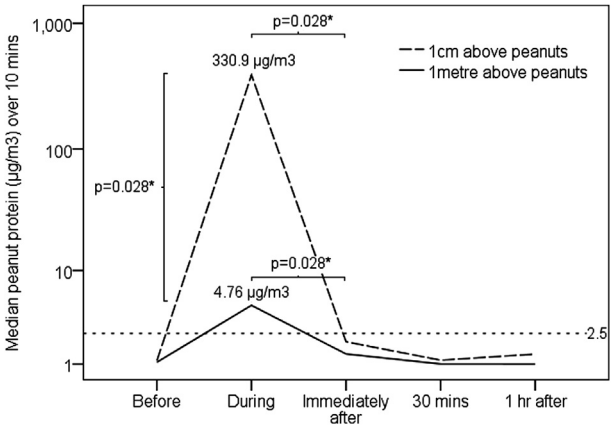

FIG 2. Time course of airborne peanut during peanut deshelling. The air sampling monitor was run for 10 minutes before, during, immediately after, and 30 and 60 minutes after deshelling peanuts at 1 cm and 1 m above the peanuts (n = 6). The *y-axis* is displayed in logarithmic scale. The LLQ (2.5 µg/m^3) of the VPPE is depicted by a *horizontal dotted line*. Median peanut protein levels (in micrograms per cubic meter) are depicted during peanut deshelling at 1 cm versus 1 m above peanuts (P = .028*), with different peanut protein levels during peanut deshelling versus immediately after deshelling both at 1 cm and 1 m (P = .028*).

range, 2.50-7.19 µg/m^3; Fig 2). Median peanut protein levels decreased to less than the LLQ immediately after peanuts stopped being deshelled, even at 1 cm above the peanut shells.

Peanut protein on hands and saliva after peanut consumption

Peanut protein levels increased from less than the LLQ (0.025 µg/ml) to a median of 3.34 µg/mL (range, 0.03-12.19 µg/mL) for saliva (n = 6) and 0.39 µg per wipe (0.03-1.18 µg per wipe) for the right hand (n = 6) 3 hours after peanut consumption (Fig 3).

Persistence of peanut protein despite cleaning

Table surfaces. After spiking wooden, granite, and laminate tables with peanut butter, median peanut protein levels increased from less than the LLQ (0.2 µg per wipe) to a median of 7.86 to 9.21 µg of peanut protein per wipe sample (Fig 4). The highest peanut protein samples obtained after spiking were from granite tables. After a single water wipe, there was only a small reduction in peanut protein levels to a median of 6.56 to 7.92 µg per wipe. After vigorous detergent cleaning, peanut protein levels were less than the LLQ on granite table surfaces but still present on the laminate (median, 0.47 µg per wipe; range, 0.42-0.55 µg per wipe; n = 3) and wooden (median, 1.75 µg per wipe; range, 1.62-3.33 µg per wipe; n = 3) tables. Peanut protein levels were less than the LLQ after plain butter spikes.

Sofa covers and pillows. The median peanut protein level in sofa cover dust (n = 5) at baseline was 38.74 µg/g (range, 10.05-485.43 µg/g), which decreased to 0.73 µg/g (range, 0.25-30.73 µg/g) after the first machine wash. After peanut consumption, peanut protein levels in the sofa cover dust increased to 6217.74 µg/g (range, 1293.02-6460.84 µg/g) and then decreased to 6.06 µg/g (range, 0.25-829.50 µg/g) after the second machine wash, constituting a median 1000-fold reduction in peanut protein (Fig 5, *A*). In a similar experiment peanut protein levels in pillow dust (n = 5) decreased from a median of 2.40 µg/g (range, 1.77-2.78 µg/g) to 1.14 µg/g (range, 0.85-1.76 µg/g) after the first machine wash and from 76.75 µg/g (range, 24.92-183.92 µg/g) after peanut consumption to 1.80 µg/g (range, 0.66-15.19 µg/g; Fig 5, *B*) after the second machine wash, constituting an approximate 40-fold reduction. The LLQ was 0.5 µg/g.

