Increased food diversity in the first year of life is inversely associated with allergic diseases

Caroline Roduit, MD, MPH,[a] Remo Frei, PhD,[b] Martin Depner, PhD,[c] Bianca Schaub, MD,[c] Georg Loss, PhD,[d] Jon Genuneit, MD,[e] Petra Pfefferle, PhD,[f] Anne Hyvärinen, PhD,[g] Anne M. Karvonen, PhD,[g] Josef Riedler, MD,[h] Jean-Charles Dalphin, MD, PhD,[i] Juha Pekkanen, MD,[g] Erika von Mutius, MD, MSc,[c] Charlotte Braun-Fahrländer, MD,[d,k] Roger Lauener, MD,[j] and the PASTURE study group* *Zurich, Basel, and Davos, Switzerland, Munich, Ulm, and Marburg, Germany, Kuopio, Finland, Schwarzach, Austria, and Besançon, France*

Background: The role of dietary factors in the development of allergies is a topic of debate, especially the potential associations between infant feeding practices and allergic diseases. Previously, we reported that increased food diversity introduced during the first year of life reduced the risk of atopic dermatitis.

Objective: In this study we investigated the association between the introduction of food during the first year of life and the development of asthma, allergic rhinitis, food allergy, or atopic sensitization, taking precautions to address reverse causality. We further analyzed the association between food diversity and gene expression of T-cell markers and of Cε germline transcript, reflecting antibody isotype switching to IgE, measured at 6 years of age.

Methods: Eight hundred fifty-six children who participated in a birth cohort study, Protection Against Allergy Study in Rural Environments/EFRAIM, were included. Feeding practices were reported by parents in monthly diaries during the first year of life. Data on environmental factors and allergic diseases were collected from questionnaires administered from birth up to 6 years of age.

Results: An increased diversity of complementary food introduced in the first year of life was inversely associated with asthma with a dose-response effect (adjusted odds ratio with each additional food item introduced, 0.74 [95% CI, 0.61-0.89]). A similar effect was observed for food allergy and food sensitization. Furthermore, increased food diversity was significantly associated with an increased expression of forkhead box protein 3 and a decreased expression of Cε germline transcript.

Conclusion: An increased diversity of food within the first year of life might have a protective effect on asthma, food allergy, and food sensitization and is associated with increased expression of a marker for regulatory T cells. (J Allergy Clin Immunol 2014;133:1056-64.)

Key words: *Asthma, food allergy and sensitization, food diversity, children*

Nutrition is an important environmental factor in early life that influences the development of the child's immune system. The role of nutrition during infancy on the development of allergies later in childhood remains controversial. Moreover, reverse causality is always a matter of concern.

From [a]the University of Zurich, Children's Hospital, and Christine Kühne-Center for Allergy Research and Education, Zurich; [b]the Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich and Christine Kühne-Center for Allergy Research and Education, Zurich; [c]Dr von Hauner Children's Hospital, Ludwig-Maximilians-Universität, Munich; [d]the Swiss Tropical and Public Health Institute and the University of Basel; [e]the Institute of Epidemiology and Medical Biometry, Ulm University; [f]the Institute for Laboratory Medicine, Pathobiochemistry and Molecular Diagnostics, Philipps University of Marburg; [g]the Department of Environment Health, National Institute for Health and Welfare, Kuopio; [h]Children's Hospital Schwarzach; [i]the Department of Respiratory Disease, UMR/CNRS 6249 Chrono-environment, University Hospital of Besançon; [j]Children's Hospital of Eastern Switzerland St Gallen and the Christine Kühne-Center for Allergy Research and Education, Davos; and [k]the University of Basel.

*The members of the PASTURE study group are (in alphabetical order by study center): Maija-Riitta Hirvonen, Sami Remes, Marjut Roponen, and Pekka Tiittanen (Finland); Marie-Laure Dalphin and Vincent Kaulek (France); Gisela Büchele, Markus Ege, Michael Kabesch, and Harald Renz (Germany); and Gert Doekes (The Netherlands).

Supported by European Union research grants PASTURE/EFRAIM (QRLT4-CT 2001-00250, KBBE-2-2-06) and the Kühne Foundation.

Disclosure of potential conflict of interest: M. Depner has received one or more grants from or has one or more grants pending with the European Research Council. B. Schaub has received one or more grants from or has one or more grants pending with DFG, European Union. J. Genuneit has been supported by one or more grants from the European Union. J.-C. Dalphin has received one or more consulting fees or honoraria from and has received support for travel from Chiesi; has consultancy arrangements with Novartis and Intermune; has received one or more grants from or has one or more grants pending with Novartis; has received one or more payments for lecturing from or is on the speakers' bureau for Novartis, GlaxoSmithKline, AstraZeneca, and Chiesi; has received one or more payments for the development of educational presentations for Novartis, Chiesi, GlaxoSmithKline, AstraZeneca, Intermune, and Boehringer Ingelheim; and has received one or more payments for travel/accommodations/meeting expenses from Novartis, Chiesi, and GlaxoSmithKline. E. von Mutius has been supported by one or more grants from the European Commission; is a Board member for the American Academy of Allergy, Asthma & Immunology; has consultancy arrangements with GlaxoSmithKline, Novartis, Protectimmun, ALK-Abelló, and Astellas Pharma Europe; has provided expert testimony for the UK Research Excellence Framework; and has received one or more payments for lecturing from or is on the speakers' bureau for InfectoPharm and for Nestlé Research. R. Lauener has been supported by a European Union Research Grant and a Kühne Foundation Research Grant; has received support for travel from the European Union and from the Kühne Foundation; has consultancy arrangements with the National Advisory Boards of ALK-Abelló, Nestlé, Novartis, MSD, MEDA, Allergopharma, and Menarini; has received one or more grants from or has one or more grants pending with Study Nestec; and has received one or more payments for lecturing from or is on the speakers' bureau for ALK-Abelló and for Nestlé. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication April 16, 2013; revised December 16, 2013; accepted for publication December 18, 2013.

Available online February 6, 2014.

Corresponding author: Caroline Roduit, MD, MPH, Kinderspital Zurich, Steinwiesstrasse 75, 8032 Zurich, Switzerland. E-mail: Caroline.Roduit@kispi.uzh.ch.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.12.1044

Abbreviations used
Foxp3: Forkhead box protein 3
OR: Odds ratio
PASTURE: Protection Against Allergy Study in Rural Environments
T-bet: T-box transcription factor

During the early postnatal period, the infant gut is first exposed to different food antigens, and these exposures might influence the development of immune tolerance. Mechanisms could include the acquisition of the microbiota through the diet or diet-microbiota interactions.[1-3] It has been suggested that modern changes in the postnatal environment, such as early dietary exposure, might not optimally support induction of immune tolerance because the incidence of allergic diseases during the last decades shows a strong increase.[4] However, current guidelines no longer recommend food allergen avoidance or delaying introduction in the infant diet to prevent allergic diseases because no clear benefit has been shown.[5] Recent studies have suggested a protective effect of early introduction of complementary food on allergic diseases, even though the underlying mechanism on the maturation of the mucosal immune system remains unknown.[6-12] However, it is widely accepted that oral tolerance mechanisms include regulatory T cells. Forkhead box protein 3 (Foxp3) is a key transcription factor for regulatory T cells, and it was shown that depletion of Foxp3$^+$ cells inhibited oral tolerance.[13] Our recent findings on the protective effect of an increased diversity of food introduced in the first year of life on atopic dermatitis are in agreement with the hypothesis that exposure to a variety of food antigens during early life might be important for the development of immune tolerance.[14]

Here we studied whether complementary food introduced in the first year of life was associated with asthma, food allergy, allergic rhinitis, and atopic sensitization up to 6 years of age in the prospective birth cohort study Protection Against Allergy Study in Rural Environments (PASTURE/EFRAIM).[15] Similar to our previous analyses on atopic dermatitis, we took into account potential reverse causality. We further examined whether food diversity introduced in the first year of life had an effect on gene expression of the T-cell markers T-box transcription factor (T-bet) and Gata-3, transcription factors related to the development of T_H1 and 2 T_H2 cells, respectively, and Foxp3, a transcription factor driving the development of regulatory cells T, and expression of Cε germline transcript, a marker for antibody isotype switching to IgE, at 6 years of age.

