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Introduction of various allergenic foods during infancy reduces risk of IgE sensitization at 12 months of age: a birth cohort study

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BACKGROUND: In this study, we aimed to determine whether introducing various allergenic foods during infancy is associated with IgE sensitization at 12 months of age.

METHODS: Detailed information on feeding practices regarding six possible allergenic foods (fruits, egg white, egg yolk, fish, shellfish, and peanuts) was obtained by administering age-specific questionnaires to parents of infants at ages 6 and 12 months. Fecal secretory IgA (sIgA), fecal eosinophil cationic protein (ECP), and serum levels of total IgE and IgE specific to 20 foods, and IgE specific to 20 inhalant allergens were also quantified at 12 months of age.

RESULTS: At 12 months of age, infants with IgE sensitization had been introduced to fewer allergenic food items during infancy (3.2 ± 1.4 vs. 3.7 ± 1.3 items). Compared with infants who were given 0–2 allergenic food items, infants introduced to 3–4 or ≥ 5 allergenic food items showed a significantly lower risk of IgE sensitization (odds ratios (ORs) 0.62 and 0.61, respectively) and lower total IgE levels. In addition, non-introduction of egg white or egg yolk was significantly related to IgE sensitization (ORs 1.41 and 1.26, respectively).

CONCLUSION: Increasing the diversity of allergenic foods in infancy, including fruits, egg white, egg yolk, fish, shellfish, and peanuts, may protect infants from IgE sensitization at 12 months of age.

The prevalence of atopic diseases and food allergies continues to rise around the world (1,2). Many parents experience anxiety regarding dietary choices for their child. Although the previously recommended guideline to delay the introduction of allergenic foods into the diet of infants has been withdrawn (3), the timing and possible preventive effects of allergenic-food introduction on allergy remain unclear. Some studies suggest that delayed introduction of solid foods, including those regarded as allergenic, such as peanuts, eggs, and cow milk may increase the risk of food allergies or atopic diseases regardless of infant risk (4–6), whereas others suggest that the effect of early introduction on long-term oral tolerance is unknown (7,8). Thus, additional studies are needed to address this issue more conclusively.

In young children, the diagnosis of atopic diseases is mainly based on clinical evaluation. Although assessment of total and allergen-specific IgE antibodies provides helpful information to the clinician (9), the complicated interaction between infant-feeding practices and gut immunity is still difficult to explore. Therefore, two non-invasive biomarkers—fecal secretory IgA (sIgA) and eosinophil cationic protein (ECP)—were used to elucidate intestinal immune responses in this study. Secretory IgA is the most abundant immunoglobulin in the gastrointestinal tract of infants (10). As a neutralizing antibody, sIgA contributes to mucosal homeostasis by limiting the uptake of antigens from the gut, thereby preventing primary sensitization or the triggering of allergic reactions to food antigens, and sIgA is also thought to play a critical role in oral tolerance (11,12). ECP is an excellent marker of eosinophil activation in various allergic and gastrointestinal diseases (13). As a marker of intestinal inflammation, fecal ECP can help with the diagnosis and monitoring of food hypersensitivity reactions (14,15).

In the present study, we first aimed to assess the relation between characteristics of feeding practices during infancy and IgE sensitization to potential food and inhalant allergens in 12-month-old infants. Our second aim was to explore the introduction of various allergenic foods in relation to the risk of IgE sensitization. Lastly, we monitored the intestinal immune response by examining fecal sIgA and ECP levels in infants with and without IgE sensitization, allergenic food introduction, and variable diversity of allergenic foods among our study subjects.

METHODS

Study Subjects

Our study subjects were enrolled from the Prediction of Allergies in Taiwanese Children (PATCH) study. This study's protocol was approved by the Ethics Committee of Chang Gung Memory Hospital

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(decision numbers 101-4361C and 104-7015C). A total of 637 infants were born in the delivery rooms of Chang Gung Memorial Hospital, Keelung, Taiwan, between March 2012 and July 2014 and were enrolled in the PATCH study after we obtained written informed consent from their parents soon after birth. To avoid potential confounding factors, 37 infants who dropped out during the 1-year follow-up period, 112 infants who did not regularly return for follow-up visits to the clinic or whose parents did not complete the questionnaires, 183 infants who did not provide blood samples for the measurement of allergen-specific IgE at 1 year of age, and 33 infants who had gestational age of <35 weeks, any perinatal insult, or a major congenital anomaly were not included in the analysis. Eventually, 272 infants were enrolled.

