Food allergy: Update on prevention and tolerance

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Of the many possible hypotheses that explain the recent increase in childhood food allergy (FA), the dual-allergen exposure hypothesis has been the most extensively investigated. This chapter serves as a review and update on the prevention of FA and focuses on recently published randomized controlled trials exploring the efficacy of oral tolerance induction in infancy for the prevention of FA. As a result of these RCTs, National Institutes of Health recommendations now actively encourage the early introduction of peanut for the prevention of peanut allergy, and other countries/settings recommend the inclusion of potential common food allergens, including peanut and egg, in complementary feeding regimens commencing at approximately 6 months but not before 4 months of age. Further studies that explore the efficacy of oral tolerance induction to other common food allergens and that focus on optimal timing, duration, and adherence are required. (J Allergy Clin Immunol 2018;141:30-40.)

Key words: *Food allergy, peanut allergy, egg allergy, allergy prevention*

Abbreviations used

EAT:	Enquiring about Tolerance Study
FA:	Food allergy
ITT:	Intention to treat
LEAP:	Learning Early about Peanut Allergy
LEAP-On:	Twelve-month extension of the LEAP study: Persistence of Oral Tolerance to Peanut
PETIT:	Two-step egg introduction for prevention of egg allergy in high-risk infants with eczema
RCT:	Randomized controlled trial
RR:	Relative risk
SPT:	Skin prick test
STAR:	Solids Timing for Allergy Research
STEP:	Starting Time for Egg Protein
UK:	United Kingdom

"An ounce of prevention is worth a pound of cure" is an appropriate adage to describe much research into food allergy (FA) over the past decade. Given that there is currently no cure, research has focused increasingly on interventions aimed at FA prevention. These interventions are generally applied early in life and 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.

This chapter will discuss possible reasons for the increase in FA, review current knowledge around methods for primary prevention from recently published research, describe statistical issues in FA prevention studies, and briefly outline potential directions for future research. The main focus will be on lessons learned from the recently published Learning Early about Peanut Allergy (LEAP), Persistence of Oral Tolerance to Peanut (LEAP-On), and Enquiring about Tolerance (EAT) randomized controlled trials (RCTs),[1-3] but other published FA prevention research is also included.

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Disclosure of potential conflict of interest: G. Du Toit reports income from grants from the National Institute of Allergy and Infectious Diseases (NIAID, NIH), Food Allergy & Research Education (FARE), MRC & Asthma UK Centre, UK Department of Health through NIHR, National Peanut Board (NPB), and grants from UK Food Standards Agency (FSA); these grants part funded salary over period of this submitted work. H. A. Sampson has received grants from the National Institute of Allergy and Infectious Diseases (AI-44236, CoFar, ITN); has consultant arrangements with Allertein Therapeutics, LLC, Hycor, and UCB; is Chief Scientific Officer of DBV Technologies; has received royalties from UpToDate; and has stock/stock options with DBV Technologies. A. W. Burks reports personal fees from NIH AITC Review Panel, Allertein, American Society for Microbiology, Elsevier, FARE, World Allergy Organization, Adept Field Solutions, Aimmune Therapeutics, Inc, Astellas Pharma Global Development, Inc, Biomerica, Inc, Evelo Biosciences, Inc/Epiva Biosciences, Inc, First Manhattan Co, Genentech, GLG Research, Inc, Insys Therapeutics, Intrommune Therapeutics, PPD Development, LP, Regeneron Pharmceuticals, Inc, Sanofi US Services, SRA International, Stallergenes, UKKO, Inc, and Valeant Pharmaceuticals North America, LLC and reports grants from Food Allergy Research and Education (FARE), National Institutes of Health, and Wallace Research Foundation. C. A. Akdis has received grants from Actellion, the European Union projects Medall and Predicta, Allergopharma, the Swiss National Science Foundation, and the Christine Kühne Center for Allergy Research and Education. G. Lack has received grants from the National Institutes of Allergy and Infectious Diseases (NO1-AI-15416 [contract] and UM1AI109565 [grant]), Food Allergy Research and Education (FARE), MRC & Asthma UK Centre, UK Department of Health through the National Institute for Health Research, the National Peanut Board, and Osem; and has consultant arrangements and stock/stock options with DBV Technologies. M. Plaut declares no relevant conflicts of interest.

Received for publication September 22, 2017; revised November 21, 2017; accepted for publication November 22, 2017.

Available online November 27, 2017.