METHODS

Study design and population

The PASTURE/EFRAIM study is a prospective birth cohort involving children from rural areas in 5 European countries (Austria, Finland, France, Germany, and Switzerland) designed to evaluate risk factors and preventive factors for atopic diseases.[15] Pregnant women were recruited during the third trimester of pregnancy between August 2002 and March 2005 and divided into 2 groups. Women who lived on family-run farms where any kind of livestock was kept were assigned to the farm group. Women from the same rural areas not living on a farm were in the reference group. In total, 1133 children were included in this birth cohort. The study was approved by the local research ethics committees in each country, and written informed consent was obtained from all parents.

Children with data available on allergic diseases up to 6 years of age, farming status, parental allergic history, maternal educational status, number of siblings, and feeding practices in the first year of life (n = 856) were included in the current study.

Definitions

Questionnaires were administered in interviews or self-administered to the mothers within the third trimester of pregnancy and when the children were 2, 12, 18, and 24 months of age and then yearly up to age 6 years. Children were defined as having asthma when the parents reported at least once that the child had either doctor-diagnosed asthma or at least 2 doctor-diagnosed episodes of obstructive bronchitis in the last 12 months in the year 4, 5, or 6 questionnaires independent of a diagnosis reported in the first 3 years of life. Obstructive bronchitis is commonly used to define the first occurrence of asthmatic symptoms. Food allergy was defined when the parents reported up to age 6 years that the child had at least once been given a diagnosis of food allergy by a doctor. Allergic rhinitis was defined by the presence of symptoms (itchy, runny, or blocked nose without a cold and associated with red itchy eyes) or doctor-diagnosed allergic rhinitis ever reported in the 6-year questionnaire. Levels of allergen-specific IgE antibodies (*Dermatophagoides pteronyssius*, *Dermatophagoides farinae*, alder, birch, hazel, grass pollen, rye, mugwort, plantain, cat, horse, dog, *Alternaria* species, hen's egg, cow's milk, peanut, hazelnut, carrot, and wheat flour) were measured in blood among children at age 4.5 and 6 years, as well as their mothers and fathers. Sensitization was defined as a specific IgE level of 3.5 kU/L or greater and as being strongly associated with allergic diseases.

Parents indicated the food item that was given to the child in the last 4 weeks in each monthly diary between the 3rd and 12th months of life. For the introduction of complementary food, we used the same diversity score, as previously described, based on major food items, which were defined as the items introduced in the first year of life to at least 80% of the children.[14] The food diversity score is a total count of the number of different food items included in the child's diet. Diversity scores were calculated as follows: (1) with major food items introduced in the first year of life (n = 6, including vegetables or fruits, cereals, bread, meat, cake, and yogurt); (2) with the same major food items but introduced in the first 6 months of life; and (3) with all food items introduced in the first year of life (n = 15, including any cow's milk, yogurt, other milk product, eggs, nuts, vegetables or fruits, cereals, bread, meat, fish, soy, margarine, butter, cake, and chocolate) reported in the monthly diary. The latter was calculated to include potentially allergenic food items.

Farmer children were defined as children who were living on a farm where livestock was held and whose family ran the farm, according to parental reports. Information on parental atopic status, maternal education, smoking during pregnancy, mode of delivery, birth weight, gestational age, sex, number of siblings, and duration of breast-feeding was recorded in questionnaires during pregnancy, 2 months after birth, and at 1 year of age. Positive parental history of allergies was defined as ever having asthma, allergic rhinitis, or atopic dermatitis.

Gene expression of T-cell markers

Blood samples were collected at birth (cord blood) and at 1, 4.5, and 6 years of age for assessment of mRNA expression. The method for these measurements was described in detail elsewhere.[16] The data presented are normalized values for the endogenous controls (18S rRNA and β_2-microglobulin) determined by using the comparative ($\Delta\Delta$ cycle threshold) method, according to the manufacturer's instructions (Applied Biosystems, Foster City, Calif).

Statistical analysis

Differences in characteristics of children regarding the diversity food score were tested by using the χ^2 test. Logistic regression was used to investigate the association between food exposure and asthma, food allergy, allergic rhinitis, and atopic sensitization. We performed and compared different models. Model 1 was the crude model, and model 2 included adjustment for the potential confounders: farmer, center, duration of breast-feeding (categorized according to the number of months children were breast-fed, not exclusively), parents with allergy, maternal education, sex, and number of siblings. To take into account reverse causality, we used model 3, which included the same adjustment as model 2 but additionally excluded children with doctor-diagnosed food allergy within the first year of life. For asthma, children with at least 1 episode of doctor-diagnosed obstructive bronchitis or asthma within the first year of life were also excluded. Sensitivity analysis with additional exclusions for wheeze, atopic dermatitis, or both within the first year of life was performed. Parental allergic status is potentially a strong confounder because it might influence infant feeding practices; therefore we performed additional analysis using different definitions based on atopic sensitization and/or history of allergies among the mother, father, or both. Atopic dermatitis might also be a confounder and potentially on the causal pathway between food diversity and asthma, and therefore we performed analyses with additional adjustment to control for this. Sensitivity analyses were performed with modified food scores, removing 1 of the items to determine the influence of a single item in this score. Smooth plots were performed with generalized additive models by using R statistical software to graphically display the dose-response effect of the food diversity score on allergic diseases. Test for linear trend between the food diversity score and health outcomes was performed by using the Cochran-Armitage trend test.

The gene expression data were analyzed by using the comparative threshold method of Giulietti et al.[17] This method expresses the measured number of PCR cycles of the participants relative to 1 participant. We chose as a reference the cord blood values for a nonfarmer child whose expression of all genes was greater than the detection limit. The results are expressed as a gene expression multiplication factor compared with the reference. Because the distribution of the gene expression levels was skewed, the variables were log transformed (natural logarithm), resulting in an approximately normal distribution. We used linear regressions to calculate associations between food diversity scores and mRNA expression (expressed as geometric mean ratios). Interaction terms were included in the multivariate models to test for effect modification between the diversity food score and parental history of allergy, center, and farming status on allergic diseases. Data analysis was conducted with SAS software, version 9.2 (SAS Institute, Cary, NC).

RESULTS

Characteristics and prevalence of allergic diseases

Among the 856 children included in this study, 51.5% were farmer children, and 53.6% had at least 1 allergic parent (Table I). The description of food diversity was mentioned in our previous study.[14] Farmer children received a higher number of different food items in the first year of life compared with nonfarmer children. Differences between centers were observed regarding the diversity score, with a higher proportion of French and German children having a low score. A higher proportion of children with at least 1 parent with a history of atopy had a low score compared with children with parents with no history of atopy. No association between the diversity score and sex, number of siblings, duration of breast-feeding, and maternal education was found. Approximately half of the children (47.4%) were breast-fed for more than 6 months (not exclusively), and no association between duration of breast-feeding and asthma, allergic rhinitis, food allergy, and atopic sensitization was observed (data not shown). Characteristics of children excluded from the analysis because of missing data (n = 277) did not differ from those of the included children, except for a higher proportion of nonfarmer children (58.5%) among the excluded children.

