Prevention of food allergy

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The past few decades have witnessed an increase in the prevalence of IgE-mediated food allergy (FA). For prevention strategies to be effective, we need to understand the causative factors underpinning this rise. Genetic factors are clearly important in the development of FA, but given the dramatic increase in prevalence over a short period of human evolution, it is unlikely that FA arises through germline genetic changes alone.
A plausible hypothesis is that 1 or more environmental exposures, or lack thereof, induce epigenetic changes that result in interruption of the default immunologic state of tolerance. Strategies for the prevention of FA might include *primary prevention*, which seeks to prevent the onset of IgE sensitization; *secondary prevention*, which seeks to interrupt the development of FA in IgE-sensitized children; and *tertiary prevention*, which seeks to reduce the expression of end-organ allergic disease in children with established FA. This review emphasizes the prevention of IgE-mediated FA through dietary manipulation, among other strategies; in particular, we focus on recent interventional studies in this field. (J Allergy Clin Immunol 2016;137:998-1010.)

Key Words: *Food allergy, atopic dermatitis, peanut allergy, cow's milk allergy, egg allergy, oral food challenge, specific IgE*

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Abbreviations used
AD: Atopic dermatitis
aOR: Adjusted odds ratio
CMA: Cow's milk allergy
CoFAR: Consortium of Food Allergy
EAT: Enquiring About Tolerance
FA: Food allergy
FLG: Filaggrin
HEAP: Hens Egg Allergy Prevention
ITT: Intention to treat
LEAP: Learning Early About Peanut Allergy
NNT: Number needed to treat
OFC: Oral food challenge
OR: Odds ratio
PA: Peanut allergy
RR: Relative risk
sIgE: Specific IgE
SPT: Skin prick test
STAR: Solids Timing for Allergy Research
TEWL: Transepidermal water loss
UK: United Kingdom

In many countries, food allergy (FA) is now considered a significant public health concern, affecting 3% to 6% of children in the developed world.[1,2] FA results in significant morbidity, but fatalities are rare.[3] A diagnosis of FA has been shown to negatively influence quality of life for patients and their families, and poses a significant financial burden.[4,5]

There are many risk factors associated with the development of FA, including atopic family history, male sex (at least in childhood), ethnicity, atopic dermatitis (AD), and related genetic polymorphisms. Although genetic factors are clearly important in the development of FA, its increase in prevalence has occurred over a short period of human evolution, implying that FA does not arise as a result of germline genetic changes alone. Therefore it seems plausible that 1 or more environmental exposures, or lack thereof, can induce epigenetic changes that interrupt the default immunologic state of tolerance to foods. This has stimulated ongoing research into the identification of modifiable environmental factors (including nutrition, the intrauterine environment, and lifestyle factors) that might play a role in gene expression through epigenetic modification.[6]

When explaining the increase in FA, one dominant theory is the hygiene hypothesis,[7] which posits that a lack of early childhood exposure to infectious agents, symbiotic microorganisms (such as gut flora or probiotics), and parasites increases susceptibility to allergic diseases by suppressing the natural development of the immune system. However, the recent publication of randomized trials, such as the Learning Early About Peanut Allergy (LEAP)[8] and Enquiring About Tolerance

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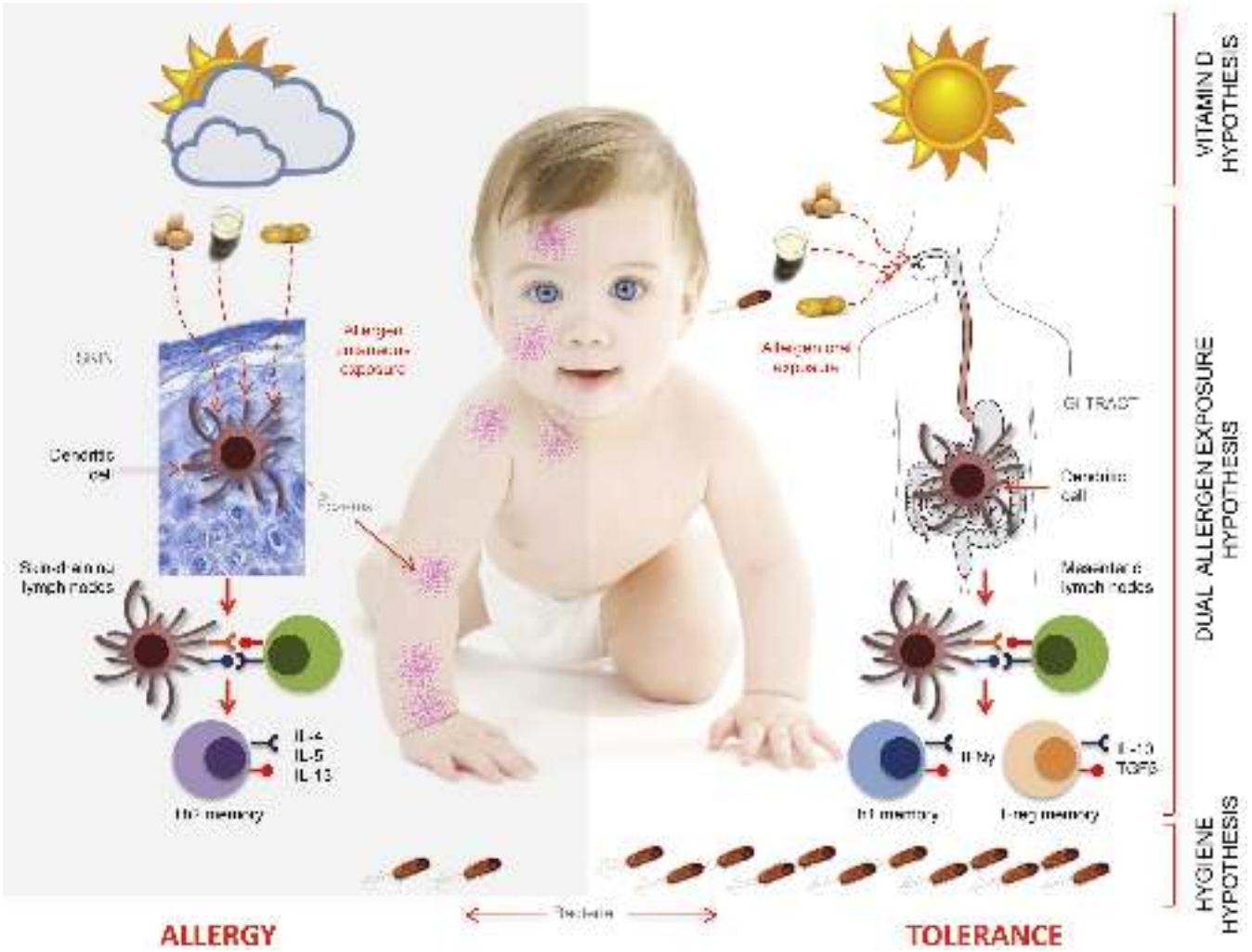

FIG 1. Integration of the vitamin D deficiency, hygiene, and dual-allergen exposure hypotheses. Sufficient levels of vitamin D, a diverse microbiota, and oral allergen exposure collectively support the development of tolerance. Conversely, allergic sensitization is promoted through cutaneous exposure, reduced diversity of microbiota, and vitamin D deficiency. Diminished microbial diversity and vitamin D deficiency are thought to interrupt the regulatory mechanisms of oral tolerance, with the latter also contributing to decreased epidermal barrier function. *GI*, Gastrointestinal; *T-reg*, regulatory T cells. Graphic modified from Lack G. Epidemiologic risks for food allergy. J Allergy Clin Immunol 2008;121:1331-6. Copyright © 2008 Elsevier. Reprinted with permission.