DISCUSSION

In this study we validated a sensitive, specific, and reliable method to quantify peanut protein levels in the home environment. Given that household peanut consumption is a risk factor for the development of peanut allergy,[5] this assay now allows direct quantitation of environmental peanut exposure. Using this commercial peanut ELISA kit, we detected high within-home correlation of peanut protein in household dust and surfaces, which suggests that peanut spreads easily around the home. We showed that hands and saliva are potential routes of peanut transfer into the environment after peanut consumption because peanut is present on both hands and saliva 3 hours after peanut consumption. Furthermore, we have shown that certain household surfaces, bedding, and furnishings retain peanut protein, even after cleaning with detergent; thus environmental peanut exposure in the home might remain after the usual cleaning methods.

Peanut is unlikely to be transferred into the environment by means of aerosolization because peanut could not be detected in a variety of aerosolization experiments, only temporarily while being propelled into the air by peanut deshelling. Previous authors were also unable to detect aerosolized peanut using air monitors strapped to the participants' heads while they ate and stamped on peanuts.[6] One could argue that the air monitor flow rate in our study was not strong enough (2 L/min); however, a previous study detected aerosolized egg at a flow rate of 1.7 to 2 L/min.[16] Additionally, we did not wish to artificially increase the flow rate to greater than a small child's minute volume because this would not have biological plausibility with regard to environmental peanut exposure and possible sensitization through the inhalational route. We ran the air samplers for as long as possible (22 hours) so as to be comparable with other studies detecting aerosolized egg (8 hours)[16] and fish (4-37 hours).[17] The fact that we could measure peanut protein at 1 cm and 1 m above deshelling peanuts proves that the Casella TUFF Air Monitor was able to capture aerosolized peanut protein. Similar findings have been reported by another group while deshelling peanuts using a SpinCon 3000 air collector (InnovaPrep, Drexel, Mo).[18] In our study median aerosolized peanut protein levels at 1 cm above peanut deshelling (330.9 µg/m^3) were similar to those found for aerosolized egg protein in egg factory transfer (644 µg/m^3) and egg-breaking rooms (255 µg/m^3)[16] but much higher than aerosolized fish protein in fish markets (2-25 ng/m^3).[17] Our study importantly showed that aerosolized peanut protein disappeared immediately after peanut deshelling stopped; thus we believe that the physical action of deshelling peanuts propelled peanut particles into the air, but these rapidly settled and did not remain airborne. Formation of large peanut protein aggregates after roasting could render the peanut particles less airborne, which might explain this observation.[19,20] Thus peanut protein is unlikely to cause either peanut sensitization or allergic manifestations in patients with peanut allergy through inhalation unless the peanuts are deshelled in close proximity to them. However, it is possible that a different cooking or handling method that was not tested here could lead to detectable airborne peanut levels. Additionally, these findings might not be applicable to pressurized and recirculated air systems, such as those found in commercial airliners; thus further studies are warranted in this field.

