

Detection of Peanut Allergens in Breast Milk of Lactating Women

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THE PREVALENCE OF PEANUT ALLERGY has increased markedly in recent years.¹ Approximately 1% of British and US preschool children are sensitized to peanuts.^{2,3} Peanut allergy is a significant public health problem as it starts early in life, is often associated with severe or life-threatening reactions, and rarely resolves.^{4,5} Peanuts account for the majority of food-induced anaphylactic fatalities.⁵⁻⁸

Between 72% and 81% of individuals who have reactions to peanuts do so on their first known exposure.^{6,9} Since IgE-mediated allergic reactions require prior exposure resulting in sensitization, earlier peanut exposure must have been occult in most cases. Potential but unproven routes of occult exposure include exposure in utero and exposure to peanuts transferred from the maternal diet via breast milk during lactation.^{6,10}

Delineation of routes of occult exposure leading to sensitization would be helpful in guiding the formulation of strategies to prevent sensitization of children at risk. In this study, we investigated the ability of maternal dietary peanut protein to pass into breast milk during lactation.

METHODS

This study was approved by the Research Ethics Committee of St Michael's Hospital, University of Toronto. Healthy, lactating women, ranging in age from 21 to 35 years (median, 31.1 years), volunteered for the study in response to

Context Most individuals who react to peanuts do so on their first known exposure. A potential but unproven route of occult exposure resulting in sensitization to peanut is via breast milk during lactation.

Objective To investigate the ability of maternal dietary peanut protein to pass into breast milk during lactation.

Design and Setting Clinical investigation conducted at 2 North American hospitals from March 1999 to October 2000.

Patients Twenty-three healthy, lactating women aged 21 to 35 years.

Intervention Each woman consumed 50 g of dry roasted peanuts, after which breast milk samples were collected at hourly intervals.

Main Outcome Measures Presence in breast milk of total peanut protein, analyzed by a sandwich enzyme-linked immunosorbent assay, and 2 major peanut allergens, *Ara h 1* and *Ara h 2*, detected by immunoblot analysis.

Results Peanut protein was detected in 11 of 23 subjects. It was detected in 10 subjects within 2 hours of ingestion and in 1 subject within 6 hours. The median peak peanut protein concentration in breast milk was 200 ng/mL (mean, 222 ng/mL; range, 120-430 ng/mL). Both major peanut allergens *Ara h 1* and *Ara h 2* were detected.

Conclusions Peanut protein is secreted into breast milk of lactating women following maternal dietary ingestion. Exposure to peanut protein during breastfeeding is a route of occult exposure that may result in sensitization of at-risk infants.

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posted notices at breastfeeding centers. Inclusion criteria included informed consent and ability to express breast milk at timed intervals on the day of protocol. Exclusion criteria were known peanut or tree nut allergy.

Subjects were instructed to avoid ingestion of legumes for 24 hours prior to breast milk collection, and fasted overnight. Participants collected breast milk at time 0 on the morning of collection. After the initial breast milk collection, they consumed 50 g (approximately ½ cup) of dry roasted peanuts. Strict precautions were taken to avoid any cross-contamination. Samples of breast milk were collected at 1, 2, 3, 4, 6, 8, and 12 hours after peanut ingestion. Aliquots of 5 mL of breast milk were collected, centrifuged to remove cellular elements, and stored at -20°C

until assay. Timing of subsequent meals and infant nursing were standardized to minimize confounding.

Samples were thawed and centrifuged at 8000g, 15 minutes at 4°C to remove fat and cellular debris. Peanut protein was analyzed quantitatively by sandwich enzyme-linked immunosorbent assay with the Veratox peanut allergen test kit (Neogen Corp, Lansing, Mich). Breast milk was added to polyclonal rabbit anti-peanut IgG-coated wells and incubated for 10 minutes at

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22°C. Unbound protein was removed and horseradish peroxidase-conjugated detection antibody was added. Substrate was added and the reaction was allowed to develop for 10 minutes at 22°C. Red stop reagent was added and the color of the resulting solution was measured at 620 nm.