There were 848 and 809 subjects with available data for allergic rhinitis and food allergy, respectively. The cumulative prevalence of asthma between 3 and 6 years of age was 8.6%, the cumulative prevalence of allergic rhinitis up to 6 years of age was 7.6%, and the cumulative prevalence of food allergy up to 6 years was 7.4%. These proportions were significantly higher in children with 2 allergic parents than among children with nonallergic parents (asthma, 10.7% vs 6.3%; allergic rhinitis, 11.0% vs 3.6%; and food allergy, 10.6% vs 3.7%, respectively).

Data on atopic sensitization were available for 596 children at age 4.5 years, 6 years, or both. Sensitization to any allergen was present in 25.5% of children, sensitization to food allergens was present 10.7%, and sensitization to inhalant allergens was present in 22.1%, as measured at 4.5 or 6 years.

Association between food diversity score and allergic diseases and atopic sensitization

Dividing the diversity score into 3 categories with the highest (all 6 food items) as reference, we observed a significant inverse dose-response association with asthma in crude analysis (model 1) and after adjustment for potential confounders (model 2, Table II). The test for linear trend was significant for asthma, food allergy, and food sensitization ($P < .001$, $P < .001$, and $P = .006$, respectively). We observed a significant reduction of 26% for the development of asthma, with each additional food item introduced in the first year of life. This inverse association between the food diversity score and asthma remained stable after exclusion of children with a doctor's diagnosis of food allergy and defined as having asthma, obstructive bronchitis, or both within the first year of life (model 3). Sensitivity analyses with additional exclusion of children with wheezing (n = 223 [26.1%]), atopic dermatitis (n = 119 [14.4%]), or both within the first year of life were performed and showed similar results (crude analysis only among children with no wheeze and no atopic dermatitis in the first year of life: odds ratio [OR] for 0-3 vs 6 items of 3.83 [95% CI, 1.02-14.41] and OR for 4-5 vs 6 items of 1.63 [95% CI, 0.74-3.61]). Moreover, analysis excluding children (n = 30) among those parents who reported that they avoided introducing food within the first year of life because of child's allergy showed similar associations.

Additional adjustment for atopic dermatitis showed similar results (see Table E1 in this article's Online Repository at www.jacionline.org), and the negative association between food diversity score and asthma was stronger for children having both diseases (n = 29) compared with those having neither disease (OR for food diversity score, continuous: 0.61 [95% CI, 0.49-0.76]). For children with asthma but no atopic dermatitis up to 6 years (n = 40), a similar association was observed with an increasing diversity food score (OR for food diversity score, continuous: 0.80 [95% CI, 0.62-1.04]). Additional analyses were performed for children defined as having asthma based only on a doctor's diagnosis of asthma between 3 and 6 years of age (n = 36), which showed similar results (see Table E2 in this article's Online Repository at www.jacionline.org). Recently published results from this birth cohort showed that late-onset and persistent wheeze phenotypes were best correlated with clinical phenotypes of asthma.[18] We observed that the strongest association between the food diversity score and different phenotypes of wheeze was with late-onset and persistent wheeze phenotypes (OR for transient wheeze, 1.08 [95% CI, 0.86-1.35]; OR for intermediate wheeze, 0.91 [95% CI, 0.68-1.21]; OR for late-onset wheeze, 0.75 [95% CI, 0.53-1.08]; and OR for persistent wheeze, 0.76 [95% CI, 0.55-1.07]).

A smoothed plot of the relationship between the food diversity score and asthma was performed, which showed a decrease in the log odds of asthma with an increasing score (Fig 1, *A*).

TABLE I. Characteristics of the study population and prevalence of allergic diseases

	All		Food diversity score						
			0-3 items		4-5 items		6 items		
	No.	Percent	No.	Percent	No.	Percent	No.	Percent	*P* value*
Characteristics									
All	856	100	37	4.3	263	30.7	556	65.0	
Farmer									<.001
Yes	415	51.5	9	24.3	112	57.4	294	52.8	
No	441	48.5	28	75.7	151	42.6	263	47.2	
Center									.01
Austria	161	18.8	1	2.7	58	22.1	102	18.3	
Switzerland	187	21.9	9	24.3	57	21.7	121	21.8	
France	150	17.5	12	32.4	48	18.2	90	16.2	
Germany	203	23.7	12	32.4	63	23.9	128	23.0	
Finland	155	18.1	3	8.1	37	14.1	115	20.7	
Sex									.66
Girls	424	49.5	20	54.0	125	47.5	279	50.2	
Boys	432	50.5	17	46.0	138	52.5	277	49.8	
Siblings									.10
0	310	36.2	9	24.3	100	38.0	201	36.1	
1-2	459	53.6	27	73.0	140	53.2	292	52.6	
≥3	87	10.2	1	2.7	23	8.8	63	11.3	
Parents with atopy history									.005
Yes	459	53.6	26	70.3	156	59.3	277	49.8	
No	397	46.4	11	29.7	107	40.7	279	50.2	
Breast-feeding									.70
Never	80	9.4	5	13.5	26	9.9	49	8.8	
>0-2 mo	132	15.4	5	13.5	40	15.2	87	15.7	
3-6 mo	238	27.8	6	16.2	75	28.5	157	28.2	
7-9 mo	180	21.0	9	24.3	48	18.3	123	22.1	
≥10 mo	226	26.4	12	32.4	74	28.1	140	25.2	
Maternal education									.16
Low	140	16.4	11	29.7	40	15.2	89	16.0	
Mid	370	43.2	10	27.0	109	41.4	251	45.1	
Mid-high	251	29.3	10	27.0	82	31.2	159	28.6	
High	95	11.1	6	16.2	32	12.2	57	10.3	
Outcome prevalences									
Asthma (doctor-diagnosed asthma and/or ≥2 obstructive bronchitis episodes 3-6 y)	74/856	8.6	7	18.9	33	12.6	34	6.1	<.001
Allergic rhinitis (doctor-diagnosed hay fever or allergic rhinitis OR symptoms [nasal AND eye] ever at age 6 y)	64/848	7.6	4	10.8	24	9.2	36	6.6	.31
Food allergy up to 6 y (doctor-diagnosed food allergy)	60/809	7.4	7	21.9	23	9.2	30	5.7	.001
Any sensitization at 4.5 or 6 y (cutoff: 3.5 kU/L)	152/596	25.5	12	46.2	44	24.2	96	24.7	.05
Food sensitization at 4.5 or 6 y (cutoff: 3.5 kU/L)	62/580	10.7	7	26.9	23	13.1	32	8.5	.006
Inhalant sensitization 4.5 or 6 y (cutoff: 3.5 kU/L)	131/594	22.1	9	34.6	39	21.4	83	21.5	.29

*Based on the χ^2 test.

The analysis restricted to children for whom no food avoidance was reported within the first year of life because of child's allergy showed the same pattern (Fig 1, *B*). To also include allergenic foods, we additionally analyzed the association between another food diversity score with all the food items (n = 15) and asthma. The same inverse dose-response effect was observed (Fig 1, *C*). The same analyses were performed with food allergy (Fig 2), and similar patterns were observed. Additionally, we calculated a diversity score with food items introduced within the first 6 months of life and observed the same tendency of a negative association between a higher score compared with a lower score on asthma risk (OR for food diversity score, continuous: 0.83 [95% CI, 0.68-1.03]), although with a test for linear trend of borderline significance (*P* = .08, see Fig E1 in this article's Online Repository at www.jacionline.org).