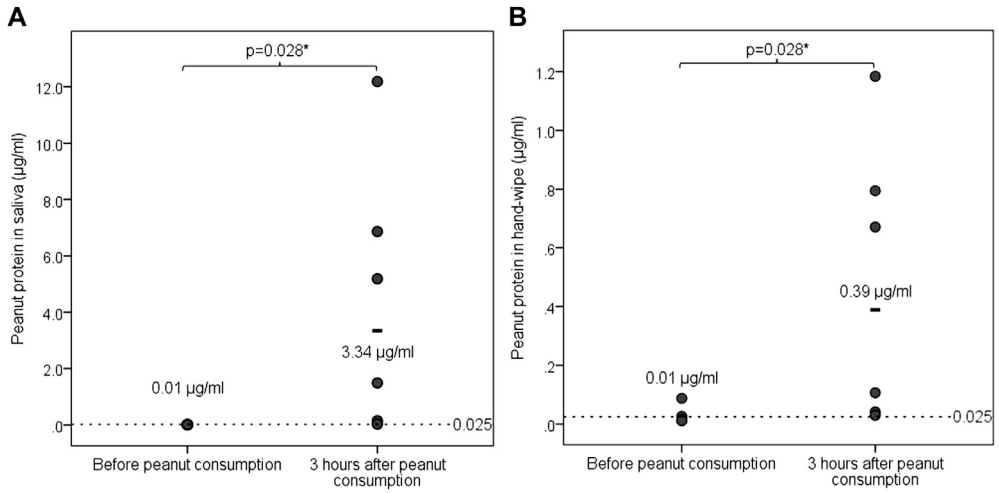

FIG 3. Peanut protein levels (in micrograms per milliliter) in saliva **(A**; n = 6) and on hands **(B**; n = 6) before and 3 hours after consumption of 50 g of peanut. Median peanut protein levels (in micrograms per milliliter) are displayed before and after peanut consumption (P = .028*). The LLQ (0.025 µg/mL) is displayed as a *horizontal dotted line*.

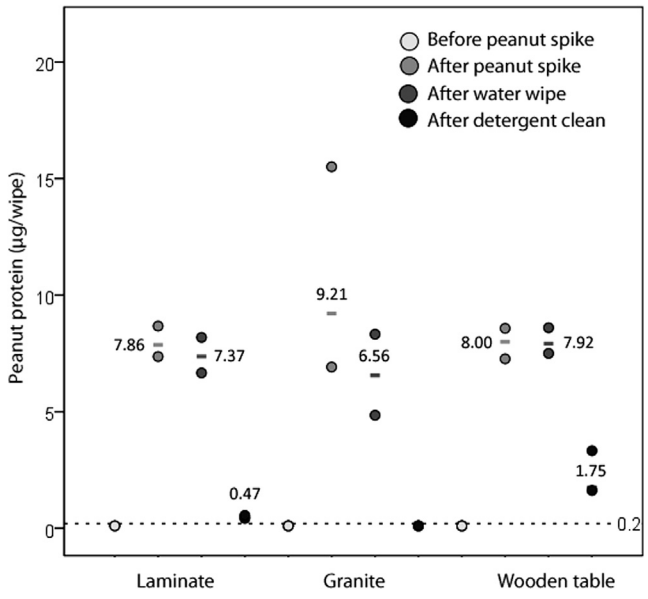

FIG 4. Peanut protein levels (in micrograms per wipe) on 3 different table surfaces (granite, laminate, and wood) before and after a 0.5-mL peanut butter spike, water wipe, and vigorous detergent cleaning (n = 3). Median peanut protein levels (in micrograms per wipe) are displayed as a *short horizontal line*. The LLQ (0.2 µg per wipe) is displayed as a *dotted line*.

Fox et al[5] postulated that environmental peanut exposure might occur through hand transmission because high household consumption of peanut butter (which is sticky and easily transferred on hands) was associated with a higher risk of peanut allergy than household consumption of covered peanuts (eg, chocolate). In this study we found peanut protein on hands and in saliva for a longer time period and at higher concentrations than found in other studies using a monoclonal ELISA against Ara h 1.[6,14] Peanut protein levels in saliva were almost 10 times higher than those in hand-wipe samples after peanut consumption. This might be because participants washed their hands during the 3-hour period but did not brush their teeth.[14] Vigorous cleaning with detergent did not remove peanut protein from laminate and wooden table surfaces, which is in contrast to previous literature.[6] This is likely due to the sensitivity of the VPPE used in this article. However, peanut levels were less than the LLQ on granite tables after detergent cleaning, presumably because it was easier to remove peanut from a smooth uncorrugated surface, such as granite, as shown by the high initial peanut result found on granite after peanut spiking.

We assessed the efficacy of detergent on removing peanut from the dust of sofa covers and pillows as a reflection of an infant's play and sleeping environment, respectively. Although machine washing significantly reduced peanut protein levels, there remained microgram quantities of peanut protein per gram of dust. Similar machine wash experiments have reduced house dust mite allergen levels but have had less success with cat allergen.[21] Persistence of environmental peanut protein after cleaning was still 1000 times lower than that required to elicit a peanut-induced allergic reaction (11.9-65.5 mg)[22]; however, this could be high enough to sensitize young children.