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0091-6749/$36.00

https://doi.org/10.1016/j.jaci.2017.11.010

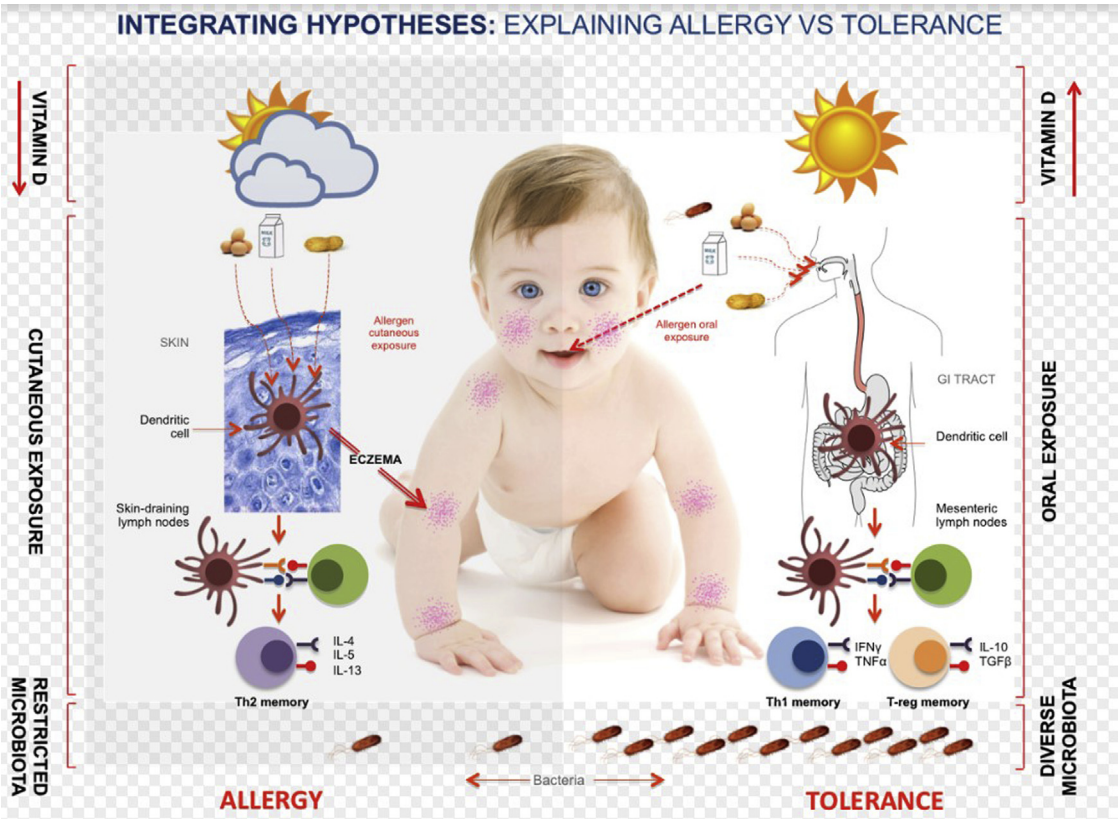


FIG 1. Integration of the vitamin D deficiency, hygiene, and dual-allergen exposure hypotheses. Sufficient levels of vitamin D, a diverse microbiota, and oral allergen exposure support the development of tolerance. Conversely, allergic sensitization is promoted through cutaneous exposure, reduced diversity of the 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.[4]

HYPOTHESIZING THE INCREASE IN FA

Various hypotheses have been put forward to explain the increase in FA. Integration of the vitamin D deficiency, hygiene, and dual-allergen exposure hypotheses (which is the focus of this chapter) are shown in Fig 1.[4] This article focuses on the dual-allergen exposure hypothesis, which suggests that allergic sensitization to food occurs through low-dose cutaneous sensitization, whereas early consumption of food protein induces oral tolerance.[4] This hypothesis was developed after publication of studies demonstrating a strong association between dietary exposure, eczema, and the development of FA.

Studies demonstrating the role of cutaneous sensitization in patients with FA

Animal and human observational and *in vitro* studies demonstrate transcutaneous sensitization to food allergens through inflamed eczematous skin. In human subjects the topical application of *Arachis* species (peanut) oil onto eczematous skin during infancy was significantly associated with peanut allergy in eczematous children.[5] Environmental exposure to peanut during infancy (assessed by household peanut consumption) increased the risk of peanut allergy; however, if infants consumed peanut in the first year of life, they were protected against peanut allergy.[6]

More recent studies found that eczema severity amplifies the risk of peanut sensitization and likely allergy resulting from exposure to peanut antigen in household dust.[7] A similar increase in peanut sensitization and allergy risk was seen in children with filaggrin loss-of-function mutations exposed to high levels of peanut allergens in household dust.[8] This provides a good example of gene-environment interactions leading to the development of peanut allergy in young infants.

A cross-sectional study assessed the route of peanut exposure in the development of allergy and captured maternal peanut consumption during pregnancy, breast-feeding, and the first year of life through a questionnaire. Household peanut consumption was also quantified. Median weekly household peanut consumption in the patients with peanut allergy was significantly increased (18.8 g) compared with that in control subjects without allergy (6.9 g) and high-risk control subjects (1.9 g).[6] These findings suggest that high levels of environmental exposure to peanut during infancy can promote sensitization and support the hypothesis that peanut sensitization occurs as a result of environmental exposure.

Studies demonstrating the role of tolerance induction in early childhood

An ecological study exploring the prevalence of peanut allergy in infants in Israel compared with infants in the United Kingdom