(EAT)[9] studies, has given support to the notion of oral tolerance induction, consistent with the dual-allergen exposure hypothesis (Fig 1).[10] The latter suggests that early cutaneous exposure to food protein through a disrupted skin barrier leads to allergic sensitization, whereas early oral exposure to food allergen induces tolerance. Additional theories relate to other environmental factors; for example, vitamin D might be required for regulatory immunologic mechanisms that are important in preventing FA and establishing oral tolerance. These integrated hypotheses provide a framework for research focused on the prevention of FA. However, investigations in this field are often hindered by methodological limitations (Table I).

In this review, we briefly cover the role of nonmodifiable genetic factors before highlighting cross-sectional studies and recent interventional studies in the field of allergy prevention through dietary manipulation.

NONMODIFIABLE FACTORS

Genetics

A family history of FA is itself a risk factor for FA. For instance, a child has a 7-fold increase in risk of peanut allergy (PA) if there is a parent or sibling with PA.[11]

The complex interplay between genetic and environmental factors giving rise to FA is perhaps best demonstrated by comparing concordance rates for allergy between genetically identical (monozygotic) and nonidentical (dizygotic) subjects. Although previous twin studies have estimated a high degree of heritability for atopic diseases, such as asthma (87%),[12] and AD (74%),[13] a study by Sicherer et al[14] of 58 twin pairs estimated the heritability for PA to be as high as 82% to 87%. In a recent review, Hong et al[15] highlighted more than 10 genes (several involved in allergen presentation, a T_H2-skewed immune system, or both) that have been associated with FA or food sensitization. However, genetically determined skin barrier

dysfunction—associated with mutations in the gene encoding filaggrin *(FLG)*—has attracted most interest, as this is known to predispose to multiple systemic atopic diseases.

Race

Racial differences have also been associated with a higher prevalence of FA. Sicherer et al[16] found a higher prevalence of self-reported seafood allergy among African Americans responding to a telephone survey using random-digit dialing. However, other studies have reported no significant trends, or found differences only in food sensitization.[17-20] More recently, Panjari et al[21] demonstrated that the high PA prevalence among infants of Asian-born parents in Australia appears to have occurred in a single generation and is not present among infants with parents migrating from other countries. These findings highlight the importance of gene-environment interactions—in other words, genetic changes alone cannot explain the increasing prevalence of FA or why only some predisposed subjects have FA.

Sex

Studies enrolling infants at high risk of FA (usually with AD, egg allergy, or both) reflect an overrepresentation of male participants. For example, the high-risk cohorts of the Consortium of Food Allergy Research (CoFAR; n = 213, multicenter recruitment)[22] and LEAP (n = 640, single-center recruitment)[8] studies reveal a male bias of 64.18% and 60%, respectively. In the HealthNuts study (n = 2848, recruited from multiple vaccine centers in the community setting),[23] a male bias was not apparent in the overall cohort (52.8%); however, when different allergic phenotypes were identified by using latent class analysis,[24] infants with the following FA phenotypes were more often male: multiple FAs but predominantly peanut (adjusted odds ratio [aOR], 2.00; 95% CI, 1.38-2.9; $P < .001$; 63% male) and multiple FAs but predominantly egg (aOR, 2.27; 95% CI, 1.37-3.71; $P = .001$; 66% male). The only phenotype without a male predominance was egg allergy alone.

MODIFIABLE FACTORS

Window of opportunity

Strategies for the prevention of FA might include *primary prevention*, which seeks to prevent the onset of IgE sensitization, and *secondary prevention*, which seeks to interrupt the development of FA in IgE-sensitized children. It is important to establish when sensitization to foods and allergy occurs because this will define the window of opportunity in which prevention strategies will have the greatest success.

Although it has been suggested that food allergens and aeroallergens can be transmitted through the placenta,[25] 2 large birth cohort studies[26,27] were unable to demonstrate measurable food-specific IgE (sIgE) levels in cord blood, even in children who subsequently developed food sensitization or allergy. However, sensitization may present early in life, and there is not always concordance between skin prick test (SPT) responses and sIgE levels, the markers of IgE sensitization. For example, when considering all infants screened for participation in the LEAP study,[28] a trial enrolling infants aged 4 to 11 months with severe AD, egg allergy, or both, 17% of those with negative SPT responses to peanut were unexpectedly found to have peanut sIgE sensitization (≥0.35 kU/L; Fig 2, *A* and *B*). Importantly, sensitization to peanut was present even in infants with mild AD (Fig 2, *C*). Similarly, when considering oral food challenge (OFC)–proven FA rates in the HealthNuts cohort,[29] a high degree of reactivity was noted at 12 months of age: the prevalence of challenge-proven PA was 3.0% (95% CI, 2.4% to 3.8), that of raw egg allergy was 8.9% (95% CI, 7.8% to 10.0%), and that of sesame allergy was 0.8% (95% CI, 0.5% to 1.1%).

Given that *in utero* IgE sensitization (if it occurs at all) does not predict the development of FA and that initial manifestations of FA typically occur in infancy, the optimal time for primary prevention appears to be early infancy. However, identifying those at risk and implementing primary prevention strategies during the window of opportunity might not be feasible. Instead, it may be more effective to focus on strategies for secondary prevention.

Transcutaneous sensitization

Preclinical data. It has been well established in animal studies that transcutaneous sensitization can occur to food allergens and give rise to IgE-mediated hypersensitivity responses. An early study by Saloga et al[30] showed that application of ovalbumin to the skin of BALB/c mice resulted in increased anti-ovalbumin IgE levels. Importantly, this only occurred in the presence of abraded skin (achieved by means of skin stripping, which results in skin barrier impairment and inflammation) and not through normal skin. Similarly, there are studies demonstrating that epicutaneous peanut exposure can induce a potent allergic T_H2-type response and anaphylaxis after a single oral antigen challenge; again, this was only achieved if skin stripping had been performed before antigen application.[31-33] Furthermore, in flaky tail mice carrying a mutation in the murine *Flg* gene, topical application of ovalbumin led to a cellular infiltrate and ovalbumin sIgE response, even without physical skin stripping.[34] In summary, transcutaneous sensitization has been found to be allergen specific, and the IgE response is exclusive to the allergen that is applied to abraded skin.