Breast milk samples were mixed with sodium dodecyl sulfate (SDS) buffer containing 2-mercaptoethanol, treated at 100°C for 2 minutes. Total protein (20 µg) was applied to each lane of a vertical slab SDS polyacrylamide gel. Resolving gels were 12% wt/vol polyacrylamide, with 4% wt/vol stacking gels. Gels were run at 170 V for 55 minutes in Tris-glycine buffer.

Proteins were electrophoretically transferred to Hybond ECL nitrocellulose membrane (Amersham Pharmacia Biotech, Piscataway, NJ) in a semi-dry Transblot apparatus (Bio-Rad Laboratories, Hemel Hempstead, England) for 40 minutes at 18 V in Tris-glycine buffer containing 20% methanol. Protein transfer was monitored by transfer of prestained marker proteins and by staining the remaining gel with coomassie brilliant blue and the membrane with ponceau solution.

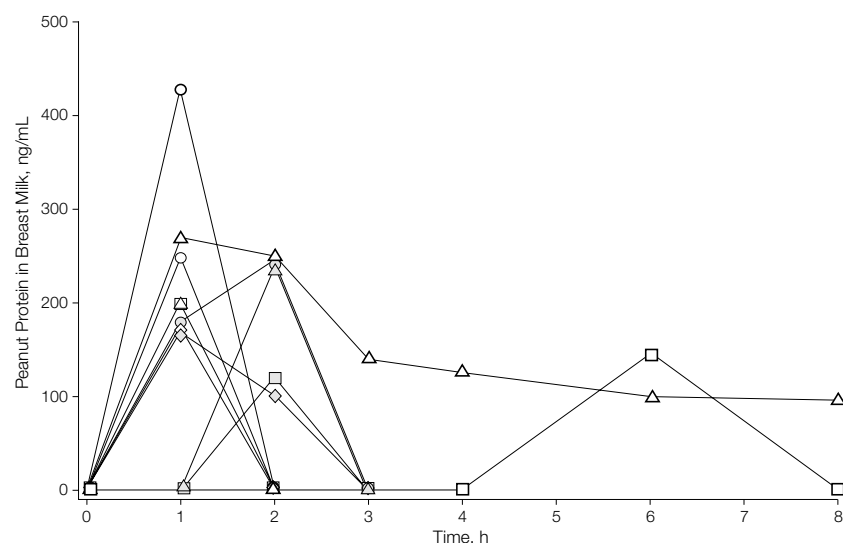
Blots were probed with rabbit anti-peanut antibody. After blocking with a solution of 3% bovine serum albumin in Tris-buffered saline the immunoblots were incubated with a 1:1000 dilution of rabbit polyclonal anti-peanut antibody for 2 minutes at 22°C. After washing with phosphate-buffered saline with Tween, protein G was added and the blots were rotated for 2 hours at room temperature. After washing, the blots were then exposed to Bio Max film (Eastman Kodak Co, Rochester, NY) and developed.

RESULTS

Authentic peanut protein was added to breast milk and assayed as described above. The assay was linear to a peanut protein concentration of 1000 ng/mL. Addition of peanut standard to breast milk yielded an absorbance curve identical to that for peanut protein in buffer.

The results of analyses of breast milk samples from 23 subjects are shown in

Figure 1. Time Course of Appearance of Peanut Protein in Breast Milk



Subjects ingested 50 g of peanuts. Individual curves represent results in each of 11 subjects in whom peanut protein was detected after challenge.

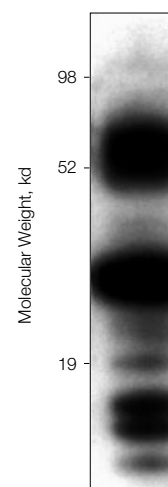
FIGURE 1. Peanut protein was detected in 11 of 23 subjects. Peanut protein appeared within 1 hour of ingestion in 8 of 11 subjects, within 2 hours in 2 of 11 subjects, and was delayed for 6 hours in 1 subject. Median peak peanut protein concentration in breast milk was 200 ng/mL (mean, 222.3 ng/mL; range, 120-430 ng/mL). Peanut protein was cleared rapidly from breast milk except in 1 subject, who showed protracted presence of peanut protein.