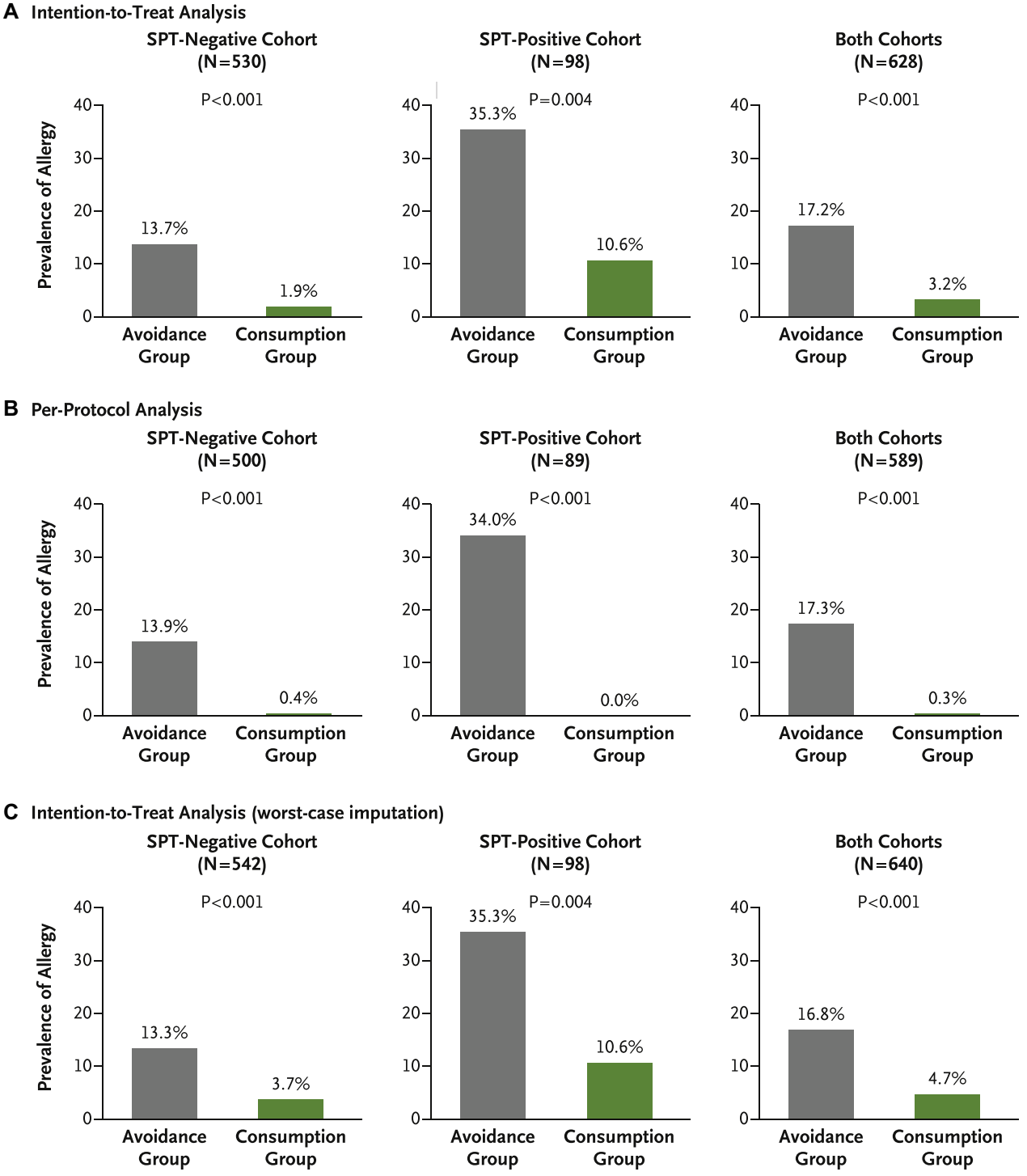


FIG 2. LEAP study primary outcome findings. **A** and **B,** The prevalence of peanut allergy at 60 months of age is shown among participants with a negative SPT response at baseline, among participants with a positive response at baseline, and in both groups combined in the ITT (Fig 2, *A*) and per-protocol (Fig 2, *B*) analyses. Among the 640 participants who underwent randomization, peanut allergy status was determined by means of an oral food challenge in 617 (96.4%) and by means of a diagnostic algorithm in 11 (1.7%). Peanut allergy could not be evaluated by using the diagnostic algorithm in 2 participants (0.3%). A total of 10 participants (1.6%) withdrew voluntarily or were lost to follow-up. **C,** The worst-case imputation analysis assumes that participants with missing data in the peanut consumption group would have been allergic to peanuts and that participants with missing data in the peanut avoidance group would have been nonallergic. *P* values are based on χ^2 analyses. From Du Toit et al.[1]

TABLE I. Summary of RCTs with hen's egg

Trial name	Country	Type	Population	Intervention group (hen's egg protein per week)	Control group	No.	Intervention period (age [mo])	Outcome assessed (age [mo])	Primary outcome	Outcome in ITT (*P* value)
Enquiring About Tolerance (EAT) study	UK	RCT, open label	General population	Cooked whole hen's egg (4 g)	Hen's egg avoidance until 6 mo of age	1303	3-6	12-36	Hen's egg allergy (OFC)	RR, 0.69 (95% CI, 0.40-1.18); $P = .17$
Hens' Egg Allergy Prevention (HEAP) study	Germany	RCT, blinded	General population	Pasteurized raw hen's egg white powder (7.5 g) Hen's egg–free diet	Placebo powder (rice) Hen's egg–free diet	298	4-12	12	Hen's egg sensitization (specific IgE)	RR, 2.20 (95% CI, 0.68-7.14); $P = .24$
Solids Timing for Allergy Research (STAR) study	Australia	RCT, blinded	High risk (infants with moderate/severe eczema)	Pasteurized raw whole hen's egg powder (6.3 g)	Placebo powder (rice)	86	0-8	12	Raw hen's egg allergy (OFC) and sensitization (SPT)	RR, 0.65 (95% CI, 0.38-1.11); $P = .11$
Starting Time for Egg Protein (STEP) study	Australia	RCT, blinded	Moderate risk (atopic mothers)	Pasteurized raw whole hen's egg powder (2.8 g)	Placebo powder (rice)	820	4-10	12	Raw hen's egg allergy (OFC) and sensitization (specific IgE)	Adjusted RR, 0.75 (95% CI, 0.48-1.17); $P = .20$
Beating Egg Allergy (BEAT) study	Australia	RCT, blinded	Moderate risk (first-degree relative with allergy)	Pasteurized raw whole hen's egg powder (2.45 g) Hen's egg–free diet	Placebo powder (rice) Hen's egg–free diet	254	4-8	12	Hen's egg sensitization (SPT)	OR, 0.46 (95% CI, 0.22-0.95); $P = .03$
Two-step egg introduction for prevention of egg allergy in high-risk infants with eczema (PETIT)	Japan	RCT, blinded	Moderate risk (with atopic dermatitis)	Heated hen's egg powder (0.175 g for 3 mo and then 0.875 g for 3 mo)	Placebo powder (squash)	121	4-12	12	Hen's egg allergy (OFC)	RR, 0.222 (95% CI, 0.08-0.61); $P = .0012$

OFC, Oral food challenge; *OR*, odds ratio.

(UK) found a significantly higher rate in the UK (1.85% vs 0.17%, $P < .001$).[9] This 10-fold increase in the prevalence of peanut allergy in UK children remained after accounting for confounding factors. One explanation for this difference is that peanut was introduced at an earlier age and consumed in larger quantities in Israeli infants: 7.1 g of peanut protein per month compared with no exposure (0 g) to peanut protein in children in the UK ($P < .001$).