Clinical data: Skin barrier dysfunction. The Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort study demonstrated that infants who had PA by the age of 5 years were more likely to have had severe AD in the first 6 months of life, and to have been treated with *Arachis* (peanut) oil for dry skin.[27] Of children with both PA and AD, 90% had been exposed to topical therapies containing *Arachis* oil in the first 6 months of life. It is unclear whether AD is a modifiable risk factor for FA, but the possibility is emerging that we could one day prevent the development of AD. It is important to note that there is a preceding history of AD in the majority of children with PA, but not all. In healthy control subjects as well as patients with AD, the outermost layer of skin (the stratum corneum) contributes greatly to skin barrier function. Transepidermal water loss (TEWL) is a noninvasive *in vivo* measurement of water loss across the stratum corneum, and is increased in patients with AD.[35,36] Increased TEWL is seen at both lesional and nonlesional skin sites.[37,38] Interestingly, recent studies of atopic subjects showed that increased TEWL can precede the clinical manifestation of AD in those at high risk of atopy.[39] Increased skin barrier permeability, as measured by increased TEWL, not only allows water loss through the skin, but also facilitates allergen penetration and subsequent sensitization.[40] A cross-sectional study of children has reported that increased TEWL is associated with increased aeroallergen sensitization.[41] Taken together, this demonstrates that the *FLG*-null mutation

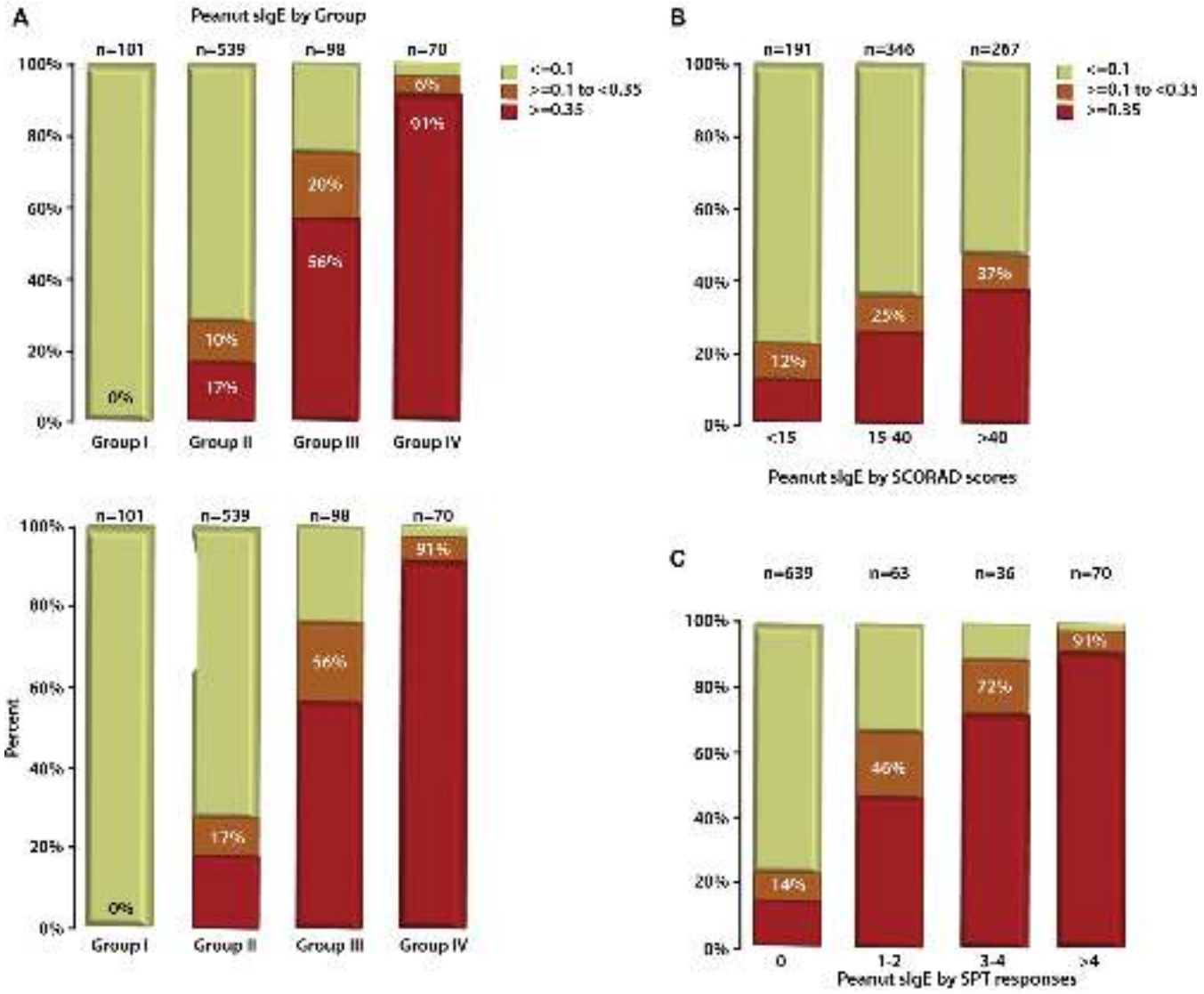

FIG 2. Peanut sIgE levels by group **(A)**, SCORAD scores **(B)**, and SPT responses **(C)**. Each *bar* represents the percentage of infants in each range of peanut sIgE. *Numbers within bars* represent the percentage of participants in each group with sIgE levels of 0.35 kU/L or greater. From du Toit G, Roberts G, Sayre PH, Plaut M, Bahnson HT, Mitchell H, et al. Identifying infants at high-risk of peanut allergy: the Learning Early About Peanut Allergy (LEAP) screening study. J Allergy Clin Immunol 2013;131:135-43. Copyright © 2013 Elsevier. Reprinted with permission.

significantly impairs skin barrier function, and that this can be measured as increased TEWL.

Because TEWL correlates with skin dryness, it is possible to identify reduced skin barrier function in patients with no history or clinical evidence of AD. Other factors known to influence skin integrity and permeability might also play a role, including the use of "wet wipes" and more frequent washing of babies. With progressively smaller family size, there is now a tendency toward the latter. Thus it is conceivable that skin permeability to foreign proteins has changed.[42-44]

Our increasing understanding of the importance of the skin barrier in the development of sensitization and FA may help to identify new preventative strategies. Two small intervention studies[45,46] have suggested that the regular application of emollients from birth reduces the risk of AD development and might thus affect FA, although the study investigating egg sensitization did not find a significant reduction.[45] Larger studies of more rigorous design are required to determine whether these initial findings can be replicated.