The VPPE had a more sensitive recovery rate of an independent peanut standard than the monoclonal or polyclonal Ara h 1 ELISA. This would be expected because a polyclonal anti-peanut ELISA has a greater diversity of antibodies than a monoclonal ELISA, and Ara h 1 is only one of the peanut proteins detected by using the VPPE.[23] We found similar peanut protein levels in sieved dust versus residual "fluff," which has also been found for Der p 1.[24] However, our experience was that the fluff was more difficult to centrifuge down, and thus we continued to use sieved dust for extraction and analysis. The nylon collection filter did not retain peanut protein, and thus dust could be tipped out of the filter without losing peanut protein. Freezing at −80°C and different durations of thawing did not affect peanut protein levels in extracted dust, which would be expected given that Ara h 1 and 2 are stable allergens resistant to other forms of environmental stress, such as heating and gastric digestion.[25,26]

The shortcomings of this study included the lack of an internationally recognized peanut reference standard to compare the peanut ELISA kits. However, steps are being taken toward having a suitable peanut reference standard to use as quality control material.[27] We addressed this by quantifying the peanut protein concentration and Ara h 1, 2, and 3 levels in the ALK-Abelló independent peanut standard.

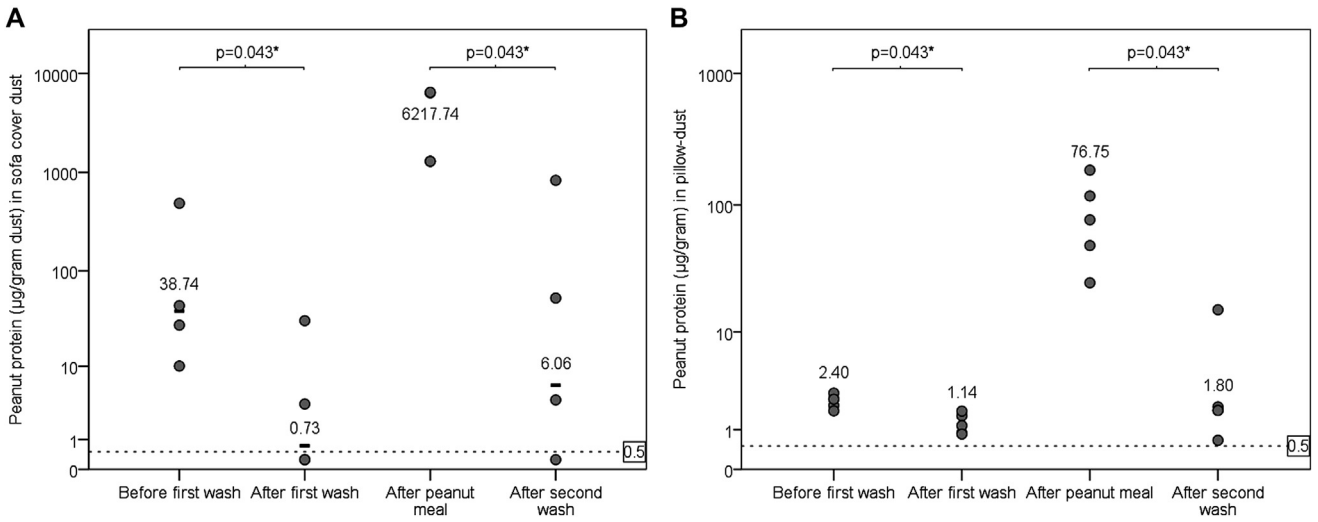

FIG 5. Peanut protein levels (in micrograms per gram of dust) in sofa covers **(A)** and pillows **(B**; n = 5 each) at baseline and after a machine detergent wash and then after peanut consumption and repeat machine detergent wash. Median peanut protein levels (in micrograms per gram of dust) are shown for each time point. The LLQ (0.5 µg/g dust) is displayed as a *horizontal dotted line*.

In conclusion, we have shown that peanut in the environment is measurable and transferrable and might persist despite usual detergent cleaning methods. Peanut protein can be transferred into the environment through hands or saliva but is unlikely to become airborne. Further research into the significance of environmental peanut exposure is required. We plan to further evaluate this in multicenter cohort studies.

We thank Dr Mohammed H. Shamji for advice on validation of assays, Ms Anjeli Chadha for collecting saliva and hand-wipe samples, and ALK-Abelló for providing the independent peanut standard.