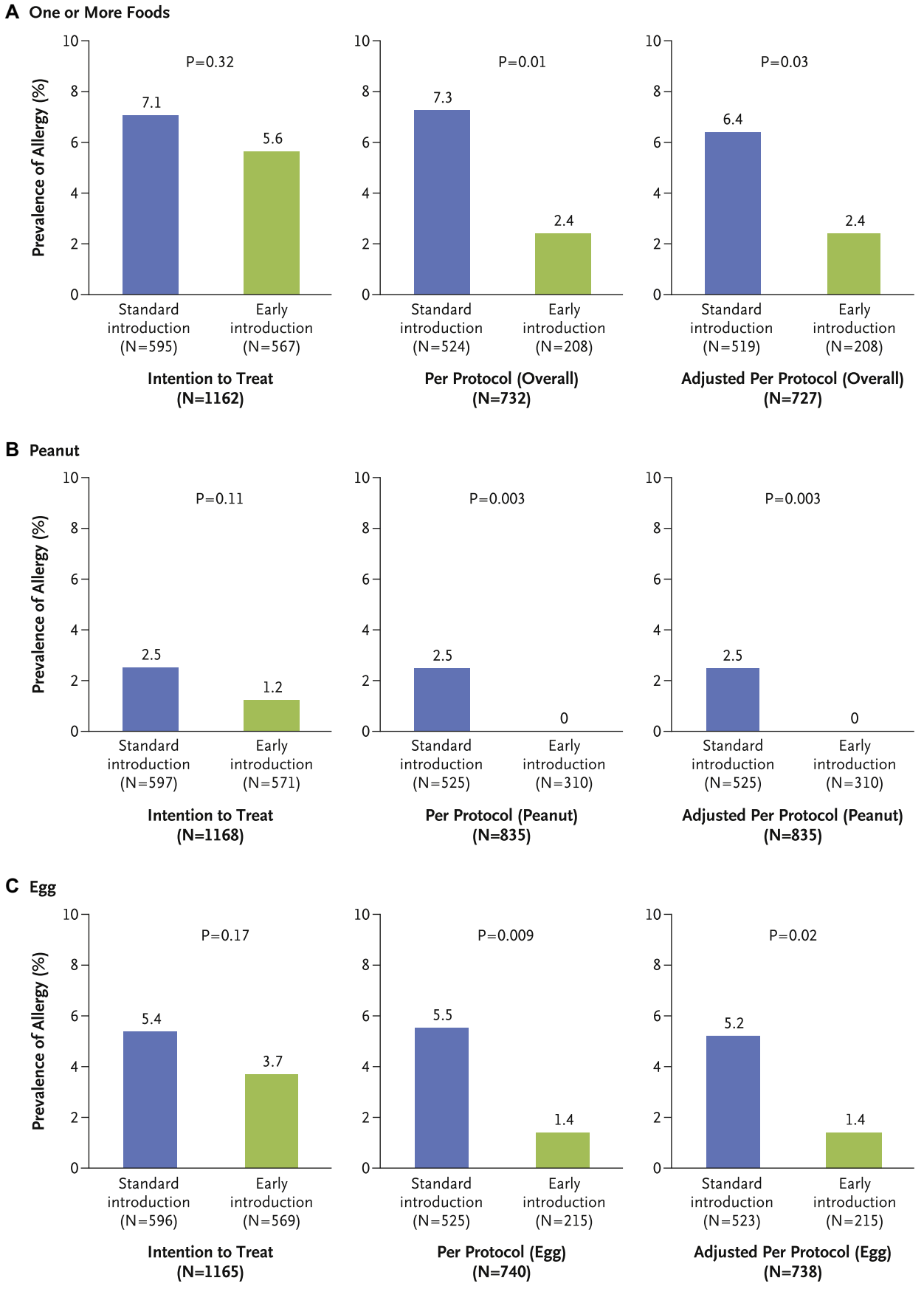
The dual-allergen exposure hypothesis combines observational data exploring cutaneous sensitization and early tolerance induction and proposes that the balance of exposures during the first year of life (depending on whether the initial exposure to peanut is through the skin or gut) primes the immune system to allergy or tolerance (respectively). A window of opportunity exists during the child's first year of life within which to influence a tolerogenic response. The dual-allergen exposure hypothesis, predominantly under the guise of oral tolerance induction, has been tested in several RCTs, which are discussed below.

RCTs OF ORAL TOLERANCE INDUCTION

For the purposes of this chapter, we consider tolerance to be a state of clinical unresponsiveness to a known allergen. Later in this chapter, we discuss the evidence that tolerance can be enjoyed without the need for ongoing exposure to that allergen after oral tolerance induction programs.

Peanut allergy

The LEAP study was developed after publication of observational data showing that early and regular consumption of peanuts was associated with prevention of peanut allergy, particularly in children at higher risk because of a compromised skin barrier.[8,9] The LEAP study was an RCT that assessed oral tolerance induction of peanut in high-risk children (with severe eczema, egg allergy, or both) aged between 4 and 11 months in the UK. Infants were randomized to consuming peanut products at least 3 times a week (average of 6 g of peanut protein a week) or completely avoiding any peanut until 60 months of age.[1]


A One or More Foods
Prevalence of Allergy (%)
P=0.32
7.1
5.6
Standard introduction (N=595)
Early introduction (N=567)
Intention to Treat (N=1162)
P=0.01
7.3
2.4
Standard introduction (N=524)
Early introduction (N=208)
Per Protocol (Overall) (N=732)
P=0.03
6.4
2.4
Standard introduction (N=519)
Early introduction (N=208)
Adjusted Per Protocol (Overall) (N=727)
B Peanut
P=0.11
2.5
1.2
Standard introduction (N=597)
Early introduction (N=571)
Intention to Treat (N=1168)
P=0.003
2.5
0
Standard introduction (N=525)
Early introduction (N=310)
Per Protocol (Peanut) (N=835)
P=0.003
2.5
0
Standard introduction (N=525)
Early introduction (N=310)
Adjusted Per Protocol (Peanut) (N=835)
C Egg
P=0.17
5.4
3.7
Standard introduction (N=596)
Early introduction (N=569)
Intention to Treat (N=1165)
P=0.009
5.5
1.4
Standard introduction (N=525)
Early introduction (N=215)
Per Protocol (Egg) (N=740)
P=0.02
5.2
1.4
Standard introduction (N=523)
Early introduction (N=215)
Adjusted Per Protocol (Egg) (N=738)

LEAP results showed that early introduction and regular ongoing consumption of peanut resulted in a significant reduction in the number of children with peanut allergy at 60 months of age compared with those who avoided peanut. The intention-to-treat (ITT) analysis showed that in the peanut avoidance group 17.2% of the children had challenge-proved peanut allergy at 60 months of age compared with 3.2% in the peanut consumption group (81% relative reduction). Furthermore, the LEAP study demonstrated both primary and secondary prevention: there was a reduction in peanut allergy at 60 months of age in those children who had peanut introduced early, regardless of their sensitization status at baseline (based on skin prick test [SPT] and specific IgE levels, Fig 2).[1]

Early introduction of peanut was also found to be effective at preventing peanut allergy in a per-protocol but not ITT analysis of children who participated in the EAT study, an RCT in which exclusively breast-fed children from the general population were randomized to consume peanut (alongside 5 other allergenic foods) from 3 months of age or continue exclusive breast-feeding until approximately 6 months of age, after which parents introduced allergenic foods as they wished.[3] Children who introduced peanut from 3 months of age per protocol were significantly less likely to have peanut allergy than those who followed UK Department of Health advice to delay solid-food introduction until approximately 6 months of age (per-protocol analysis: 0% vs 2.5%, $P = .003$). It is important to acknowledge that per-protocol analyses can introduce hidden bias unless the probability of receiving the intervention is random with respect to all predictors of a study's outcome. However, an instrumental variable analysis of the EAT study data showed no evidence that the per-protocol estimate of efficacy was biased,[10] suggesting that early introduction of peanut is an effective prevention strategy, even in the general population.

In a recently published meta-analysis of oral tolerance induction, Ierodiakonou et al[11] note "moderate certainty" of evidence that introducing peanut between 4 and 11 months of age reduced the risk of peanut allergy (relative risk [RR], 0.29; 95% CI, 0.11-0.74) based on 2 RCTs (LEAP and EAT studies) investigating early peanut introduction in 1550 children.