Clinical data: Environmental allergen exposure. The case-control study by Fox et al[47] showed that children with PA had been exposed to significantly higher levels of peanut in the home environment in the first year of life, compared with children who had allergy to egg but not peanut. Peanut consumption was determined by questionnaire among all household members, allowing quantification of environmental household exposure. A clear dose-response relationship was demonstrated between household peanut consumption (using a validated food frequency questionnaire) and the risk of PA in young children (Fig 3). Environmental peanut levels were not directly measured in this

study. However, other studies have demonstrated quantifiable levels of egg, milk, fish, and peanut protein (Ara h 2) in vacuumed household dust.[48-50] In one report, egg and milk protein levels in dust were high enough to elicit positive sIgE responses in the sera of patients with egg and milk allergy, respectively.[49]

In collaboration with the CoFAR cohort, Brough et al[51] showed that high levels of peanut in household dust were associated with an increased risk of sensitization and likely PA in children with AD. Importantly, this risk was augmented in children with more severe AD. Brown et al[52] had already shown that *FLG*-null mutations were associated with an approximately 4-fold increase in the risk of PA in 4 separate cohorts in different countries. In collaboration with the Manchester Asthma and Allergy cohort, Brough et al[53] also reported that increased peanut in dust from the infant's play area was associated with an increased risk of PA at school age in children with an *FLG*-null mutation but not in children with the normal genotype. Thus there is an increasing body of circumstantial clinical evidence that transcutaneous sensitization to environmental peanut allergen can occur through an inflamed and impaired skin barrier.

FIG 3. Proportion of allergic children with PA as a function of household peanut consumption during infancy and as a function of maternal peanut consumption during pregnancy. Adapted from 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. Copyright © 2009 Elsevier. Reprinted with permission.

Dietary factors

Maternal diet. Despite numerous observational studies, there are few data supporting the manipulation of the maternal diet during pregnancy, breast-feeding, or both for the prevention of FA. Although dietary restrictions during pregnancy do not appear to have a role in sensitization *in utero*, there is arguably a stronger rationale to look at the maternal diet during breast-feeding because FA often arises during infancy.

In 2012, Kramer and Kakuma[54] published a Cochrane review assessing the effects of an allergen avoidance diet during pregnancy, lactation, or both on maternal and infant nutrition and on the prevention or treatment of atopic disease in childhood. Evidence was extracted from 5 trials involving 952 participants. Unfortunately, these studies did not directly evaluate FA as an outcome; instead, AD was a major focus, and although this cannot be considered a surrogate of FA, it often coexists with FA. In terms of maternal allergen avoidance during pregnancy, there were no data to suggest a protective effect on the incidence of AD during the first 18 months of life (risk ratio, 1.01; 95% CI, 0.57-1.79). Of note, the restricted diet during pregnancy was associated with a slight but significantly lower mean gestational weight gain, a nonsignificant increase in risk of preterm birth, and a nonsignificant reduction in mean birth weight. In terms of maternal allergen avoidance during lactation, evidence from 2 trials[55,56] involving 523 participants did not show a significant protective effect on the incidence of AD during the first 18 months or on positive SPT responses to cow's milk, egg, or peanut allergen at 1, 2, or 7 years.

Several cross-sectional studies have assessed the association between maternal peanut consumption during pregnancy and breast-feeding and a diagnosis of PA in offspring. Sicherer et al's atopic cohort of 503 infants (aged 3-15 months with likely milk or egg allergy but no previous diagnosis of PA) in the CoFAR study[57] demonstrated that maternal ingestion of peanut during pregnancy was strongly associated with a high level of peanut sensitization. Although the frequency of peanut consumption during both pregnancy and breast-feeding showed a dose-response association with peanut IgE levels of 5 kU/L or greater, only consumption during pregnancy was a significant predictor. However, it should be noted that this study did not control for household peanut consumption. As discussed above, environmental exposure is likely to be more relevant, and when Fox et al[47] adjusted for household exposure, the effect of increased peanut consumption during pregnancy on PA was no longer significant. Likewise, the ALSPAC study[27] showed no effect of maternal peanut consumption during pregnancy or lactation on the development of an immunologic or clinical reaction to peanut by 4 to 6 years of age.

Infant diet: General measures. *Breast-feeding.* The potential benefits of breast-feeding have long been a focus of interest. Conceptually, breast-feeding could work in a variety of ways: breast milk might harbor antiallergic properties; prolonged breast-feeding effectively delays allergen introduction; and there may be factors in breast milk (such as IgA or IgG_4) that combine with allergen to induce tolerance. However, studies have not consistently shown a protective effect of breast-feeding on the development of atopy or FA,[58-62] and some observational studies have in fact demonstrated an increased risk of FA in breast-fed infants.[63-65] The latter is likely due to reverse causality.

Modified infant formula. Because not all infants are breast-fed and because exclusive breast-feeding has no proven effect on the prevention of FA, attention has turned to the possible protective effects of different types of formula—especially cow's milk–based hydrolysates. In a Cochrane review, Osborn and Sinn[66] found no evidence to support feeding with a hydrolyzed formula for the prevention of allergy compared with exclusive breast-feeding. For high-risk infants unable to be exclusively breast-fed, there was limited evidence that prolonged feeding with a hydrolyzed formula compared with cow's milk formula reduced infant and childhood allergy and infant cow's milk allergy (CMA).

Infant diet: Allergen-specific measures. *Allergen-specific avoidance.* In a study by Zeiger and Heller[67] of 165 mother/infant pairs, participants were randomized to either a prophylactic group (maternal avoidance of cow's milk, egg, and peanut during the last trimester of pregnancy and lactation; infant's diet free from cow's milk ≤1 year, egg ≤2 years, and peanut and fish ≤3 years) or a control group (mothers had unrestricted diets; infants followed American Academy of

Pediatrics feeding guidelines). Cow's milk sensitization and AD before the age of 2 years were significantly reduced in the prophylactic group compared with the control group, but no significant reduction was observed in FA, food sensitization, serum IgE levels, or atopic disease at 7 years of age.

In another study, Arshad et al[68] randomized 120 infants to a prophylactic or control group. In the first year of life, children in the prophylactic group were either given an extensively hydrolyzed formula or breast-fed by mothers who themselves excluded dairy products, egg, fish, and nuts. In this group, exposure to house dust mite was reduced by the use of acaricides and mattress covers. By contrast, the control group followed standard United Kingdom (UK) Department of Health advice. Follow-up was at 1, 2, 4, and 8 years of age. A preventive effect on asthma, AD, rhinitis and atopy was demonstrated in the prophylactic group, but study powering did not allow for an assessment of FA.

Allergen-specific introduction: Observational studies. Numerous studies have explored associations between the timing of introduction of food allergens and the development of FA. The study by Saarinen and Kajosaari[69] found no difference in the cumulative incidence of fish allergy at 3 years of age between children in whom fish was introduced early or late. In a cohort with a cumulative IgE-mediated CMA incidence of 0.5% (66/13,019), Katz et al[70] reported lower CMA rates in infants who were introduced to cow's milk earlier in life. Only 0.05% of infants started on regular cow's milk protein formula within the first 14 days had IgE-mediated CMA versus 1.75% of infants started on formula between 105 and 194 days ($P < .001$).