The children with a low food diversity score (with the major food items) had an increased risk of food allergy up to 6 years of age and sensitization to food allergens at age 4.5 or 6 years compared with children with the highest score (Table II). The analysis with children having doctor-diagnosed food allergy combined with positive food sensitization (n = 18) showed an even stronger negative association with food diversity (OR for food diversity score, continuous: 0.55 [95% CI, 0.40-0.76]). After exclusion of children with food allergy within the first year of life, the associations were no longer statistically significant, even though estimates for the continuous variable remained similar (model 3). There was a tendency toward a negative association between the food diversity score and allergic rhinitis or sensitization to inhalant allergens, although this was not statistically significant. There was no interaction between the food diversity score and parental allergies or farming status on

TABLE II. Association between diversity score within the first year of life and allergic diseases and atopic sensitization

	Model 1			Model 2			Model 3		
	No.	OR	95% CI	No.	OR	95% CI	No.	OR	95% CI
Asthma									
Food diversity score within 1st year									
0-3 items	7/37	**3.58**	**1.47-8.75**	7/37	**3.15**	**1.24-8.05**	5/32	**3.77**	**1.23-11.53**
4-5 items	33/263	**2.20**	**1.33-3.64**	33/263	**2.01**	**1.24-3.46**	16/215	1.60	0.80-3.20
6 items, reference	34/556	1		34/556	1		21/487	1	
Diversity score, continuous	74/856	**0.73**	**0.61-0.87**	74/856	**0.74**	**0.61-0.89**	42/734	**0.76**	**0.60-0.96**
Allergic rhinitis									
Diversity score major food within 1st year									
0-3 items	4/37	1.73	0.58-5.15	4/37	1.78	0.56-5.67	2/34	0.94	0.21-4.26
4-5 items	24/261	1.45	0.84-2.48	24/261	1.35	0.77-2.38	20/252	1.14	0.63-2.06
6 items, reference	36/550	1		36/550	1		36/547	1	
Diversity score, continuous	64/848	0.83	0.68-1.01	64/848	0.80	0.64-1.00	58/833	0.93	0.71-1.22
Doctor-diagnosed food allergy									
Food diversity score within 1st year									
0-3 items	7/32	**4.65**	**1.86-11.61**	7/32	**4.43**	**1.62-12.10**	4/29	2.61	0.77-8.88
4-5 items	23/249	1.69	0.96-2.97	23/249	**1.85**	**1.02-3.35**	13/239	1.13	0.56-2.31
6 items, reference	30/528	1		30/528	1		26/524	1	
Diversity score, continuous	60/809	**0.71**	**0.58-0.85**	60/809	**0.70**	**0.57-0.86**	43/792	0.79	0.60-1.03
Sensitization to food allergens at 4.5 or 6 y									
Food diversity score within 1st year									
0-3 items	7/26	**3.99**	**1.56-10.19**	7/26	**5.47**	**1.91-15.67**	6/23	**5.22**	**1.70-16.04**
4-5 items	23/176	1.63	0.92-2.87	23/176	1.52	0.83-2.76	22/167	1.53	0.83-2.84
6 items, reference	32/378	1		32/378	1		31/376	1	
Diversity score, continuous	62/580	**0.76**	**0.62-0.93**	62/580	**0.72**	**0.57-0.90**	59/566	**0.72**	**0.56-0.91**
Sensitization to inhalant allergens at 4.5 or 6 y									
Food diversity score within 1st year									
0-3 items	9/26	1.93	0.83-4.49	9/26	1.79	0.74-4.37	7/23	1.50	0.57-3.95
4-5 items	39/182	1.00	0.65-1.53	39/182	0.87	0.56-1.37	36/172	0.84	0.53-1.34
6 items, reference	83/386	1		83/386	1		83/384	1	
Diversity score, continuous	131/594	0.94	0.78-1.13	131/594	0.97	0.80-1.18	126/579	1.01	0.82-1.25

Diversity scores with major food items are shown. Boldface values are significant ($P < .05$).
Model 1, Crude; *Model 2*, model 1 plus adjusted for center, farmer, parents with allergy, sex, breast-feeding, siblings, and maternal education; *Model 3*, model 2 plus exclusion of food allergy at 1 year (n = 17) and only with asthma: exclusion of at least 1 episode of obstructive bronchitis and/or asthma, both doctor diagnosed and reported at 1 year (n = 102).

allergic diseases. Moreover, similar results were obtained in separate analyses stratified by parental history of allergy or by farming status, as well as after additional adjustment for consumption of unboiled farm milk (data not shown).

The analysis with different definitions of asthma, both atopic and nonatopic (defined with or without positive sensitization to inhalant allergens at 4.5 or 6 years of age: n = 23 and n = 30, respectively) showed the same tendencies (adjusted OR for each additional food items introduced in the first year of life, 0.70 [95% CI, 0.52-0.94] and 0.77 [95% CI, 0.59-1.00], respectively). Analysis for a subgroup excluding children with a low score of food diversity showed similar results (see Table E3 in this article's Online Repository at www.jacionline.org). Sensitivity analyses with modified scores, excluding 1 of the food items to evaluate the influence of the excluded item in this score, showed similar associations between reduced scores and allergic diseases (data not shown).

To analyze the association between single food items and allergic outcomes, we also performed different models to take into account the reverse causality effect. The results from the analyses between single food items and asthma showed a strong negative association with milk products, such as yogurt and butter, introduced within the first year of life, compared with reference children who did not consume these foods in the first year of life (see Table E4 in this article's Online Repository at www.jacionline.org). The analysis among a subgroup excluding children with food allergy or asthma within the first year of life (model 3) showed similar results, although these were weaker for introduction of yogurt. The analysis with the 2 predictor variables of yogurt and butter in the model showed the same protective effect, as well as stratified analysis by parental history of allergy (data not shown). The risk of food allergy was reduced by a factor of 0.5 among children who consumed fish within the first year of life compared with children who did not (see Table E5 in this article's Online Repository at www.jacionline.org). This effect remained significant after adjustment for potential confounders, such as atopic dermatitis, and after exclusion of children with food allergy within the first year of life. The stratified analysis showed an inverse association only among the children with allergic parents (data not shown). The negative association between fish introduced within the first year of life and sensitization to food allergens was only significant in the crude analysis (see Table E6 in this article's Online Repository at www.jacionline.org).