Questionnaires

The parents and study participants returned for a follow-up clinical visit regularly and were checked by a neonatologist at 2, 4, 6, and 12 months of age. Standardized questionnaires were administered to parents under the guidance of well-trained research assistants at all follow-up visits to the clinic. Detailed information about one or both parents having a physician-confirmed history of asthma, rhinoconjunctivitis, atopic eczema, urticarial, and/or food allergy and data regarding the infants' demographic characteristics, living conditions (such as the presence of cigarette smoke or household pets, e.g., dogs, cats, or birds), general health information, and clinical atopic symptoms were also collected.

The infants' diet was assessed by administering PATCH study group-designed, age-specific dietary questionnaires to parents at 0, 2, 4, 6, and 12 months of infants' age (10,16). Data about feeding practices were also collected: current feeding regimens (e.g., human milk, infant formula, or a mix), the timing for introduction of solid foods, and which potentially allergenic foods have been introduced into their diet, such as (1) fruits, (2) egg white, (3) egg yolk, (4) fish, (5) shellfish, and (6) peanuts. The parents were asked to record the exact month that the food was introduced to infants with an accuracy of one decimal place (e.g., age 6.5 months).

Quantification of Serum Allergen-Specific IgE and Total IgE

Blood samples (3–5 ml) were collected from each study subject at 12 months of age. The total serum IgE was quantified by Immuno CAP (Uppsala, Sweden) in 251 infants, and allergen-specific IgE was determined by means of an automated microfluidic-based multiplexed immunoassay system (BioIC Allergen-specific IgE Detection Kit-AD40 Panel; Agnitio Science and Technology, Hsinchu, Taiwan) in 272 infants. BioIC screening represents a high-density multitarget screening technique that requires minimal amounts of serum (25–100 µl). This relaxed requirement is especially crucial for allergen screening among small children, where large blood draws are difficult and serum supply is limited (17,18). The following 40 allergens were included in the Allergen-specific IgE Detection Kit: (1) 20 food allergens: cow milk, goat milk, egg white, egg yolk, crab, shrimp, codfish, salmon, blue mussel, soybean, wheat, potato, peanut, almond, garlic, cheese, baker's yeast, kiwi, tomato, and carrot and (2) 20 inhalant allergens in the following six categories: (a) mites: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Blomia tropicalis*; (b) animals: dog dander, cat dander, chicken feathers and skin, and duck feathers and skin; (c) cockroaches: German cockroach and Oriental cockroach; (d) mold: *Candida albicans*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Penicillium notatum*, and *Alternaria alternata*; (e) pollen: timothy grass, bermuda grass, ragweed, mugwort, and goldenrod; and (f) others: latex (17). The cutoff levels achieved with the BioIC microarray were arbitrarily defined as 1 AU. Class scores from 0 to 6 were determined by ranges: <1, 1–2, >2–4, >4–8, >8–16, >16–32, and >32 AU, respectively (18,19).

Definitions Used in this Study

Maternal/paternal allergy. Parents who self-reported that they have a physician's confirmation for an allergic disease, such as asthma, rhinoconjunctivitis, eczema, or urticarial or food allergies, in conjunction with a response to antiallergic treatment.

Infants with IgE sensitization. Infants who got sensitized to one or more allergens determined by BioIC (class score 1–6).

Infants without IgE sensitization. Infants who did not get sensitized to any allergens as determined by BioIC (class score = 0).

Allergenic food diversity. This parameter was calculated by means of six food items (fruits, egg white, egg yolk, fish, shellfish, and peanuts) and categorized as "0–2 food items," "3–4 food items," and "5–6 food items" in our study.

Non-introduced food. A food item that was never introduced during the first year of life.

Infants with doctor-diagnosed atopic dermatitis. Infants who had been confirmed by any physician to have atopic dermatitis by 12 months of age.