A cross-sectional study[71] of Israeli (n = 5615) and UK (n = 5171) Jewish children found that the prevalence of PA was 10-fold higher in the UK (1.85%) than in Israel (0.17%, $P < .001$). Despite accounting for atopy, the adjusted risk ratio for PA between countries was 9.8 (95% CI, 3.1-30.5) in primary school children. This study showed that peanut was introduced earlier and eaten more frequently and in larger quantities in Israel than in the UK. The median monthly consumption of peanut in Israeli infants aged 8 to 14 months was 7.1 g of peanut protein compared with 0 g in the UK ($P < .001$), and the median number of times peanut was eaten per month was 8 in Israel and 0 in the UK ($P < .0001$).

Studies have also suggested that introducing egg at an early age in small amounts may be beneficial. Nwaru et al[72] demonstrated an increased risk of egg sensitization at 60 months of age if egg was introduced after 10.5 months. Similarly, Koplin et al[73] reported an aOR of 3.4 (95% CI, 1.8-6.5) in children introduced to egg after 12 months compared with those introduced after 4 to 6 months. Interestingly, introduction of cooked egg was more protective than introduction of egg in baked goods. However, differential atopic predisposition caused by genetic and environmental factors could explain these differences.

Allergen-specific introduction: Randomized trials. The LEAP study[8] was a randomized controlled trial designed to assess oral tolerance induction to peanut, recruiting 640 high-risk UK children between 4 and 11 months of age. Infants either consumed peanut products at least 3 times a week (total of 6 g of peanut protein weekly, which is equivalent to 24 g of peanuts or 3 teaspoons of peanut butter) or completely avoided peanut until 60 months of age. The LEAP study demonstrated that in high-risk atopic infants, sustained peanut consumption initiated in the first 11 months of life resulted in a substantial reduction in the proportion of children with PA at 60 months of age compared with children who avoided peanut.

The LEAP study[8] included 542 infants with negative peanut SPT responses and 98 infants with peanut SPT wheal diameters of 1 to 4 mm (minimally positive) at study entry. The intention-to-treat (ITT) analysis found that 17.2% of the peanut avoidance group had food challenge–proven PA at 60 months of age compared with 3.2% of the peanut consumption group. This corresponded to an absolute risk reduction of 14%, a number needed to treat (NNT) of 7.1, and a relative risk reduction of 80%. Overall, the risk of allergic reactions during early introduction in this group was low; however, 7 of the 319 children randomized to the consumption group reacted to peanut at baseline food challenge. These reactions were not severe and epinephrine was not required for their treatment, suggesting that the introduction of peanut in young infants is achievable and safe—even in infants with low-positive SPT responses to peanut. Six participants in the consumption group had PA during the study, indicating that PA can still develop despite primary and secondary prevention strategies. The LEAP study findings have prompted a review of current recommendations, and numerous allergy, dermatology, and pediatric societies are now publishing interim consensus statements that adopt an encouraging approach with respect to early introduction of peanut in high-risk infants.[74-76]

The EAT study[9] was designed to assess whether early introduction of food allergens would prevent the development of FA in infants recruited from the general population; by comparison, the LEAP study enrolled at-risk infants. One thousand three hundred three exclusively breast-fed 3-month-old infants were randomly assigned either to the early introduction group (introducing 6 allergenic foods: peanut, cooked egg, cow's milk, sesame, white fish, and wheat) or to the standard introduction group (following UK recommendation of exclusive breast-feeding to around 6 months of age). The primary outcome was the proportion of participants with FA to 1 or more of the 6 foods by 36 months of age. In the ITT analysis, 7.1% (42/595) of the standard introduction group and 5.6% (32/567) of the early introduction group had FA to 1 or more of the 6 intervention foods, but this difference was not significant ($P = .32$). The per-protocol analysis showed significant differences of 7.3% versus 2.4% ($P = .01$) for any FA, 2.5% versus 0% ($P = .003$) for PA, and 5.5% versus 1.4% ($P = .009$) for egg allergy in the standard and early introduction groups, respectively.

Efficacy of the intervention in the EAT study was related to the duration of specific food consumption and quantity of food consumed between 3 and 6 months of age.[9] Modeling determined that 2 g or more of peanut protein per week might prevent PA, which is comparable to the 7.1 g per month consumption by Israeli infants in the study by du Toit et al,[71] in which PA was 10-fold lower (0.17% in Israel compared with 1.85% in Jewish children in the UK). In the EAT study[9] consumption of 2 g of peanut protein per week for at least 4 weeks also reduced PA 10-fold, from 2.5% to 0.2%. With respect to hen's egg, modeling showed that consumption of 2 g of egg white protein per week could have a similar effect in preventing egg allergy.

Despite these promising data, the EAT study did not show efficacy in an ITT analysis.[9] However, the data do lean toward significance in the ITT analysis for peanut and egg: PA occurred in 1.2% of the early introduction group compared with 2.5% of the standard introduction group, a nonsignificant relative reduction of 51% ($P = .11$); egg allergy occurred in 3.7% of the

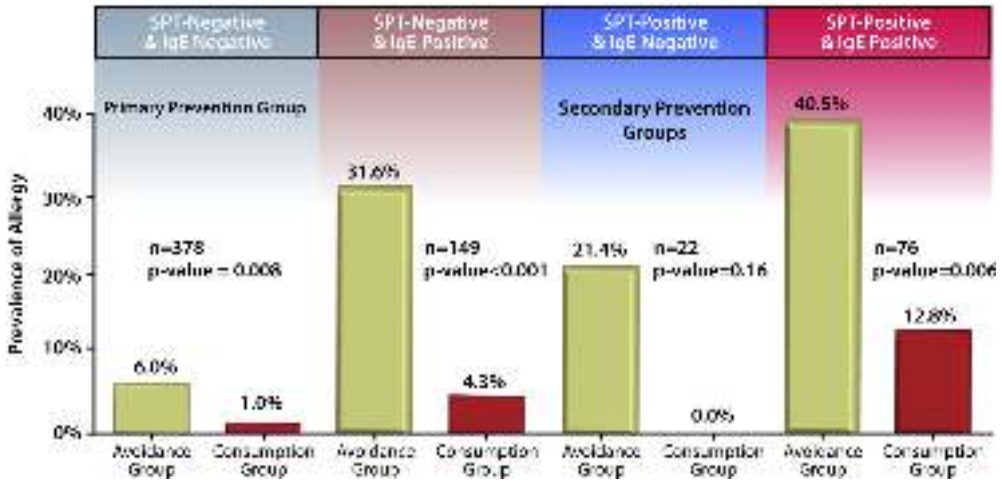

FIG 4. LEAP study: primary and secondary prevention PA. From du Toit G, Roberts G, Sayre PH, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med 2015;372:803-13. Copyright © 2015 Massachusetts Medical Society. Reprinted with permission (figure generated from data in Table S3). The primary prevention group comprised participants who had both negative peanut SPT responses and negative sIgE levels (<0.1 kU/L) at baseline. The overall secondary prevention group comprised participants who had either positive peanut SPT responses, positive sIgE levels (≥0.1 kU/L), or both at baseline.

early introduction group compared with 5.4% of the standard introduction group, a nonsignificant relative reduction of 31% ($P = .17$).