Association between food diversity score and gene expression for marker of IgE antibody isotype switching and for T-cell markers

Among children with a low food diversity score within the first year of life, we observed a significantly increased level of Cε germline transcript at age 6 years by a factor of 1.8 compared with

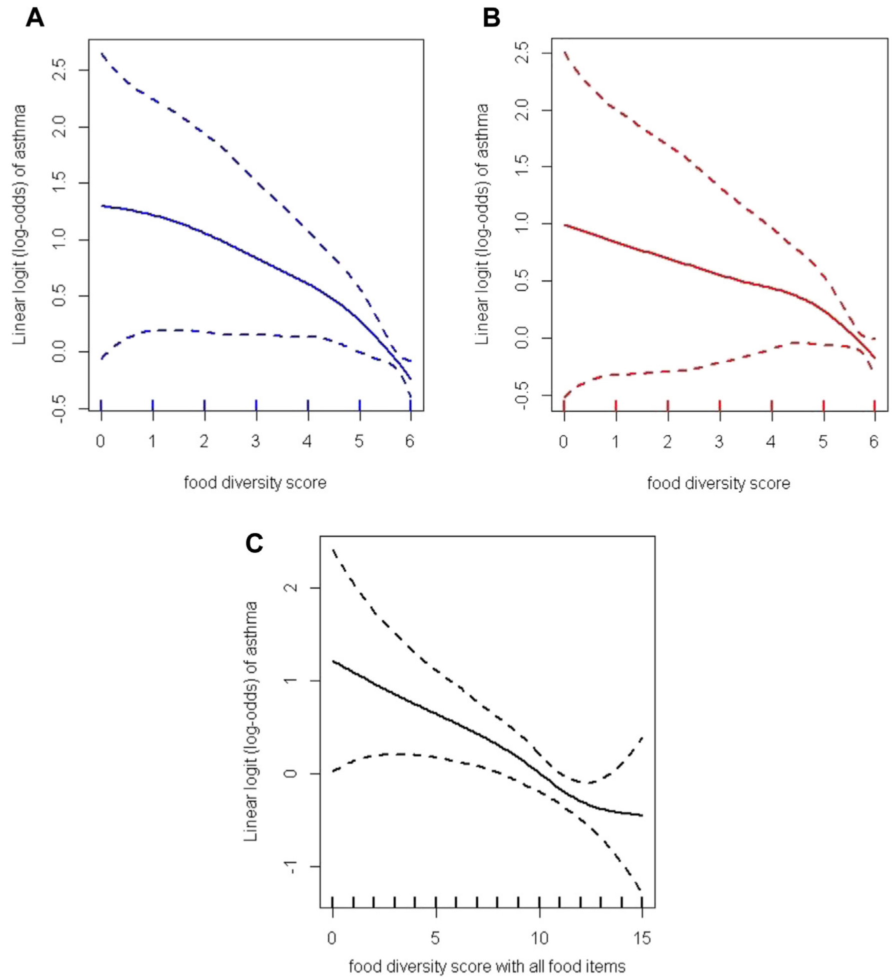

FIG 1. Association between increasing diversity of food introduced within the first year of life and asthma. **A,** Diversity score with major food for the entire study population. **B,** Diversity score with major food restricted to children without food avoidance within the first year of life because of allergies. **C,** Diversity score with all different food items for the entire study population. The *solid line* represents the predicted value of asthma as a function of the score, and *dashed lines* represent the CI. The *y-axis* is the linear logit of asthma, and the values are centered on 0 (50/50 odds) and extended to both positive and negative values. All models are adjusted for farmer, center, duration of breast-feeding, parents with allergy, maternal education, sex, and siblings.

children with the highest score (Table III). Moreover, those children with a low food diversity score showed a significantly lower level of gene expression for Foxp3, a transcription factor driving development of regulatory T cells, measured at 6 years after adjustment for potential confounders and the value measured in cord blood (Table IV). Analysis stratified by parental allergic status showed similar results. We did not find an indirect effect of Foxp3 or Cε germline transcript expression on the associations between food diversity and allergic diseases or sensitization (data not shown). We did not observe an association between the food diversity score and mRNA expression of T-bet and Gata-3, transcription factors related to the development of T_H1 and T_H2 cells, respectively.

DISCUSSION

Our data show that an increased diversity of food introduced in children's diet within the first year of life is negatively associated with the development of asthma and food allergy up to 6 years of age and on sensitization to food allergens at 4.5 or 6 years of age. Moreover, among children with a low food diversity score, we found an increased expression of marker for antibody isotype switching to IgE and a reduced expression of the regulatory T cell–associated gene Foxp3 measured at 6 years of age.

To our knowledge, the present findings are the first showing an inverse association of an increased diversity of exposures to food antigens in the first year of life on the development of allergic diseases later in childhood. Previous studies on the diversity of food introduced in infants' diets and allergies focused on the first 4 or 6 months of life.[19-21] Only 1 study reported an increased risk of doctor-diagnosed eczema up to 6 years of age in association with an increased diversity of solid food introduced within the first 4 months.[20] However, this effect was seen neither with eczema up to 4 years nor with a definition based on symptoms, and a tendency of protective effect on asthma up to 6 years was observed with an increased diversity of food introduced within the first 4 months. In our study a diversity score with food items introduced within the first 6 months of life was calculated, and

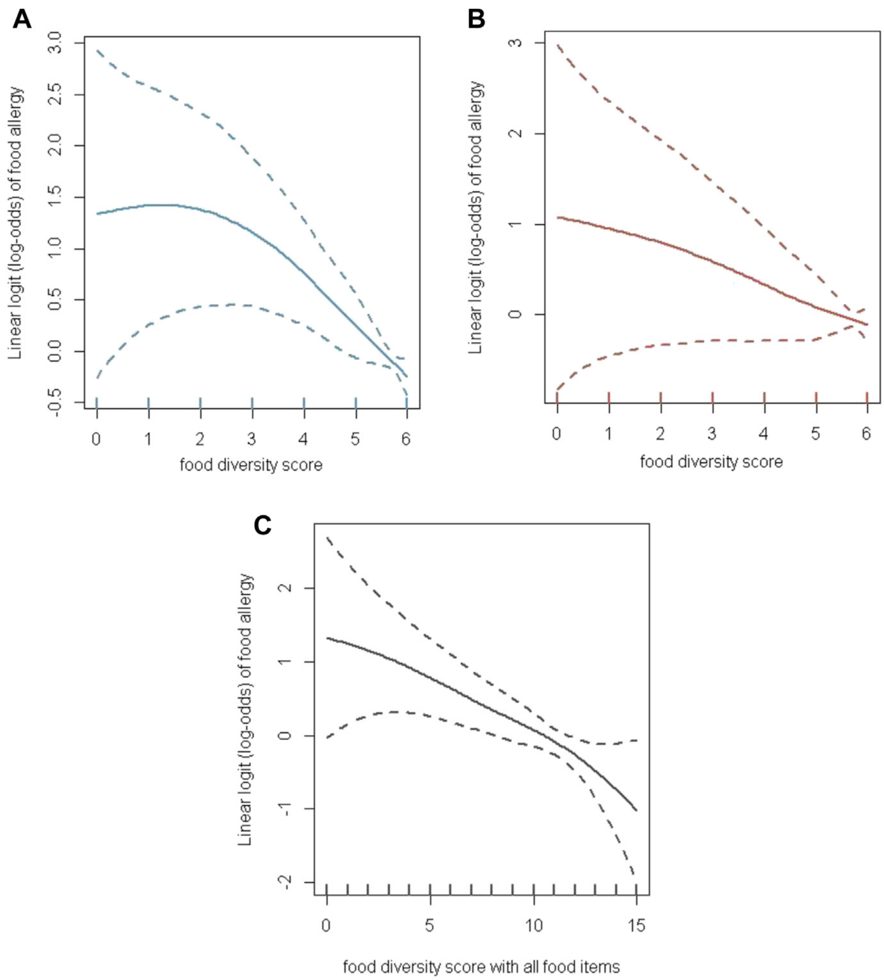

FIG 2. Association between increasing diversity of food introduced within the first year of life and food allergy. **A,** Diversity score with major food for the entire study population. **B,** Diversity score with major food restricted to children without food avoidance within the first year of life because of allergies. **C,** Diversity score with all different food items for the entire study population. The *solid line* represents the predicted value of asthma as a function of the score, and *dashed lines* represent the CI. The *y-axis* is the linear logit of food allergy, and the values are centered on 0 (50/50 odds) and extended to both positive and negative values. All models are adjusted for farmer, center, duration of breast-feeding, parents with allergy, maternal education, sex, and siblings.