A representative rabbit polyclonal anti-peanut immunoblot is shown in FIGURE 2. As can be seen in this example, there were recognizable amounts of peanut protein that coincided with both allergens *Ara h 1* and *Ara h 2* in the breast milk samples. Although the number of subjects was small, there were no differences between the 11 secretors and 12 nonsecretors in age (median, 31.1 and 31.0 years, respectively), time postpartum (median, 17 and 21 weeks, respectively), or history of atopy (3 vs 2 subjects).

COMMENT

Many studies have documented subjects' reactions to foods on the first known exposure.^{6,9,11} A study of 8 exclusively breastfed infants described

Figure 2. Rabbit Polyclonal Anti-Peanut Immunoblot



A representative immunoblot of breast milk collected at 1 hour following peanut ingestion. The IgE-binding bands of molecular weight 17 kD and 66 kD correspond to the major peanut allergens *Ara h 2* and *Ara h 1*, respectively.

multisystem allergic reactions to milk, eggs, or peanuts occurring on the first known exposure.¹¹ More recent studies have shown that such reactions are increasingly commonplace.⁶ In 2 studies^{9,12} of peanut and tree nut allergy, reactions on first known exposure oc-

curred in 72% to 81% of all cases. Since sensitization requires prior exposure to generate allergen-specific IgE, the sensitizing exposure must be occult in many cases.

Exposure to food allergens in breast milk originating from the maternal diet is thought to be responsible for occult sensitization.^{6,10,13-15} Some food allergens have been detected in breast milk of lactating women, including the major cow's milk allergen, β -lactoglobulin.¹⁶⁻¹⁹ The time from ingestion to peak concentration of β -lactoglobulin in breast milk was 1 to 6 hours. Two major egg allergens, ovalbumin and ovomucoid, are readily transferred to breast milk,^{16,20,21} with peak concentrations attained 2 to 6 hours after ingestion. Similarly, gliadin from dietary wheat is detectable within 2 to 4 hours of ingestion.^{22,23} Concentrations of bovine β -lactoglobulin (5-800 ng/mL), egg ovalbumin (200 pg/mL-6 ng/mL) and ovomucoid (0-2.88 ng/mL), and wheat gliadin (5-95 ng/mL) are dependent on the amount ingested, and peak concentrations in breast milk are generally in the nanogram per milliliter range.^{16,20-23}

Peanut proteins have long been suspected to be secreted but have never

been identified in breast milk. We now provide definitive evidence for secretion of peanut protein into breast milk of lactating women. Peanut protein concentrations found in breast milk ranged from 120 to 430 ng/mL, comparable with the levels of β -lactoglobulin, ovalbumin, ovomucoid, and gliadin detected in breast milk.

The time courses of appearance of proteins from peanuts, eggs, milk, and wheat are similar. Peanut proteins appeared within 1 to 3 hours following oral ingestion and were quickly cleared from breast milk. Both low- and high-molecular-weight proteins with mobilities corresponding to *Ara h* 1 and *Ara h* 2 were secreted intact into breast milk with no evidence for degradation. Ovalbumin, β -lactoglobulin, and gliadin also appear in undegraded form in breast milk.^{16,21,23}

Only a portion of lactating women (48%) secreted peanut protein in their milk. Similarly, only 53% to 63% of women secreted β -lactoglobulin and 59% to 74% of women secreted ovalbumin into breast milk after ingestion of cow's milk and eggs, respectively.^{16,17,20,21}

Atopic status and other demographic characteristics of lactating women have

not accounted for the variable secretion of food proteins into breast milk in previous studies, nor in this study, although the number of women investigated was small.^{18,19}

Several studies have documented an epidemiologic relationship between increased consumption of peanut by pregnant and breastfeeding mothers and the likelihood of allergic sensitization of their children.^{1,9,10} These studies, in conjunction with our data, suggest that transfer of maternal dietary peanut protein to breast milk may predispose at-risk children to occult sensitization.

Author Contributions: Study concept and design: Vadas.

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Analysis and interpretation of data: Vadas, Burks, Perelman.

Drafting of manuscript: Vadas, Wai, Burks.

Critical revision of the manuscript for important intellectual content: Vadas, Burks, Perelman.

Statistical expertise: Vadas, Perelman.

Obtained funding: Vadas, Wai.

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Study supervision: Vadas.

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