Stool Sample Collection and Processing

The infants' stool samples were collected into plastic containers within 2 days before the study participants returned to the clinic for a follow-up at 12 months of age. The methods used for stool sample collection and processing were described in our previous studies (10,16). Briefly, our research assistants weighed each stool sample and added extraction buffer containing citrate. The stool samples were then mixed for homogeneity and centrifuged for 15 min at 3,000 rpm and 4 °C. The supernatant was collected and frozen at − 80 °C until use.

Determining the Levels of Fecal sIgA and ECP by ELISAs

After we thawed the supernatants from infant stool samples, fecal sIgA and ECP concentrations (ng/ml) were determined using the sIgA ELISA kit (Immundiagnostik, Bensheim, Germany) and eosinophil cationic protein ELISA kit (MyoBioSource, San Diego, CA). To calculate sIgA and ECP concentrations in the stool of infants (per gram), the measured fecal sIgA and ECP units were adjusted and expressed in milligrams per gram of stool (mg/g) and nanograms per gram of stool (ng/g), respectively (10,16).

Statistical Analysis

Data used for analyses of the study population included the questionnaire data on 6-month and 12-month infants, fecal sIgA, ECP levels, and total serum and allergen-specific IgE data at the age of 12 months. Numerical variables were summarized as mean ± standard deviation (SD) or as frequencies and percentages. Associations between categorical variables were studied by the chi-square test. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated to describe the relation of the timing of allergenic food introduction, diversity, or introduction of selected allergenic foods with IgE sensitization to any allergens contained in the BioIC test or doctor-diagnosed atopic dermatitis at 12 months of age. The differences in continuous variables with non-normal distribution such as fecal ECP and sIgA levels were evaluated by the Kruskal–Wallis test. *P*-values < 0.05 were assumed to denote significance. Statistical analysis was performed using SPSS 20.0 software for Mac (Chicago, IL).

RESULTS

Feeding Practices and Clinical Characteristics

A total of 272 infants were enrolled in our study. Examination of feeding practices showed that there was high variation in exclusive breastfeeding duration (median: 2 months, mean: 4.2 months), and most of the participants (84.8%) started

Table 1. Demographic data and characteristics of feeding practices in infants that were or were not IgE-sensitized at 12 months of age

Characteristics	Infants with IgE sensitization ($N = 83$)	Infants without IgE sensitization ($N = 189$)	*P*-value
Neonatal and environmental factors			
Sex of infants, male (%)	49 (59.0)	102 (54.0)	0.229
Gestational age (week)	37.9 ± 1.6	37.9 ± 2.2	0.888
Cesarean section, *n* (%)	32 (38.6)	82 (43.3)	0.370
Birth body weight (kg)	3.0 ± 0.6	3.0 ± 0.5	0.939
Environmental tobacco smoking after birth, *n* (%)	51 (69.0)	120 (58.7)	0.891
Household pets at 12 months, *n* (%)	21 (25.3)	53 (28.0)	0.372
Maternal factors			
Maternal age (y)	31.2 ± 5.1	31.9 ± 8.0	0.490
Maternal education level			
High school or below, *n* (%)	26 (31.3)	53 (29.0)	0.563
College or above, *n* (%)	57 (68.7)	136 (71.0)	0.770
Parental history of allergy			
Maternal allergy, *n* (%)	38 (45.7)	73 (38.6)	0.088
Paternal allergy, *n* (%)	36 (43.3)	71 (37.6)	0.129
Infant-feeding practices			
Exclusive breastfeeding duration[a] (months)	5.4 ± 4.9	4.3 ± 4.6	0.078
Age at introduction of solid foods (months)	5.3 ± 1.6	5.5 ± 1.5	0.207
Timing at allergenic food introduction			0.248
< 6 months old, *n* (%)	23 (27.7)	45 (23.8)	
≥ 6–12 months old, *n* (%)	56 (76.4)	140 (74.1)	
Never, *n* (%)	4 (4.8)	4 (2.1)	
Allergenic food diversity, allergenic food items at 12 months	3.2 ± 1.4	3.7 ± 1.3	0.006[b]