The LEAP-On study[77] is a 12-month extension of the LEAP study, investigating whether participants who consumed peanut remain protected against PA, even after cessation of peanut consumption for 12 months. The study design represented an opportunity to investigate mechanisms of loss of allergic responses, with potential implications for other FAs and immune-mediated diseases. The LEAP-On study's findings are covered below (question 3).

Key clinical questions generated by the LEAP,[8] LEAP-On,[77] and EAT[9] studies are now addressed:

Question 1. The LEAP study only included high-risk infants with severe AD, egg allergy, or both. Would this strategy be protective for infants without these risk factors? The EAT study[9] enrolled infants from the general population, the majority of whom would have been considered low risk for PA; indeed, only 9 of 1303 EAT study participants would have been eligible to enroll in the LEAP study (based on the LEAP study's high-risk enrollment criteria). Notably, 76% of the EAT study's standard introduction group did not have AD at 3 months of age; however, these participants accounted for 38% of the overall burden of FA. Such data raise the question of whether recommendations for early introduction of allergenic foods should be extended to all infants, regardless of AD, in countries with a high prevalence of FA.

Question 2. In the LEAP study, 76 children were excluded before randomization based on a peanut SPT response of 5 mm or greater, because a high likelihood of reacting to an oral peanut challenge was assumed. If we had intervened in these children at an earlier window of opportunity, such as 3 to 4 months of age, would we have been able to prevent the development of PA? The LEAP study[8] design excluded 9.1% (76/834) of the infants who were screened because of a large average wheal (>4 mm in diameter) at baseline peanut SPT. The safety and effectiveness of early peanut consumption in this population of infants is therefore not known because the intervention was not applied. However, in the Peanut Allergy Sensitisation (PAS) study, 49 of these 76 infants were assessed for PA at 60 months of age using OFCs. Seventy-eight percent (38/49) had a positive OFC result, and 11 participants had a negative outcome (unpublished data).[78] These findings suggest that a cutoff peanut SPT response of greater than 4 mm in this population of high-risk infants is predictive of PA at 60 months of age. The 11 participants with a negative OFC result might never have had PA, or they might have outgrown PA. It remains unknown whether peanut consumption would have been safe, tolerated, and protective in these infants.

In addition, analyses reveal that LEAP and PAS study participants were more likely to have positive SPT sensitization to peanut if they were older at age of screening and had more severe AD (as measured by SCORAD scores, unpublished data). Taken together, these data suggest that preventive dietary interventions should take place early in infancy.

Question 3. Are the benefits of allergy protection sustained, or are they dependent on ongoing peanut consumption? The LEAP study[8] did not tell us whether participants achieved transient desensitization or long-term tolerance; this question was central to the subsequent LEAP-On study.[77] This reported that the reduction in PA achieved through early peanut introduction and consumption (until 60 months of age) persists at 72 months of age, even after 12 months of peanut avoidance. There was a 74% relative reduction in the prevalence of PA in the previous LEAP consumers compared with the previous LEAP avoiders, demonstrating longer-lasting unresponsiveness to peanut after 12 months of peanut avoidance. Immunologic findings (small SPT wheal size, continued decrease in Ara h 2 sIgE levels, and high peanut-specific IgG_4/IgE ratios) noted in nonallergic LEAP consumers at month 60 were maintained after 12 months of peanut avoidance.

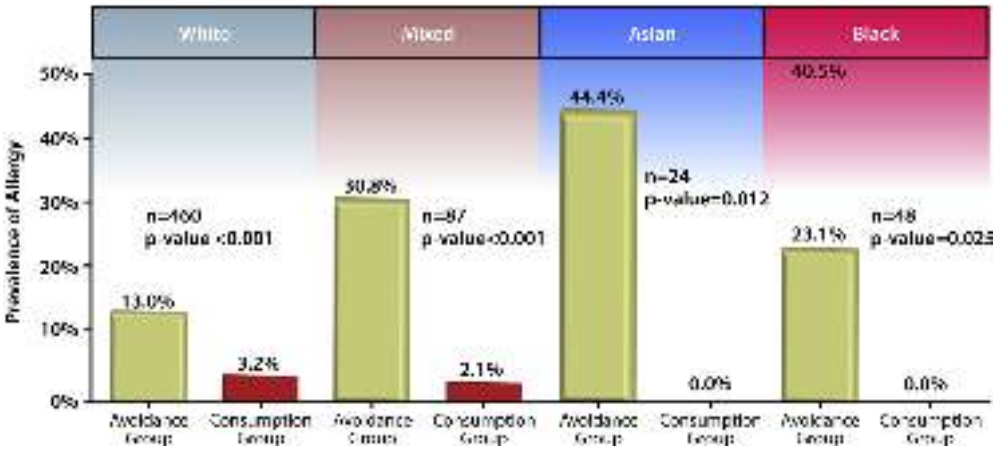

FIG 5. LEAP study: primary outcome by race. From du Toit G, Roberts G, Sayre PH, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med 2015;372:803-13. Copyright © 2015 Massachusetts Medical Society. Reprinted with permission (figure generated from data in Table S12).

In summary, the LEAP and LEAP-On studies together demonstrate that early introduction of peanut induces unresponsiveness to peanut that persists for 12 months without ongoing consumption. Follow-on studies over a longer duration are planned to determine whether unresponsiveness persists during a prolonged period of *ad libitum* consumption.

Question 4. Does the LEAP intervention protect against the development of PA even in infants who were sensitized (based on SPT responses, sIgE levels, or both) at baseline; that is, did the study demonstrate both primary and secondary allergy prevention? The LEAP results show that early peanut consumption prevented PA both in participants who were sensitized to peanut and in those who were not sensitized at baseline. Therefore the LEAP study demonstrates both primary and secondary prevention.[8] This reduction translates to an NNT of 8.5 among participants with a negative peanut SPT response and an NNT of 4.0 among participants with a low-positive peanut SPT response (Fig 4).

Question 5. Are these findings applicable to children of different ethnic groups or in different geographic settings? Secondary analyses of the LEAP data showed similar levels of prevention in white, black, and Asian (Indian and Pakistani) children, suggesting that these findings were not limited to one racial group and would likely be as efficacious in other geographic locations (Fig 5).[8] The EAT study lacked power to assess the efficacy of the intervention in different ethnic groups; however, FA was significantly associated with AD at enrollment, nonwhite ethnicity, having siblings, and difficulty adhering to the early introduction intervention.[9] Thus if early dietary intervention is adopted as a prevention strategy, a greater benefit may be achieved through targeted interventions focusing on infants of nonwhite ethnicity and those with AD.