Egg allergy

Six RCTs from different countries have now published their findings assessing introduction of egg during infancy for the prevention of egg allergy, as detailed in Table I. There is great variability in the populations enrolled (high-risk vs population cohorts) and in the form of egg used in these studies (ranging from pasteurized raw whole egg to less allergenic extensively heated egg), which makes it difficult to compare the findings. Nonetheless, there are some commonalities between the outcomes of the studies.

Two RCTs made use of egg sensitization as the primary study outcome; although no significant effect on egg white–specific IgE levels was noted in the Hens' Egg Allergy Prevention (HEAP) study, the Beating Egg Allergy (BEAT) study showed a significant difference between groups for egg white SPTs.[12,13]

Four RCTs assessed egg allergy by means of oral food challenge. No significant difference was noted in the STEP[14] or STAR studies (but recruitment was discontinued early in the STAR study).[15] The EAT study found a significant difference in egg allergy, and this was only true for the per-protocol population.[3]

The 2-step egg introduction for the prevention of egg allergy in high-risk infants with eczema (PETIT) study is the only RCT to demonstrate a statistically significant decrease in allergy to egg in the ITT analyses.[16] In the PETIT study infants with eczema at age 4 to 5 months (n = 147) were recruited and assigned to either the placebo or intervention group. Uniquely, this trial made use of heated egg powder and extremely low starting doses (25 mg of egg protein, equivalent to 0.2 g of whole egg boiled for 15 minutes). At completion, the prevalence of egg allergy (as determined by oral food challenge [OFC] to a cumulative dose of 7 g of heated whole egg powder) was significantly lower in the intervention group compared with the control group (8% and 38%, respectively; RR, 0.22; 95% CI, 0.09-0.54; $P = .0001$). This interim finding prompted an early cessation in enrollment, according to the study stopping rules. Because many of the participants were egg sensitized at baseline, it might well be that this study reflects secondary as opposed to primary prevention of egg allergy.

Although individual studies can show conflicting or inconclusive results, a meta-analysis by Ierodiakonou et al[11] found "moderate certainty" of evidence that introducing egg between 4 and 6 months of age reduced the risk of egg allergy (RR, 0.56; 95% CI, 0.36-0.87) based on 5 RCTs, including 1915 children.

Other foods: EAT study

The EAT study examined oral tolerance through early introduction of 6 allergenic foods in more than 1000 exclusively breast-fed children.[3] In addition to egg and peanut (discussed earlier), the intervention group had cow's milk, wheat, sesame, and fish introduced into their diets from 3 months of age. The control group followed standard UK government advice of exclusively breast-feeding until introduction of solid food at approximately 6 months of age. The randomized sequence of food introductions for the early introduction group was cow's milk (yogurt) first, followed by peanut, egg, sesame, and whitefish in random order; wheat was introduced last. The main outcome

FIG 3. EAT study outcome findings. The prevalence of IgE-mediated FA is shown with respect to 1 or more of the 6 early intervention foods (peanut, cooked egg, cow's milk, sesame, whitefish, and wheat; **A**), to peanut **(B)**, and to egg **(C)**. The *first column* shows the ITT analysis, the *second column* shows the per-protocol analysis, and the *third column* shows an adjusted per-protocol analysis. The ITT analysis included all the participants with data that could be evaluated; the per-protocol population included all participants who adhered adequately to the assigned regimen. The adjusted per-protocol analysis was a conservative per-protocol analysis that adjusted the prevalence of FA in the standard introduction group by subtracting the number of participants in the early introduction group with a positive result on the challenge at enrollment and who completed the trial with a confirmed FA from both the numerator (number of participants with allergy in the standard introduction group) and the denominator (number of participants in the standard introduction group who adhered to the protocol). *P* values are based on χ^2 analyses or the Fisher exact test, as appropriate. From Perkin et al.[3]

was a challenge-proved diagnosis of allergy to 1 or more of the 6 foods at 1 and 3 years of age.

In the ITT analysis 7.1% of the infants in the standard group had FA to 1 or more of the 6 potentially allergenic foods compared with 5.6% in the intervention group (not statistically significant, $P = .32$). However, in the per-protocol analysis the prevalence of any FA was significantly lower in the early introduction group compared with the standard introduction group (2.4% vs 7.3%, $P = .01$). The risk of having a positive SPT response to any food was 22% lower in the early introduction group compared with the standard introduction group at 12 ($P = .07$) and at 36 ($P = .47$) months of age. Primary outcome data of the EAT study are shown in Fig 3.[3]

In conclusion, RCTs of oral tolerance induction to a range of foods have shown variable results. Nonetheless, the finding of "moderate certainty" in the meta-analysis by Ierodiakonou et al[11] for introduction of peanut and hen's egg between 4 and 11 months of age is reassuring. Their findings for fish and early introduction of milk or hydrolyzed formula were of "low certainty" and "no evidence," respectively.

Importantly, both the LEAP and EAT studies demonstrated that early introduction of allergenic foods into the infant's diet was achievable and safe and that this did not affect breast-feeding rates or later nutrition and growth. However, in all studies adverse event data show that children experienced allergic reactions during the initial baseline food challenge, and thus, especially in high-risk populations, children can have pre-existing FA, despite never having knowingly consumed the food. This is discussed further in the following section exploring windows of opportunity for oral tolerance induction.

CONCEPT OF DIFFERENT WINDOWS OF EXPOSURE, POSSIBLY RELATING TO DIFFERENT FOODS, AGE, AND IMMUNOLOGIC MARKERS

Typically, FA has its genesis early in infancy, and although the age of onset of different food allergies is variable, the body of evidence suggests that the pathogenesis of common food allergies starts early in life. Several RCTs examining oral tolerance induction found infants to have a high level of sensitization or to be allergic to the food at baseline and, importantly, before any known oral exposure to the food.[12,15] Thus, to maximize the effectiveness of oral tolerance induction, it is important to understand the age at which oral tolerance induction programs should be commenced.