Values present numbers (%) or mean ± SD.
[a]Exclusive breastfeeding duration: duration during which infants received only breast milk and no infant formula.
[b]Statistically significant: $P < 0.05$.

eating solid foods between 4 and 6 months of age (median: 6 months, mean: 5.4 months); only 25% of participants were introduced to allergenic foods earlier than 6 months of age, 72.1% were introduced between 6 and 12 months, and 2.9% were never introduced by 12 months of age. Additionally, 64 (23.5%) infants were introduced to greater than five allergenic food items, 151 (55.5%) were given 3–4 food allergenic food items, and 58 (21.3%) were given 0–2 allergenic food items by the age of 12 months. Non-introduction of peanuts was observed in 231 (84.9%) infants, shellfish in 218 (80.1%), egg white in 101 (37.1%), egg yolk in 57 (21.0%), fish in 35 (12.8%), and non-introduction of fruits in 20 (7.3%) infants by the age of 12 months. There were no reports of severe adverse reactions to allergenic foods in the infants. Sixty-nine infants (25.3%) became IgE-sensitized to at least one food allergen and 45 (16.5%) infants to at least one inhalant allergen. As for food allergens, sensitization to egg yolk was observed in 13.6% of infants, to peanuts in 13.2%, to cow milk in 10.6%, to egg white in 10.3%, to cheese in 6.6%, to codfish/or salmon in 5.5%, to goat's milk in 3.7%, to shrimp in 3.3%, to crabs in 2.6%, to wheat in 2.6%, to kiwi in 0.7%, and to baker's yeast in 0.4% of the infants. Sensitization to inhalant allergens was as follows: to *Dermatophagoides pteronyssinus* (house dust mite) in 15% of the infants, to *Dermatophagoides farina* in 8.4%, to cat dander in 5.5%, to dog dander in 2.9%, to timothy grass in 3.3%, and to Bermuda Grass in 0.7% of the infants. A total of 189 (69.5%) infants did not get sensitized to any allergen.

The demographic characteristics of our participants categorized by IgE sensitization status are shown in **Table 1**. Among our participants, we found no association between IgE sensitization and sex, birth history, environmental tobacco

smoking, household pets, maternal age, education levels, and family history of allergies. Besides, there were little or no differences in exclusive breastfeeding duration and the age at which solid food was introduced between infants with and without maternal/paternal allergies (data not shown). The timing of introduction of allergenic foods was not significantly different between the two groups ($P = 0.248$). At age 12 months, infants with IgE sensitization had been introduced to fewer allergenic food items during infancy in comparison with controls (3.2 ± 1.4 vs. 3.7 ± 1.3 items; $P = 0.006$).

Allergen-Specific IgE Sensitization and Doctor-Diagnosed Atopic Dermatitis at 12 Months of Age in Relation to Exposure to Allergenic Foods

For evaluating the timing of allergenic-food introduction, we found that the risk of IgE sensitization was not different between groups with early (<6 months) and late (≥6–12 months) introduction of allergenic foods (OR: 0.83, 95% CI 0.54–1.27; $P = 0.441$; **Table 2**). Overall, our results indicated that the greater the diversity of allergenic foods introduced by 12 months of age, the lower was the likelihood of allergen-specific sensitization, indicating a possible protective effect. Compared with infants who were given 0–2 allergenic food items, the infants introduced to 3–4 or ≥5 allergenic food items showed a significantly reduced risk of IgE sensitization (OR 0.62, 95% CI 0.40–0.93, $P = 0.032$ and OR: 0.61, 95% CI 0.43–0.86, $P = 0.012$, respectively). Non-introduction of egg white by 12 months of age was significantly related to IgE sensitization (OR 1.41, 95% CI 1.11–1.79; $P = 0.002$). Similarly, a positive association with IgE (OR 1.26, 95% CI 1.07–1.48; $P = 0.002$) was observed among the infants not introduced to egg yolk. Non-introduction of fruits, fish, shellfish, or peanuts by 12 months of age was not significantly related to the risk of IgE sensitization.