Clinical implications: Environmental peanut protein exposure might be an important route of peanut sensitization. This study validates quantification of environmental peanut exposure for use in future studies.

REFERENCES

1. Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. J Allergy Clin Immunol 2001;107:191-3.
2. 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.
3. 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.
4. 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.
5. 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.
6. Perry TT, Conover-Walker MK, Pomes A, Chapman MD, Wood RA. Distribution of peanut allergen in the environment. J Allergy Clin Immunol 2004;113:973-6.
7. Witteman AM, van LJ, van der ZJ, Aalberse RC. Food allergens in house dust. Int Arch Allergy Immunol 1995;107:566-8.
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.
9. 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.
10. Brough HA, Stephens AC, Turcanu V, Lack G. Distribution of peanut in the home. Allergy 2009;64(suppl 90):543.
11. Sicherer SH, Furlong TJ, DeSimone J, Sampson HA. Self-reported allergic reactions to peanut on commercial airliners. J Allergy Clin Immunol 1999;104:186-9.
12. Simonte SJ, Ma S, Mofidi S, Sicherer SH. Relevance of casual contact with peanut butter in children with peanut allergy. J Allergy Clin Immunol 2003;112:180-2.
13. Jones RT, Stark DF, Sussman GL, Yunginger JW. Recovery of peanut allergens from ventilation filters of commercial airliners. J Allergy Clin Immunol 1996;97(suppl 1):423.
14. Maloney JM, Chapman MD, Sicherer SH. Peanut allergen exposure through saliva: assessment and interventions to reduce exposure. J Allergy Clin Immunol 2006; 118:719-24.
15. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Validation of analytical procedures: text and methodology Q2 (R1). Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf. Accessed May 11, 2012.
16. Boeniger MF, Lummus ZL, Biagini RE, Bernstein DI, Swanson MC, Reed C, et al. Exposure to protein aeroallergens in egg processing facilities. Appl Occup Environ Hyg 2001;16:660-70.
17. Taylor AV, Swanson MC, Jones RT, Vives R, Rodriguez J, Yunginger JW, et al. Detection and quantitation of raw fish aeroallergens from an open-air fish market. J Allergy Clin Immunol 2000;105(suppl 1):166-9.
18. Johnson R, Barnes C, Dowling P. Airborne concentrations of peanut protein. Ann Allergy Asthma Immunol 2010;105(suppl 1):A15.
19. van Boxtel EL, van Beers MM, Koppelman SJ, van den Broek LA, Gruppen H. Allergen Ara h 1 occurs in peanuts as a large oligomer rather than as a trimer. J Agric Food Chem 2006;54:7180-6.
20. Schmitt DA, Nesbit JB, Hurlburt BK, Cheng H, Maleki SJ. Processing can alter the properties of peanut extract preparations. J Agric Food Chem 2010;58:1138-43.
21. Tovey ET, Marks G. Methods and effectiveness of environmental control. J Allergy Clin Immunol 1999;103:179-91.
22. Taylor SL, Crevel RW, Sheffield D, Kabourek J, Baumert J. Threshold dose for peanut: risk characterization based upon published results from challenges of peanut-allergic individuals. Food Chem Toxicol 2009;47:1198-204.
23. Nogueira MC, McDonald R, Westphal C, Maleki SJ, Yeung JM. Can commercial peanut assay kits detect peanut allergens? J AOAC Int 2004;87:1480-4.
24. Mason K, Crane J, Fitzharris P, Siebers R. The effect of sieving on Der p 1 levels. Allergy 2001;56:1012-3.
25. Maleki SJ, Kopper RA, Shin DS, Park CW, Compadre CM, Sampson H, et al. Structure of the major peanut allergen Ara h 1 may protect IgE-binding epitopes from degradation. J Immunol 2000;164:5844-9.
26. Sen M, Kopper R, Pons L, Abraham EC, Burks AW, Bannon GA. Protein structure plays a critical role in peanut allergen stability and may determine immunodominant IgE-binding epitopes. J Immunol 2002;169:882-7.
27. Zeleny R, Schimmel H. Towards comparability of ELISA results for peanut proteins in food: a feasibility study. Food Chem 2010;123:1343-51.