At inclusion of the EAT study, 5.1% (33/652) of the early introduction group had a positive SPT response to 1 of the 6 allergenic foods being introduced. EAT study infants were all enrolled at 3 months of age, highlighting that sensitization to foods can begin in very early infancy.[3] In the LEAP study 76 of the 899 patients screened were excluded from enrollment because they had an SPT response of greater than 4 mm at between 4 and 11 months of age.[17] This group was older than those participants who were eligible for enrollment in the LEAP study and who had negative SPT responses at the time of screening (8.3 [SD, 1.88] vs 7.7 [SD, 1.74] months of age), and the median peanut SPT wheal diameter in this group was suggestive of peanut allergy at 7.5 mm (IQR, 6.0-9.0).[17]

Data from the LEAP and EAT studies suggest that, for oral tolerance to be effective, it should be commenced early, when high-level sensitization is less likely to have occurred. To this end, recently published allergy prevention recommendations suggest that introduction be targeted to early infancy but not before 4 months of age.[18-20] However, as demonstrated in the EAT study, early-life dietary interventions present logistical challenges because weaning must be balanced with the infant's developmental ability to consume solid food. Further studies exploring the effect of age on food-induced allergic sensitization and the efficacy of oral tolerance induction in very young infants are needed.

Although evidence suggests that oral tolerance induction might be most effective in very young infants who are not yet sensitized to foods, it is also important to understand whether oral tolerance induction is effective in children who are already sensitized either because they did not introduce allergenic foods in early infancy or because they became sensitized very early in life. The LEAP study excluded children with an SPT response of greater than 4 mm. This *a priori* decision was based on the assumption that such children would very likely have peanut allergy. Although including children with larger SPT responses in the study would have been useful scientifically, several other studies have shown that using a greater than 4-mm cutoff as a surrogate marker for existing peanut allergy is reasonable, regardless of the age or risk profile of the child. In the HealthNuts Study around 80% (95% CI, 73.0% to 87.4%) of high-risk infants with an SPT wheal size of greater than 4 mm had challenge-confirmed peanut allergy as 12 months of age, and the Basophil Activation Validation study found the optimal cutoff for the diagnosis of peanut allergy in a UK cohort was greater than 4 mm.[21,22]

There is a clear need for robust scientific data assessing the outcome of oral tolerance induction in infants who are sensitized (particularly high level sensitized) to food allergens. However, until these data are available, current studies suggest that 4 mm is an appropriate cutoff for clinical use.

DOSAGE ISSUES

In addition to the window of exposure, the efficacy of oral tolerance induction appears to be influenced by the dose of food used. There seems to be a critical level of protein consumption required for the development of oral tolerance. In a murine model a single high dose of peanut flour (100 mg) promoted oral tolerance and prevented subsequent IgE sensitization and T-cell proliferation. However, there is a paucity of data in the human population as to the optimal dosage of an allergenic food protein for the development of long-lived oral tolerance. The LEAP study peanut consumption recommendations were based on upper quartiles of those noted for Israeli infants who appeared to be protected against peanut allergy.[9] In the LEAP study a dose of 6 g of peanut protein per week was recommended, and on average, consumption of 7.1 g of peanut protein per week was achieved. This LEAP study intervention achieved an overall 81% reduction in the level of peanut allergy.

Because adherence was excellent in the LEAP study, it was not possible to explore a dose-response relationship, but this was explored in the EAT study, in which 31.9% of the early introduction group were able to adhere, and within those who were nonadherent, the level of food-specific adherence in the early consumption group was variable. It is of interest that the statistically derived protective level for oral tolerance to peanut in the EAT study mirrors the median consumption of peanut protein per week in the Israeli population (1.7 g in Israel): statistical

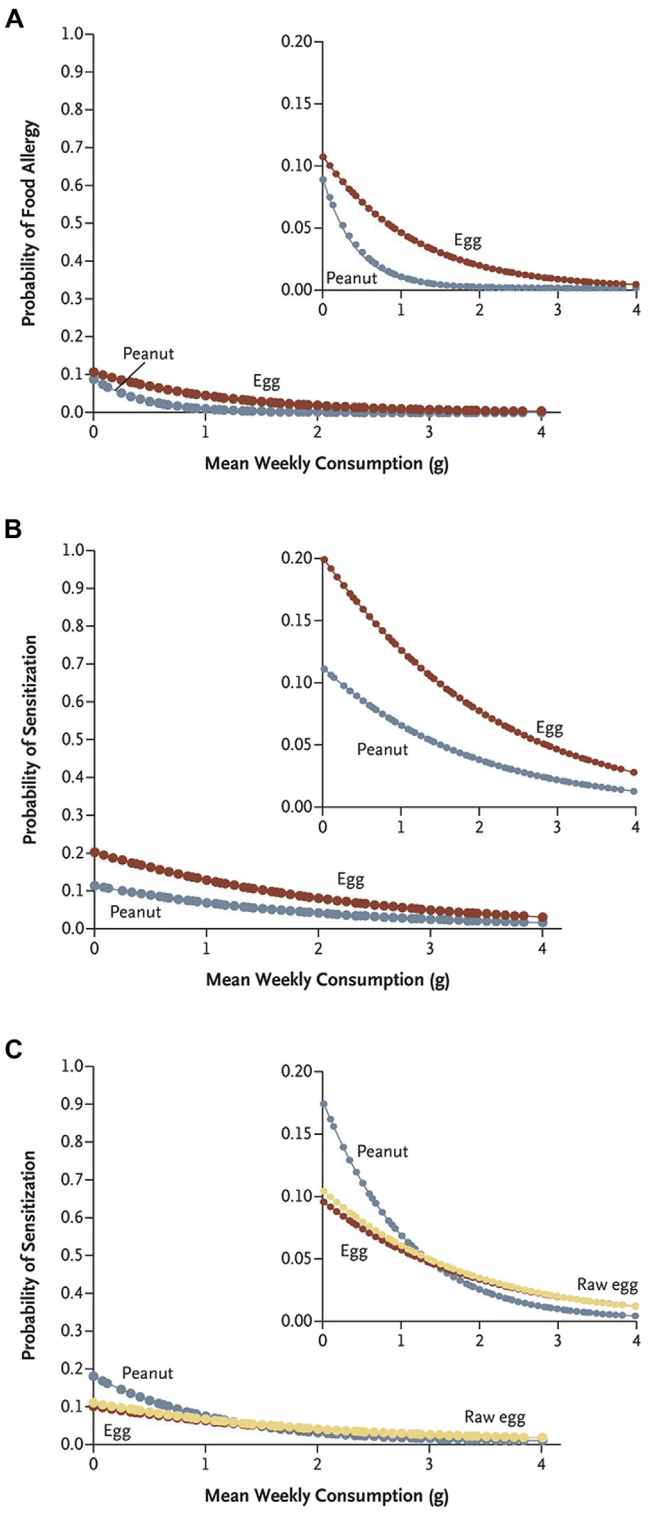


FIG 4. EAT dose-response analysis of the relationship between mean weekly dose of peanut or egg protein consumed and allergy or positive result on SPTs to peanut, egg, and raw egg white. Shown are predictive probability plots generated from statistical models of the prevalence of peanut and egg allergy **(A)** and of positive SPT responses to peanut and egg at 12 months **(B)** and to peanut, egg, and raw egg white at 36 months **(C)**, according to the mean weekly consumption of peanut and egg protein between enrollment and 6 months of age. The prevalence of both FA and a positive SPT response diminishes with increasing levels of mean weekly consumption. *Insets* show the same data on an enlarged *y-axis*. From Perkin et al.[3] Copyright © 2016 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