By 12 months of age, 37 (13.6%) infants had a physician-confirmed diagnosis of atopic dermatitis, and we found that the prevalence of IgE sensitization was much higher among the infants with atopic dermatitis compared with the controls (62.2% vs. 26.2%, $P < 0.001$). Nonetheless, no statistically significant associations were observed between the timing, diversity, or non-introduction of allergenic foods and doctor-diagnosed atopic dermatitis at 12 months of age (**Table 2**).

Feeding Practices in Relation to Fecal sIgA and ECP and Serum IgE Levels

A total of 210 stool samples were collected from 272 infants at the age of 12 months. **Table 3** shows fecal sIgA, ECP, and total serum IgE levels in relation to the various feeding practices used by 12 months of age. During the comparison of fecal immune markers, although without statistical significance, we found that fecal sIgA levels were lower whereas fecal ECP levels were higher in IgE-sensitized infants as compared with controls. In addition, fecal sIgA levels showed an upward trend, whereas fecal ECP levels showed a downward trend in infants as greater diversity of allergenic foods was introduced (**Table 3**).

Total serum IgE levels were different among groups with different allergenic foods diversity (≥5, 3–4, 0–2 food items; $P = 0.014$). Infants who were introduced to ≥5 allergenic food items had significantly lower serum IgE levels when compared with the infants who were introduced to 0–2 allergenic food items (43.46 ± 48.02 vs. 94.08 ± 148.93 kU/l, $P = 0.014$) and 3–4 food items (43.46 ± 48.02 vs. 61.28 ± 88.07 kU/l, $P = 0.049$). In addition, we found that infants in the egg yolk non-introduction group had significantly higher serum IgE levels than those in the egg yolk introduction group (93.11 ± 147.86 vs. 56.28 ± 79.87 kU/l, $P = 0.009$).

DISCUSSION

This study explored infant-feeding practices involving allergenic foods in Taiwan. To the best of our knowledge, this is the first study to examine the relation between the diversity of introduced allergenic foods and IgE sensitization in infants. We found that more infants got sensitized to food allergens than to inhalant allergens at 12 months of age (20,21). Among the food allergens in this study, eggs, peanuts, and cow milk were the most common allergens (22), and mites were the most common inhalant allergen linked to IgE sensitization in infancy. In addition, we found that the introduction of higher diversity of allergenic foods during infancy might reduce the risk of IgE sensitization at 12 months of age.

Although there is mounting evidence that avoidance or delayed introduction of commonly allergenic foods may cause unfavorable atopic outcomes, no clear recommendations are officially provided about the optimal timing and method of introduction of these allergenic foods into an infant's diet with the aim of reducing the risk of allergy (9). Recently, both animal and human studies have shown that induction of oral tolerance can be achieved by regular exposure to food allergens (23–25). Therefore, food avoidance strategies may increase the risk of an adverse immune response to allergens (23). In our study, parents often seemed hesitant to introduce allergenic foods into their child's diet during infancy, regardless of whether the parents had allergic diseases. Notably, peanut and shellfish non-introduction rates were more than 80, and 21.3% of infants were introduced to fewer than two allergenic food items by 12 months of age.

A review of the literature reveals that infants with allergic sensitization are at a higher risk of allergic diseases in early childhood (21,26). In addition, studies have uncovered a quantitative relation of elevated levels of specific IgE antibodies or total serum IgE with various symptoms of allergic diseases (16,27). According to our results, there were no differences in the demographic data, parental allergy history, exclusive breastfeeding duration, and the age of introduction of solid foods between infants with and without IgE sensitization (**Table 1**). Our data revealed that the higher the diversity of allergenic foods introduced into the diet of infants within the first year of life, the lower is the likelihood of IgE sensitization (ORs ranged from 0.61 to 0.62; **Table 2**). Likewise, serum IgE levels were significantly lower in infants who were introduced to ≥5 allergenic foods than in infants

Table 2. The prevalence of IgE sensitization and doctor-diagnosed atopic dermatitis at 12 months of age in relation to allergenic food introduction during infancy