Question 6. Are similar findings demonstrated in other interventional studies using hen's egg for the prevention of egg allergy? The Solids Timing for Allergy Research (STAR) trial[79] was one of the earliest randomized controlled trials making use of the model of early-life allergen exposure for the prevention of IgE-mediated FA. The investigators randomized 4-month-old infants with moderate-to-severe AD to 1 teaspoon of either pasteurized raw whole egg powder (n = 49) or rice powder (n = 37). Egg allergy (based on both SPT and OFC results) was lower (but not significant) in the egg group than the rice group (RR, 0.65; 95% CI, 0.38-1.11; $P = .11$). Secondary outcome analyses found a lower proportion of infants in the egg group (45% [19/42]) to be sensitized to egg based on SPT responses at 12 months of age compared with the control group (63% [22/35]); however, this difference did not reach statistical significance (RR, 0.72; 95% CI, 0.47-1.09; $P = .12$). Of note, a high rate of egg sensitization among previously egg-naive infants with AD at the start of the study was observed and a high proportion (31% [15/49]) of infants randomized to receive egg had an allergic reaction to the egg powder and did not continue powder ingestion.

Early findings of the Hens Egg Allergy Prevention (HEAP) trial (investigating early egg introduction in the general population) are available in abstract form.[80] This trial found no effect of early consumption of pasteurized egg white powder starting at 4 to 6 months in preventing egg allergy up to age 12 months (8 children receiving pasteurized egg white powder had positive egg sIgE levels compared with only 4 children in the placebo group). Six percent (23/406) showed egg sensitization at screening (sIgE ≥0.35 kU/L). The majority of infants (94% [16/17]) who underwent double-blind, placebo-controlled food challenges were allergic, and 3 experienced anaphylactic reactions. Furthermore, in the active group 2 more children reacted to the pasteurized egg white powder with first exposure at home (1 with anaphylaxis). Therefore the STAR and provisional HEAP trial results highlight the importance of the form of egg used as intervention, with pasteurized egg proving more allergenic than the cooked egg used in the EAT study.

It is hoped that additional studies, such as the Starting Time for Egg Protein[81] and Beating Egg Allergy[82] trials, will provide further data on the efficacy and safety of early introduction of hen's egg.

Question 7. Acceptability of early dietary interventions: How do we maintain and bolster adherence? The overall rate of adherence to LEAP per-protocol consumption was high at 92.0%.[8] Among the 319 participants randomly

assigned to consumption, 7 were instructed not to consume peanut because they had a positive result at baseline to the OFC, and 9 terminated consumption largely because they started to have allergic symptoms to peanut. This indicates that peanut consumption is possible in the majority of children who meet the LEAP eligibility criteria.

In the EAT study,[9] early introduction group per-protocol adherence was defined as follows: continued breast-feeding to at least 5 months of age and consumption of at least 5 of the early introduction foods for at least 5 weeks between 3 and 6 months of age of at least 75% of the recommended dose (3 g/wk allergenic protein). Individual per-protocol adherence in this group was variable and food specific, as follows: egg, 43.1% (215/499); sesame, 50.7% (266/505); fish, 60.0% (297/495); peanut, 61.9% (310/501); and milk, 85.2% (415/487). Four factors accounted for 78% of the nonadherence in the dominance analysis: nonwhite ethnicity (odds ratio [OR], 2.21; 95% CI, 1.18-4.14), parentally perceived symptoms to any of the foods (OR, 1.7; 95% CI, 1.02-2.86), reduced maternal quality of life (psychological domain), and AD at enrollment (OR, 1.38; 95% CI, 0.87-2.19). These groups might benefit from additional support during the early introduction period.

There may be other reasons for nonadherence. Adherence was highest for milk (recommended as yogurt) compared with egg (a more textured food); this might have been influenced by age-related oral motor development, with some food consistencies more easily tolerated at an early age than others. In addition, yogurt can be readily served directly from the pot, whereas the other foods require preparation, such as pureeing or mixing with milk/water (and cooking in the case of egg and fish), to achieve a consistency suitable for feeding infants less than 6 months of age. The logistic demands of these processes, together with the number of foods introduced, might have influenced adherence. Finally, taste might play a role: although sesame given as tahini paste is already a suitable texture as sold, its strong taste might explain why adequate adherence was achieved by only half of early-introduction group participants. Dietary recommendations might achieve greater adherence (and therefore success, given that effective allergy prevention appears to be related to duration of consumption and dose consumed) by focusing on the introduction of fewer food allergens and by recommending that foods are given in liquid form. The food allergens selected should aim to cover food allergens that are common in the geographic setting in which the intervention is to be applied.

Question 8. Are the dietary interventions allergen specific? Given the findings of the LEAP[8] and EAT[9] studies, the question arises as to whether the benefit noted with early allergen consumption is allergen specific and indeed allergic disease specific. In other words, would such interventions influence rates of tree nut allergy and AD? These study findings are as yet unpublished. Peanut, tree nuts, and sesame contain highly conserved homologous seed storage proteins, and it is possible that cross-sensitization explains the co-occurrence of allergy to these foods in certain populations. Therefore, if the consumption of peanut in early life protects against the development of PA, it might be that peanut consumption also protects against the development of other related FAs. Indeed, it could be that the lower prevalence of PA, tree nut allergy, and sesame allergy in Israeli children compared with age-matched UK children demonstrated by du Toit et al[71] arises because of cross-tolerance induced through the early, copious, and frequent consumption of peanut in Israel.

Additional dietary considerations

Vitamin D. Observational studies examining the effects of increased dietary vitamin D levels through supplementation during infancy[83-85] suggest an increased rate of sensitization and allergy, but it has been reported that this is only true when administered in a water-soluble vehicle.[86]

Ecological observations suggest that the geographic distribution of allergy prevalence is linked to regional dietary practices[87] and/or UV exposure and its consequences on vitamin D synthesis. However, there is great variability between studies suggesting that vitamin D insufficiency can contribute to FA.[88]

Most epidemiologic investigations have focused on possible adverse immune effects of low vitamin D levels, such as low sunlight (as measured by season of birth and latitude) as a risk factor for FA.[89-94] These findings appear to be independent of longitude, socioeconomic status, or physician density. Studies have also directly assessed vitamin D status (as measured by serum 25OHD levels) and its potential role in the development of FA.[95] Although conflicting, these studies suggest a more complicated association than a linear dose response in all subjects, with some indicating different associations based on host characteristics (such as concomitant AD, genetic polymorphisms, and country of birth).[96] Although studies have generally focused on a single maternal, neonatal, or childhood 25OHD level, there are many other factors related to sunlight exposure that need consideration.

A major limitation of the vitamin D studies to date is that they have largely not considered biologically available vitamin D, because the standard measurements of vitamin D do not capture this. Biological availability can vary substantially by race, and this appears to be related to differences in polymorphisms in vitamin D binding protein.