TABLE III. Association between diversity food score within the first year of life and gene expression for marker of IgE antibody isotype switching (Cε germline transcript) measured at 6 years

	Cε germline transcript (6 y)								
	All			No allergic parents			≥1 Allergic parent		
Diversity score, major food items within 1st y	No.	GMR*	95% CI	No.	GMR*	95% CI	No.	GMR*	95% CI
0-3 items	22	**1.81**	**1.21-2.70**	7	1.81	0.84-3.88	15	**1.78**	**1.13-2.79**
4-5 items	161	1.18	0.99-1.40	61	1.26	0.94-1.69	100	1.14	0.93-1.39
6 items, reference	336	1		156	1		180	1	

Boldface values are significant ($P > .05$).
*Geometric mean ratios are adjusted for center, farmer, parents with allergy, sex, breast-feeding, maternal education, and cord blood value for the respective gene.

children with a higher score had a lower risk of asthma compared with those with a lower score. Moreover, the analysis with mutual adjustment for the score within the first 6 months and the score within the first year of life showed a persistent significant protective effect of the first year's score, meaning that exposure to food proteins in the time period between 6 and 12 months of age might be an important time window for protection against the development of later allergic diseases. The new guidelines on early nutrition and allergy prevention recommend that complementary foods should not be introduced before 4 months of age but should be introduced for all infants by 6 months of age.[5,22] Even though more evidence is required, because our

TABLE IV. Association between diversity food score within the first year of life and gene expression for T-cell markers measured at 6 years

Diversity score, major food items within 1st year	All			No allergic parents			≥1 Allergic parent		
	No.	GMR*	95% CI	No.	GMR*	95% CI	No.	GMR*	95% CI
T-bet									
0-3 items	22	0.94	0.71-1.25	7	1.13	0.61-2.12	15	0.90	0.70-1.16
4-5 items	163	0.96	0.85-1.09	63	0.98	0.77-1.24	100	0.95	0.85-1.07
6 items, reference	339	1		157	1		182	1	
Gata-3									
0-3 items	22	0.93	0.74-1.18	7	0.93	0.56-1.56	15	0.98	0.79-1.21
4-5 items	163	1.06	0.96-1.17	63	1.10	0.91-1.34	100	1.03	0.94-1.14
6 items, reference	339	1		157	1		182	1	
Foxp3									
0-3 items	22	**0.70**	**0.51-0.96**	7	0.78	0.41-1.51	15	**0.69**	**0.50-0.96**
4-5 items	163	0.99	0.87-1.14	63	0.92	0.72-1.18	100	1.03	0.89-1.20
6 items, reference	339	1		157	1		182	1	

Boldface values are significant ($P > .05$).

*Geometric mean ratios are adjusted for center, farmer, parents with allergy, sex, breast-feeding, maternal education, and cord blood value for the respective gene.

findings form the basis for a new hypothesis, an increased diversity of food in the second part of the first year of life might be an interesting strategy to prevent allergic diseases.

The strengths of this study are the prospective design and collection of data on the introduction of complementary food within the first year of life, which avoid recall bias. One major concern with the association between feeding practices and atopic diseases is the potential bias caused by reverse causality. Among children with early symptoms of the disease, those with allergic parents, or both, introduction of certain complementary foods, especially allergenic foods, tends to be delayed. With analyses performed in a subgroup of children, excluding those with food allergy, respiratory disorders, or both within the first year of life, significant results remained with asthma, and there was a similar tendency, although weaker, with food allergy and food sensitization. Moreover, all multivariate models were additionally adjusted for atopic dermatitis, and the negative association between food diversity and asthma seems to be independent of the effect on atopic dermatitis. Sensitivity analysis with models adjusted for parental allergic status using different definitions based on atopic sensitization and/or history of allergies among the mother, father, or both showed similar results, as did stratified analysis (data not shown). Our results provide strong support to conduct a randomized clinical trial, which is essential to completely exclude the reverse causality effect. Selection bias is unlikely in this study because the excluded and included children did not differ significantly. The definition of the health outcomes based on a doctor's diagnosis might lead to an underestimation of the prevalence and, despite this, could lead to an underestimation of the association by a dilution effect; however, we still found a significant association. Information on asthma medications was not included in the definition and might be considered a limitation. Moreover, a child might be defined as having asthma if the reported doctor's diagnosis was made between 3 and 4 years of age and not later and therefore related to wheeze that resolves by school age. In any case, the strongest negative association between food diversity and wheeze phenotypes was with late-onset and persistent wheeze, which both were shown to be related to clinical phenotype of asthma.[18] Another limitation of this study is the selected study population, from rural areas in Europe, so that our findings might not be applicable to other populations and also the lack of information on lactose or other food intolerance and on conditions that could affect infant feeding or could represent subclinical manifestations of food allergy, such as colic and gastroesophageal reflux. The latter could induce a reverse causality effect and is a major limitation in this study. Therefore reverse causality cannot be completely excluded. Another reason that reverse causality cannot be excluded is that reasons for the variability in food introduction in the first year of life are not well understood.

Our findings highlight the role for diversity of environmental exposures on the development of allergic diseases. We have shown previously that the diversity of exposures might play a role in the development of allergic disease, even during pregnancy, with contact with different farm animal species.[16] Similarly, recent findings showed that the increased diversity of microbial exposures had a protective effect on asthma.[23] Our results support the hypothesis that exposure in early life to diverse food antigens, such as food proteins, could increase the maturation of the mucosal immune system and induce tolerance networks.[24] Moreover, it is believed that regulatory T cells are involved in tolerance acquisition. In this study we showed decreased expression of a marker for regulatory T cells using the transcription factor Foxp3 at the age of 6 years among children with a low food diversity scores within the first year of life. However, this transcription factor does not assess the functional activity of regulatory T cells. The protective effect of exposure to an increased diversity of food in early life might be associated with the induction of regulatory T cells rather than a shift in the T_H1/T_H2 balance. One of the mechanisms that might be involved in the inhibition of allergy development by regulatory T cells is the inhibition of isotype switching to IgE[25]; interestingly, our results showing a decrease in Cε germline transcript with an increased food diversity are in agreement with this hypothesis.

The negative association was observed between increased food diversity and asthma and food sensitization. However, no significant association was found with sensitization to inhalant allergens and allergic rhinitis, even though this is difficult to evaluate because of the low numbers. One explanation might be that early food sensitization is a better predictor for allergic diseases and asthma than inhalant sensitization because this was already shown in previous studies.[26,27] Moreover, the same tendency toward a negative association with an increased food diversity was observed with both atopic and nonatopic asthma (even though it is not significant, this might be due to small numbers). Even though the underlying mechanism for the protective effect on asthma remains unclear, it might involve an induction of regulatory T cells, which are known to play a role in constraining inflammation.[28] Moreover, some dietary components, such as short-chain or long-chain fatty acids, as well as prebiotics, are known to have immunoregulatory properties.[3] A potential mechanism for the protective effect of an increased diversity in the infant's diet on allergic diseases might involve the gut microbiota, its metabolites, or both. It has been shown that diet, gut microbiota, and immune responses are connected.[2,29] Human studies examining the gut microbiota and allergies have shown conflicting results; however, an inverse association between the bacterial diversity of the gut microbiota in the first months of life and the development of eczema in early life was previously reported.[30]

In conclusion, this is the first study showing that infants exposed to an increased diversity of food items within the first year of life have a reduced risk of asthma, food allergy, and sensitization to food allergens up to age 6 years.