	Infants with IgE sensitization ($n=83$)			Doctor-diagnosed atopic dermatitis ($n=37$)		
	n (%)	OR (95% CI)	*P*-value	*n* (%)	OR (95% CI)	*P*-value
Age at introduction of allergenic foods						
≥6–12 months	56 (28.7)	0.83 (0.54–1.27)	0.441	24 (12.6)	0.95 (0.85–1.07)	0.408
<6 months	23 (34.3)	1		11 (16.1)	1	
Allergenic food diversity						
5–6 Items	14 (22.2)	0.61 (0.43–0.86)	0.012[a]	7 (11.1)	0.91(0.78–1.06)	0.307
3–4 Items	43 (28.5)	0.62 (0.40–0.93)	0.032[a]	19(12.6)	0.93 (0.81–1.07)	0.272
0–2 Items	26 (44.8)	1		11(18.9)	1	
Egg white introduction						
No	42 (41.6)	1.41 (1.11–1.79)	0.002[a]	17 (17.1)	1.44 (0.79–2.61)	0.273
Yes	41 (24.0)	1		20 (11.6)	1	
Egg yolk introduction						
No	27 (47.4)	1.26 (1.07–1.48)	0.002[a]	11 (19.3)	1.60 (0.84–3.03)	0.191
Yes	56 (26.0)	1		26 (12.1)	1	
Fruits introduction						
No	7 (35.0)	1.02 (0.95–1.09)	0.582	4 (20.0)	1.31 (0.59–2.91)	0.596
Yes	76 (30.2)	1		33 (13.1)	1	
Fish introduction						
No	10 (28.6)	0.99 (0.90–1.09)	1.000	6 (17.3)	1.31 (0.59–2.91)	0.596
Yes	73 (30.8)	1		31 (13.0)	1	
Shellfish introduction						
No	68 (31.2)	1.17 (0.67–2.04)	0.617	30 (13.8)	1.06 (0.49–2.29)	1.000
Yes	15 (27.8)	1		7 (12.9)	1	
Peanut introduction						
No	73 (31.6)	1.39 (0.69–2.80)	0.444	30 (13.3)	0.76 (0.36–1.62)	0.464
Yes	10 (24.4)	1		7 (17.0)	1	

[a]Statistically significant: $P<0.05$.

who were introduced to 3–4 ($P=0.049$) or 0–2 allergenic food items ($P=0.014$; **Table 3**). Our findings are consistent with the hypothesis that early exposure to diverse food antigens promotes maturation of the mucosal immune system and thereby induces an immune tolerance network (28). Similarly, recent studies on complementary feeding during infancy indicate that reduced food diversity may increase the risk of atopic sensitization, (29) and that higher diversity of foods within the first year of life may have a protective effect against atopic dermatitis, asthma, food allergy, and food sensitization (30,31). In contrast, we did not observe a relation between allergenic-food introduction within the first year of life and the development of atopic dermatitis at 1 year of age. A longer follow-up period will be needed to confirm these results.

To date, no study has assessed the association between the introduction of egg white and egg yolk separately and the risk of IgE sensitization in infants; thus, our study is unique. Our results indicate that the introduction of either egg white or egg yolk during infancy may protect from allergen-specific sensitization at 12 months of age ($P<0.05$; **Table 2**). In addition, serum IgE levels were lower in the egg white introduction group (58.42 ± 91.84 vs. 73.25 ± 109.42 kU/l, $P=0.212$) and significantly lower in the egg yolk introduction group than that in the control group (56.28 ± 79.87 vs. 93.11 ± 147.86 kU/l, $P=0.009$) by 12 months of age (**Table 3**).

Table 3. Fecal secretory IgA, eosinophil cationic protein, and total serum IgE levels in relation to IgE sensitization and allergenic food introduction at 12 months of age