modeling of EAT study data showed that approximately 2 g of food protein per week protected against both peanut and egg white allergy, reducing the burden of allergic disease by approximately 90%. This was also true for protection against positive SPT responses to egg white (including SPT to raw egg white). Dose-response modeling is shown in Fig 4.[3]

Low-level allergen exposure (to select aeroallergens) results in allergic responses, whereas high-level allergen exposure drives tolerance.[23,24] Current data suggest that gram rather than milligram doses of food protein will be required for oral tolerance induction, but studies that explore the effect of oral tolerance induction with differing doses are required. This is especially true in the context of prevention of multiple food allergies, in which, as seen in the EAT study, high-dose consumption of multiple foods can present logistical problems.

PERSISTENCE OF ORAL TOLERANCE INDUCTION

Although oral tolerance induction has been shown to be effective in preventing FA in the immediate term, claiming that tolerance, rather than a delay to onset of FA, has been achieved requires examination of the effects of avoidance of the food under investigation and/or of *ad libitum* consumption.

To date, the only FA prevention study to address this question is the LEAP-On study, which examined whether early consumption of peanut had a sustained effect on peanut allergy prevention after 12 months of peanut avoidance.[2] A total of 556 participants (88.5% [556/628]) from the original LEAP study were enrolled in the follow-on study. The rate of adherence to avoidance was 90.4% in the peanut avoidance group and 69.3% in the peanut consumption group. At 72 months of age, peanut allergy remained significantly higher in the peanut avoidance group compared with that in the peanut consumption group (18.6% vs 4.8% [$P < .001$], respectively). The LEAP-On study showed that the nonallergic status of children who had been consuming peanut remained stable over 12 months of subsequent peanut avoidance. Thus the key finding of the 2 LEAP studies is that early introduction and consumption of peanut until 60 months of age causes a reduction in peanut allergy that persists at 72 months of age after a 12-month period of avoidance.

Follow-on studies of the LEAP and EAT study cohorts are underway to observe whether the effects of early tolerance continue to persist approximately 7 years after the interventions were stopped and after *ad libitum* consumption. Future studies of oral tolerance induction should include long-term follow-up after *ad libitum* consumption into their design.

FACTORS AFFECTING ADHERENCE

A greater understanding of the many factors that influence adherence is of great clinical and public health importance. The lower rate of adherence in the EAT study varied between foods; egg ingestion was lower than peanut and milk consumption but higher than sesame, fish, and wheat consumption (which was always the last of the foods to be introduced).[3] However, partial adherence among early introduction group participants was not associated with any significant increase in allergy prevalence. This offers reassurance that children who are unable to comply with the intervention will not increase their risk of FA.

The LEAP study achieved a high adherence rate in the peanut introduction group (92%); however, frequent contact between

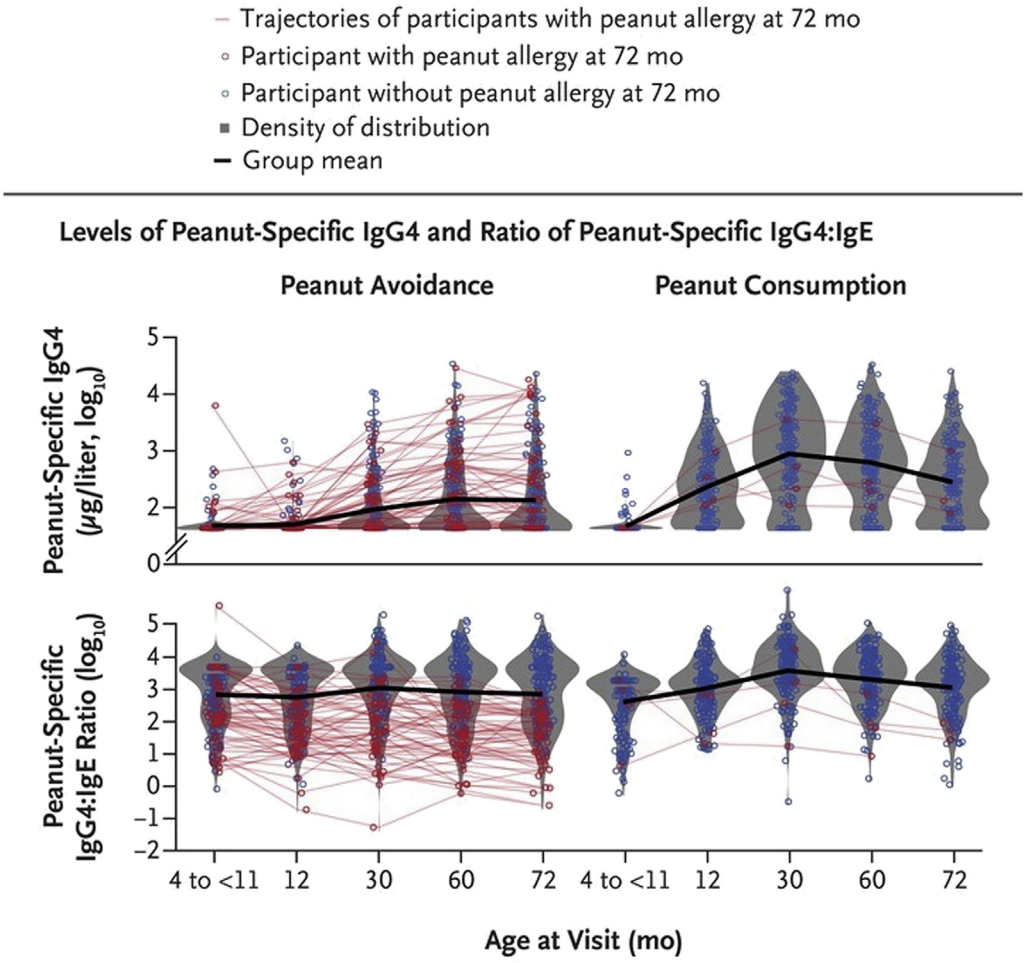


FIG 5. Changes that occur with IgE and IgG levels and IgE/IgG_4 ratios over time in children who consumed or avoided peanuts in the frame of the LEAP and LEAP-On studies. From Du Toit et al.[2] Copyright © 2016 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

study personnel and participating families was built into the protocol, and peanut introduction was successfully achieved in the LEAP study consumption group after only a few study contacts.