We thank all the fieldworkers and other PASTURE/EFRAIM team members.

Clinical implications: An increased diversity of complementary foods introduced within the first year of life might have a protective effect on the development of allergic diseases, such as asthma, in children.

REFERENCES

1. Payne AN, Chassard C, Banz Y, Lacroix C. The composition and metabolic activity of child gut microbiota demonstrate differential adaptation to varied nutrient loads in an in vitro model of colonic fermentation. FEMS Microbiol Ecol 2012;80:608-23.
2. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A 2010;107: 14691-6.
3. Frei R, Lauener RP, Crameri R, O'Mahony L. Microbiota and dietary interactions: an update to the hygiene hypothesis? Allergy 2012;67:451-61.
4. West CE, Videky DJ, Prescott SL. Role of diet in the development of immune tolerance in the context of allergic disease. Curr Opin Pediatr 2010;22:635-41.
5. Agostoni C, Decsi T, Fewtrell M, Goulet O, Kolacek S, Koletzko B, et al. Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 2008;46:99-110.
6. Nwaru BI, Takkinen HM, Niemela O, Kaila M, Erkkola M, Ahonen S, et al. Timing of infant feeding in relation to childhood asthma and allergic diseases. J Allergy Clin Immunol 2012;131:78-86.
7. Alm B, Aberg N, Erdes L, Mollborg P, Pettersson R, Norvenius SG, et al. Early introduction of fish decreases the risk of eczema in infants. Arch Dis Child 2009;94:11-5.
8. Katz Y, Rajuan N, Goldberg MR, Eisenberg E, Heyman E, Cohen A, et al. Early exposure to cow's milk protein is protective against IgE-mediated cow's milk protein allergy. J Allergy Clin Immunol 2010;126:77-82.e1.
9. Sariachvili M, Droste J, Dom S, Wieringa M, Hagendorens M, Stevens W, et al. Early exposure to solid foods and the development of eczema in children up to 4 years of age. Pediatr Allergy Immunol 2010;21:74-81.
10. Snijders BE, Thijs C, van Ree R, van den Brandt PA. Age at first introduction of cow milk products and other food products in relation to infant atopic manifestations in the first 2 years of life: the KOALA Birth Cohort Study. Pediatrics 2008;122:e115-22.
11. Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. J Allergy Clin Immunol 2008;122:984-91.
12. Koplin JJ, Osborne NJ, Wake M, Martin PE, Gurrin LC, Robinson MN, et al. Can early introduction of egg prevent egg allergy in infants? A population-based study. J Allergy Clin Immunol 2010;126:807-13.
13. Hadis U, Wahl B, Schulz O, Hardtke-Wolenski M, Schippers A, Wagner N, et al. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. Immunity 2011;34:237-46.
14. Roduit C, Frei R, Loss G, Buchele G, Weber J, Depner M, et al. Development of atopic dermatitis according to age of onset and association with early-life exposures. J Allergy Clin Immunol 2012;130:130-6.e5.
15. von Mutius E, Schmid S. The PASTURE project: EU support for the improvement of knowledge about risk factors and preventive factors for atopy in Europe. Allergy 2006;61:407-13.
16. Roduit C, Wohlgensinger J, Frei R, Bitter S, Bieli C, Loeliger S, et al. Prenatal animal contact and gene expression of innate immunity receptors at birth are associated with atopic dermatitis. J Allergy Clin Immunol 2011;127: 179-85.e1.
17. Giulietti A, Overbergh L, Valckx D, Decallonne B, Bouillon R, Mathieu C. An overview of real-time quantitative PCR: applications to quantify cytokine gene expression. Methods 2001;25:386-401.
18. Depner M, Fuchs O, Genuneit J, Karvonen AM, Hyvarinen A, Kaulek V, et al. Clinical and epidemiologic phenotypes of childhood asthma. Am J Respir Crit Care Med 2013 [Epub ahead of print].
19. Filipiak B, Zutavern A, Koletzko S, von Berg A, Brockow I, Grubl A, et al. Solid food introduction in relation to eczema: results from a four-year prospective birth cohort study. J Pediatr 2007;151:352-8.
20. Sausenthaler S, Heinrich J, Koletzko S. Early diet and the risk of allergy: what can we learn from the prospective birth cohort studies GINIplus and LISAplus? Am J Clin Nutr 2011;94(Suppl):2012S-7S.
21. Zutavern A, Brockow I, Schaaf B, von Berg A, Diez U, Borte M, et al. Timing of solid food introduction in relation to eczema, asthma, allergic rhinitis, and food and inhalant sensitization at the age of 6 years: results from the prospective birth cohort study LISA. Pediatrics 2008;121:e44-52.
22. Greer FR, Sicherer SH, Burks AW. Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics 2008;121:183-91.
23. Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrlander C, et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med 2011;364:701-9.
24. Prescott SL, Smith P, Tang M, Palmer DJ, Sinn J, Huntley SJ, et al. The importance of early complementary feeding in the development of oral tolerance: concerns and controversies. Pediatr Allergy Immunol 2008;19:375-80.
25. Palomares O, Yaman G, Azkur AK, Akkoc T, Akdis M, Akdis CA. Role of Treg in immune regulation of allergic diseases. Eur J Immunol 2010;40:1232-40.
26. Illi S, von Mutius E, Lau S, Nickel R, Niggemann B, Sommerfeld C, et al. The pattern of atopic sensitization is associated with the development of asthma in childhood. J Allergy Clin Immunol 2001;108:709-14.
27. Kjaer HF, Eller E, Andersen KE, Host A, Bindslev-Jensen C. The association between early sensitization patterns and subsequent allergic disease. The DARC birth cohort study. Pediatr Allergy Immunol 2009;20:726-34.
28. Liu F, Weng D, Chen Y, Song L, Li C, Dong L, et al. Depletion of CD4+CD25+Foxp3+ regulatory T cells with anti-CD25 antibody may exacerbate the 1,3-beta-glucan-induced lung inflammatory response in mice. Arch Toxicol 2011;85:1383-94.
29. Macia L, Thorburn AN, Binge LC, Marino E, Rogers KE, Maslowski KM, et al. Microbial influences on epithelial integrity and immune function as a basis for inflammatory diseases. Immunol Rev 2012;245:164-76.
30. Wang M, Karlsson C, Olsson C, Adlerberth I, Wold AE, Strachan DP, et al. Reduced diversity in the early fecal microbiota of infants with atopic eczema. J Allergy Clin Immunol 2008;121:129-34.