	Fecal sIgA		Fecal ECP		Total serum IgE	
	n	Mean ± SD (mg/g)	*n*	Mean ± SD (ng/g)	*n*	Mean ± SD (kU/l)
Infants with IgE sensitization						
Yes	68	4.85 ± 6.26	68	156.56 ± 121.63	73	132.51 ± 140.18
No	142	6.15 ± 14.05	138	123.71 ± 75.99	178	28.66 ± 23.10
P-value		0.498		0.125		0.000[a]
Age at introduction of allergenic foods						
≥6–12 months	158	5.82 ± 13.50	156	148.38 ± 152.21	179	55.52 ± 101.49
<6 months	44	5.88 ± 7.53	42	109.03 ± 59.70	64	69.05 ± 101.49
P-value		0.971		0.131		0.294
Allergenic food diversity at age 12 months		0.743		0.222		0.022[a]
5–6 Items	46	7.13 ± 15.74	45	118.63 ± 84.90	54	43.46 ± 48.02
3–4 Items	120	5.37 ± 7.73	119	140.52 ± 153.71	143	61.28 ± 88.07
0–2 Items	44	4.81 ± 5.59	42	160.43 ± 129.23	54	94.08 ± 148.93
P-value		0.359		0.509		0.014[a]
Egg white introduction						
No	71	6.27 ± 14.22	77	144.93 ± 111.95	94	73.25 ± 109.42
Yes	139	6.34 ± 12.28	129	137.32 ± 153.00	157	58.42 ± 91.84
P-value		0.964		0.738		0.212
Egg yolk introduction						
No	41	5.78 ± 9.24	44	164.12 ± 125.36	52	93.11 ± 147.86
Yes	169	6.45 ± 1.37	162	133.74 ± 141.96	199	56.28 ± 79.87
P-value		0.700		0.258		0.009[a]

ECP, eosinophil cationic protein; sIgA, secretory IgA.
[a]Statistically significant: $P < 0.05$.

Similar to our findings, Nwaru *et al.* (32) reported that introduction of whole eggs at or before 11 months of age is inversely associated with atopic sensitization at the age of 5 years. Recently, clinical trialists attempted to evaluate the possible preventive effect of egg exposure on egg allergies. Although more evidence indicates that early introduction (4–6 months) of eggs into the diet of infants is associated with a lower risk of egg allergies (33–35), some studies have not shown this association (36). Our results suggest that neither early (<6 months) nor late (≥6 months) introduction of allergenic foods influences IgE sensitization (OR: 0.83, 95% CI 0.54–1.27; $P = 0.441$; Table 2). Thus, the optimal timing for exposure to allergenic foods to prevent allergies remains inconclusive and needs further research. Differences in study design, study populations, and the type and dose of allergenic foods administered may all influence outcomes (37).

Among fecal immune markers, we detected changes of ECP and sIgA levels in stool samples at 12 months of age. In general, a downward trend for fecal IgA and an upward trend for fecal ECP were observed among IgE-sensitized infants and among infants provided with lower diversity of allergenic foods as compared with controls (Table 3). Fecal ECP is an intestinal inflammatory marker that can be used for monitoring intestinal inflammation in infants with food allergies (14,38), and fecal sIgA is a marker of gut maturation and plays a role in the development of oral tolerance in infants (11,12). Therefore, we believe that increased oral antigenic stimulation through increased diversity of allergenic foods does not induce overt intestinal inflammatory responses but rather promotes the gut maturation process.

A key strength of this study is its design. This is the first study to analyze the effects of allergenic-food introduction and diversity on IgE sensitization, in conjunction with analysis of fecal immune markers to evaluate the intestinal immune reaction. Detailed information about feeding practices, allergenic food introduction, and demographic characteristics of infants were obtained here on a regular basis by administering questionnaires to parents, ensuring that recall bias was minimized. A major limitation of this study is the limited number of participants, meaning that selection bias

cannot be ruled out when we restricted our analyses to infants with completed questionnaires and blood sampling tests; this approach may have partly influenced our results. Another limitation is that we did not assess the effects of frequency or form of allergenic foods introduced into the diet of infants; the effect of each food on the risk of IgE sensitization may be specific.

In summary, in this study, we investigated the effects of allergenic-food diversity (and introduction of selected allergenic foods into the diet) on the risk of IgE sensitization at 12 months of age. On the basis of our results, we suggest that an increase in oral antigenic stimulation by increasing allergenic food diversity including six potentially allergenic foods (fruits, egg white, egg yolk, fish, shellfish, and peanuts), particularly egg white and yolk introduction during infancy, may reduce the risk of IgE sensitization at 12 months of age. Additional well-designed, prospective, and randomized intervention studies are needed to further clarify this issue.

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