There are many other reasons for the differences in adherence rates between the LEAP and EAT studies, including factors relating to the food's introduction regimens and maternal and family factors, such as education, cultural, and ethnic differences. In the EAT study there was a marked influence of race on FA rates, being much higher in nonwhite participants with a stepwise increase from white (5.3%) to mixed ethnicity (9.4%) to Asian/black/Chinese participants (19.3%; $P < .0005$). Conversely, there was a statistically significant stepwise reduction in adherence that was most notable in the early introduction group with only 1 in 7 Asian/black/Chinese participants adhering to the protocol ($P = .01$).

Typically, children with FA are allergic to more than 1 food. However, single-allergen oral tolerance induction appears to be allergen specific; that is, early consumption of peanut had no effect on development or resolution of other food allergies or atopic diseases.[25] Thus if FA prevention is to be achieved through early exposure, studies that explore the many factors that influence adherence are required so as to maximize the effect of the intervention by promoting and facilitating successful introduction of multiple foods in infancy.

IMMUNOLOGIC CHANGES IN FA PREVENTION

Oral tolerance induction has proved successful in achieving clinical tolerance to specific foods, suggesting that the dual-allergen exposure hypothesis is an accurate representation of one of the mechanisms by which FA develops. In addition to clinical tolerance, the LEAP and LEAP-On studies have demonstrated immunologic changes suggestive of immune tolerance. As is now discussed, the dynamics of change are unique to each immune marker.

Changes in peanut-specific SPT responses and IgE levels against peanut and rAra h 2

In the LEAP study the mean SPT wheal size was smaller at 60 months of age in the consumption group compared with that in the avoidance group and remained smaller at 72 months of age in the LEAP-On study. In contrast, there was no difference in mean levels of IgE to peanut between groups throughout the LEAP study, but differences were noted at 72 months of age in the LEAP-On study (lower in the LEAP peanut-consuming

population). Mean levels of Ara h 2–specific IgE decreased significantly in the peanut consumption group from 30 to 60 months during the LEAP study ($P < .001$) and remained low at 72 months of age in the LEAP-On study. The inhibition of IgE synthesis is further reflected by the fact that relatively few participants in the peanut consumption group had high IgE levels to peanut and to Ara h 2 at 30, 60, and 72 months of age. Children who were allergic to peanut at 60 months of age already had higher peanut-specific IgE levels at 12 months, and differences remained at 30 and 60 months of age. These findings suggest that the elaboration of IgE antibodies to foods occurs early in infancy and might take a very long time to switch off, likely because of the presence of long-lived memory B and plasma cells committed to IgE production.

Peanut-specific IgG_4 level and IgG_4/IgE ratio changes

Peanut-specific IgG and IgG_4 levels increased over time in both LEAP groups; however, the peanut consumption group, who were largely protected against peanut allergy, had a significantly greater and earlier increase, which was already evident by 12 months of age. The overall balance between peanut-specific IgG_4 and IgE levels reflected the participants' allergic status to peanut. In the LEAP-On study peanut-specific IgG_4 levels and peanut-specific IgG_4/IgE ratios continued to be greater in the previous peanut consumption group than in the previous peanut avoidance group. However, IgG_4 levels started to slowly drift down after 30 months, even in the peanut consumption group. In the participants who became allergic in the LEAP-On study (1.1% of the peanut consumption group and 1.1% of the peanut avoidance group), the ratio of peanut-specific IgG_4/IgE levels decreased between 60 and 72 months. Children from the peanut consumption group who were able to tolerate peanut continued to have low levels of peanut-specific IgE and high IgG_4/IgE ratios at 60 months in the LEAP study, and this was maintained at 72 months. These observations indicate that IgG_4 levels are associated with protection against allergy development. Recently, peanut-specific IgG_4 levels have been shown to inhibit basophil activation *in vitro* in response to peanut (Fig 5).[2]

SPECIAL STATISTICAL CONSIDERATIONS RELATING TO PREVENTION STUDIES IN PATIENTS WITH FA

There are critical issues in the design and statistical analyses of prevention studies that differ fundamentally from treatment studies.[10] For example, in treatment studies all subjects start with the disease, and few will be cured because of the intervention. In prevention studies all subjects start without the disease, and even in high-risk studies, such as the LEAP study, less than 20% will end up with the disease. This has 2 important consequences with respect to both data imputation and analysis of changes in biomarkers of prevention. In treatment studies an ITT analysis can impute an allergic outcome to missing data because this is the most likely outcome in which allergy is assumed to persist, unless there is evidence of benefit. However, imputing an allergic outcome to all children with missing data in a prevention study could likely obscure and severely bias the treatment effect, especially if the dropout rate is comparable with or greater than the disease rate in the population. This difference in prevention studies also affects interpretation of biomarker data. If only a small subgroup of subjects (eg, 20%) are destined to have the disease, then the immunologic effects of a successful intervention can only be apparent in this subgroup of 20%. The absence of relevant biomarker changes in the 80% who are not destined to have the disease in the intervention group might obscure or dilute biomarker differences between the intervention and control groups. These problems can be overcome by using statistical methodologies to control for bias, as recently detailed by Bahnson et al.[10]

SUMMARY

Of the many possible hypotheses that explain the recent increase in childhood FA, the dual-allergen exposure hypothesis has been investigated most extensively. Recently, published RCTs provide evidence that peanut introduction (and likely hen's egg white) in early infancy offers a successful strategy for the prevention of FA. National Institutes of Health recommendations now actively encourage the early introduction of peanut for the prevention of peanut allergy, and other countries/settings recommend the inclusion of potential common food allergens, such as peanut and egg, in complementary feeding regimens commencing at around approximately 6 months of age but not before 4 months of age. Further studies that explore the efficacy of oral tolerance induction to other common food allergens and that focus on optimal timing, duration, and adherence are required.

We thank Henry T. Bahnson, Helen Fisher, and Poling Lau for support in the preparation of this manuscript.

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