Other nutritional factors. In recent years, there has been a focus on the role of vitamins, antioxidants, fruits, vegetables, and fatty acids on the prevention or treatment of allergies. Ecological data imply that increased intake of antioxidant-rich fresh fruit and vegetables is associated with a decreased prevalence of FA,[87] whereas preliminary studies suggest that foods rich in trace elements might confer protection against allergy outcomes.[97] There are also data to support an association between obesity and increased allergic sensitization to foods.[98] Studies investigating fatty acids have yielded conflicting results.[99-103]

Epigenetics

As previously discussed, numerous environmental triggers can influence allergy outcomes, including nutrition, the intrauterine environment, and lifestyle factors. Markers of these epigenetic changes include DNA methylation and histone modification.[104] There are a growing number of population-based epigenetic studies on asthma and FA, mostly focused on DNA methylation. For example, Nadeau et al[105] assessed asthma in children from areas with different pollution levels, demonstrating that forkhead box P3 *(FOXP3)* DNA methylation in regulatory T cells was increased in the blood of asthmatic children from highly polluted areas, compared with that seen in asthmatic children in less polluted areas. Regulatory T-cell impairment caused by this FOXP3 hypermethylation was strongly associated with asthma

TABLE I. Methodological issues complicating the interpretation of studies aimed at prevention of food allergy in childhood

Issue	Explanation	Recommended approach
Lack of defined strategy goals	*Primary prevention* seeks to prevent the onset of IgE sensitization, *secondary prevention* seeks to interrupt the development of FA in IgE-sensitized children, and *tertiary prevention* seeks to reduce the expression of end-organ allergic disease in children with established FA.	Study goals should be clearly defined *a priori*.
Study design	The majority of studies are observational and not interventional.	Randomized controlled trials
Reverse causality	Early signs of suspected allergic disease (eg, AD or a presumed allergic reaction) might result in altered feeding patterns.	Randomized controlled trials
Randomization	Necessary ethical restraints limit randomization to dietary interventions other than breast-feeding.	Breast-feeding should be encouraged. Studies should randomize within the breast-fed group.
Blinding of dietary interventions	Blinding of specific dietary interventions might not be possible because of safety concerns or practical limitations, such as breast-feeding or formula odor.	It might not be possible or safe to have a placebo arm to infant nutritional studies, but outcome measures can be assessed in a blinded manner.
Determination of FA	Few studies make use of OFCs for the diagnosis of FA.	Aim to perform OFCs in all participants. For children who do not undergo OFCs, *a priori* diagnostic algorithms are required.
Surrogate markers	AD, rhinitis, and asthma are often used as surrogate markers of FA.	As above
Natural history of FA	Tolerance is anticipated for many FAs in the first decade of life.	Account for natural remission and onset of FA before assessing for a study effect.
Diagnosis of allergy	Many studies use generic terms, such as allergy or atopy. Definitions and diagnostic criteria are subject to great variability.	Consensus with respect to the diagnosis of common allergic disorders is required.
Determination of diet	Determination of food consumption is usually by retrospective food frequency questionnaires. These are prone to bias and might not include important variables (eg, allergen processing, sequence of ingestion, and concomitant breast-feeding).	Use should be made of prospective food diaries that have been validated for context, language, and consistency.
Definition of weaning	Use of the term weaning is not consistent and usually limited to the introduction of solid foods only.	Adopt the World Health Organization term complementary feeding, which incorporates any nutrient-containing food or liquid other than breast milk given to young children.
Generalizability	Many studies are aimed at high-risk atopic populations.	Ideally, investigations should include entire study populations (ie, both low and high risk). At-risk populations should be defined *a priori*.
Separation of specific effects when interventions are combined	Multiple interventions are often studied at different time points.	Preliminary proof-of-concept studies need to separate out the effects of each intervention.
Introduction of complementary feeds is associated with multiple variables.	The early cessation of breast-feeding and introduction of complementary feeds has been associated with cultural, socioeconomic, and other factors.	Regression analysis should control for as many relevant confounders as possible.
Monitoring adherence	Monitoring of adherence to interventions, particularly dietary interventions, is difficult.	Better tools for monitoring dietary adherence are required.

From Tsakok T, du Toit G. Prevention of food allergy. In: Ebisawa M, Ballmer-Weber BK, Vieths S, Wood RA, editors. Food allergy: molecular basis and clinical practice. Basel (Switzerland): Karger; 2015. p. 253-62.

severity scores. In the HealthNuts cohort Martino et al[106] assessed 58 food-sensitized patients (aged 11-15 months), half of whom were clinically reactive at OFCs; 13 nonallergic control subjects were also included. Reproducibility was assessed in an additional 48 samples by using methylation data from an independent population of patients with clinical FA. Using a supervised learning approach, the investigators demonstrated a DNA methylation signature of 96 CpG sites that predict clinical outcome; diagnostic scores were derived from these 96 methylation sites, and cutoffs were determined in a sensitivity analysis. Methylation biomarkers outperformed allergen sIgE and SPT responses for predicting OFC outcomes; FA status was

correctly predicted in the replication cohort with an accuracy of 79.2%. It is hoped that the identification of modifiable trigger factors will guide the development of strategies for the prevention of FA.

CONCLUSIONS

There is evidence that a large proportion of the allergy burden is inherited, but genetic predisposition alone cannot explain the disturbing increase in FA. Studies on changes in gene function in relation to environmental influences (ie, epigenetic modifications) are beginning to provide evidence to explain the mechanisms underlying the development of FA.

Sensitization and FA can occur early in infancy, and it appears that prevention strategies should ideally commence during these early-life periods of immunologic vulnerability.

The evidence supporting use of dietary interventions in high-risk pregnant and/or lactating women for the prevention of FA is weak; noted effects are inconsistent, and furthermore, such interventions might compromise maternal and fetal nutrition. Although there are many health benefits of breast-feeding for both the mother and infant, it is not certain whether exclusive breast-feeding for any length of time offers protection against FA development. For high-risk infants who are not exclusively breast-fed, the use of hydrolyzed formula might offer some protection against allergic disease, but these effects appear weak and are generally limited to the development of AD. It might also be that any reduction in AD arises because of treatment of underlying CMA, as opposed to representing tertiary allergy prevention.

The findings of studies investigating the use of dietary interventions (such as fatty acids, antioxidants, prebiotics and probiotics, and vitamin supplements) are unconvincing, inconsistent, or not adequately tested. Furthermore, there are safety concerns surrounding some of these interventions.

Numerous questions remain about how to implement early food introduction, and which groups of infants should be targeted. However, it is clear that the paradigm has shifted from recommending avoidance of common food allergens in infancy, to consideration of early consumption strategies to prevent allergy development. The recent publication of interventional studies aimed at the prevention of FA heralds a significant advance in our quest to contain this modern-day epidemic. The LEAP study findings have already influenced recommendations across many allergy societies for the introduction of peanut in at-risk populations. The EAT study results add to these findings and strengthen the argument for a wider revision of infant feeding recommendations. It is reassuring that the interventions themselves are safe, nutritionally favorable, affordable, accessible, and—with appropriate health care support—acceptable. This strategy for FA prevention thus lends itself to practice in a variety of settings.

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