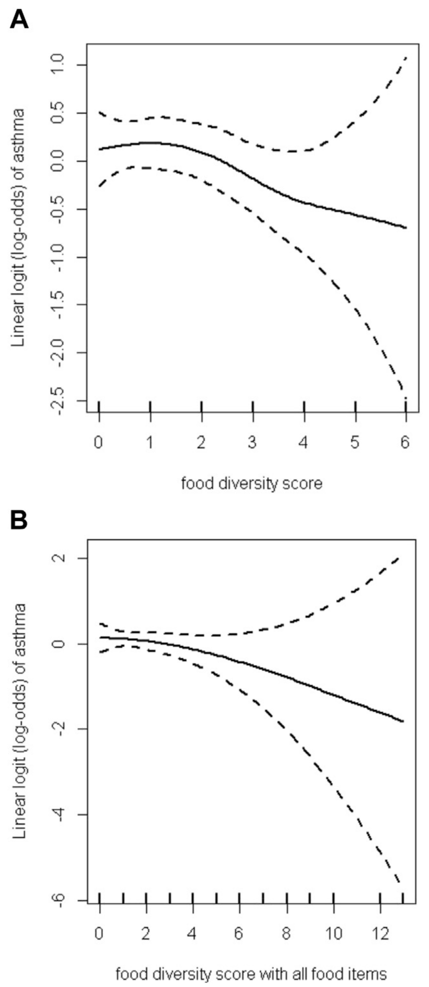

FIG E1. Association between increasing diversity of food introduced within the first 6 months of age and asthma. **A,** Diversity score with major food introduced within the first 6 months of life for the entire study population. **B,** Diversity score with all food items introduced within the first 6 months of life for the entire study population. The *solid line* represents the predicted value of asthma as a function of the score, and *dashed lines* represent the CI. The *y-axis* is the linear logit of asthma, and the values are centered on 0 (50/50 odds) and extended to both positive and negative values. All models are adjusted for farmer, center, duration of breast-feeding, parents with allergy, maternal education, sex, and siblings.

TABLE E1. Association between diversity score within the first year of life and allergic diseases and atopic sensitization, with additional adjustment for atopic dermatitis

	No.	OR*	95% CI
Asthma			
Food diversity score within 1st year			
0-3 items	7/35	**3.36**	**1.28-8.84**
4-5 items	32/243	**2.15**	**1.25-3.70**
6 items, reference	30/521	1	
Diversity score, continuous	69/799	**0.73**	**0.60-0.88**
Allergic rhinitis			
Diversity score major food within 1st year			
0-3 items	4/35	1.83	0.56-5.94
4-5 items	22/242	1.23	0.68-2.23
6 items, reference	34/518	1	
Diversity score, continuous	60/795	0.81	0.64-1.02
Doctor-diagnosed food allergy			
Food diversity score within 1st year			
0-3 items	7/32	**4.64**	**1.63-13.24**
4-5 items	22/239	1.68	0.90-3.14
6 items, reference	27/513	1	
Diversity score, continuous	56/784	**0.71**	**0.57-0.89**
Sensitization to food allergens at 4.5 or 6 y			
Food diversity score within 1st year			
0-3 items	5/24	**3.67**	**1.15-11.68**
4-5 items	21/163	1.44	0.77-2.71
6 items, reference	31/355	1	
Diversity score, continuous	57/542	**0.77**	**0.60-0.99**
Sensitization to inhalant allergens at 4.5 or 6 y			
Food diversity score within 1st year			
0-3 items	8/24	1.65	0.64-4.21
4-5 items	36/169	0.84	0.52-1.35
6 items, reference	78/362	1	
Diversity score, continuous	122/555	1.01	0.82-1.25

Boldface values are significant ($P > .05$).

*Adjusted for center, farmer, parents with allergy, sex, breast-feeding, siblings, maternal education, and atopic dermatitis up to 6 years.

TABLE E2. Association between diversity score within the first year of life and doctor's diagnosis of asthma*

	Doctor's diagnosis of asthma					
	Model 1		Model 2		Model 3	
	OR	95% CI	OR	95% CI	OR	95% CI
Food diversity score within 1st year						
0-3 items	**5.80**	**1.77-19.06**	**5.78**	**1.64-20.35**	**6.57**	**1.52-28.35**
4-5 items	**3.78**	**1.82-7.87**	**3.83**	**1.81-8.12**	2.55	0.95-6.86
6 items, reference	1		1		1	
Diversity score, continuous	**0.68**	**0.60-0.88**	**0.69**	**0.54-0.85**	**0.68**	**0.51-0.91**

Diversity scores with major food items are shown. Boldface values are significant ($P < .05$).
Model 1, Crude; *Model 2*, model 1 plus adjusted for center, farmer, parents with allergy, sex, breast-feeding, siblings, and maternal education; *Model 3*, model 2 plus exclusion of food allergy at 1 year (n = 17) and only with asthma: exclusion of at least 1 episode of obstructive bronchitis and/or asthma, both doctor diagnosed and reported at 1 year (n = 102).
*Definition of asthma based only on doctor's diagnosis of asthma between 3 and 6 years of age (n = 36).

TABLE E3. Association between diversity score within the first year of life and allergic diseases and atopic sensitization: analysis among a subgroup with exclusion of children with low diversity scores (n = 819)

		Model 1		Model 2			Model 3	
	No.	OR	95% CI	OR	95% CI	No.	OR	95% CI
Asthma								
Diversity score, continuous	67/819	**0.58**	**0.41-0.82**	**0.61**	**0.43-0.87**	37/702	0.82	0.49-1.37
Allergic rhinitis								
Diversity score, continuous	60/811	0.69	0.47-1.00	0.72	0.48-1.06	56/799	0.85	0.55-1.31
Doctor-diagnosed food allergy								
Diversity score, continuous	53/777	**0.58**	**0.40-0.86**	**0.58**	**0.39-0.86**	39/763	0.82	0.50-1.36
Sensitization to food allergens at 4.5 or 6 y								
Diversity score, continuous	55/554	0.82	0.54-1.24	0.86	0.55-1.34	53/543	0.84	0.53-1.32
Sensitization to inhalant allergens at 4.5 or 6 y								
Diversity score, continuous	122/568	1.05	0.76-1.44	1.14	0.81-1.60	119/556	1.21	0.85-1.73

Diversity scores with major food items are shown. Boldface values are significant ($P < .05$).

Model 1, Crude; *Model 2*, model 1 plus adjusted for center, farmer, parents with allergy, sex, breast-feeding, siblings, and maternal education; *Model 3*, model 2 plus exclusion of food allergy at 1 year (n = 17) and only with asthma: exclusion of at least 1 episode of obstructive bronchitis and/or asthma , both doctor diagnosed and reported at 1 year (n = 102).

TABLE E4. Association between introduction of single food items in the first year of life and asthma

	Asthma		
	Model 1	Model 2	Model 3
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Cow's milk			
3-12 mo	0.64 (0.40-1.04)	0.67 (0.40-1.14)	0.96 (0.49-1.90)
>12 mo, reference	1	1	1
Shop milk			
3-12 mo	0.69 (0.37-1.29)	0.55 (0.28-1.08)	0.96 (0.42-2.20)
>12 mo, reference (reference = no cow's milk)	1	1	1
Farm milk			
3-12 mo	0.61 (0.34-1.08)	0.82 (0.40-1.69)	0.89 (0.35-2.26)
>12 mo, reference (reference = no cow's milk)	1	1	1
Farm milk, unboiled			
3-12 mo	0.67 (0.28-1.59)	0.95 (0.37-2.40)	1.61 (0.50-5.16)
>12 mo, reference	1	1	1
Yogurt			
3-12 mo	**0.42 (0.25-0.70)**	**0.47 (0.26-0.84)**	0.55 (0.26-1.18)
>12 mo, reference	1	1	1
Other milk products			
3-12 mo	**0.40 (0.24-0.64)**	**0.37 (0.22-0.64)**	0.65 (0.32-1.33)
>12 mo, reference	1	1	1
Vegetables or fruits			
<6 mo	0.95 (0.59-1.54)	0.98 (0.53-1.82)	0.93 (0.41-2.11)
≥6 mo, reference	1	1	1
Fish			
3-12 mo	0.72 (0.44-1.15)	0.76 (0.44-1.31)	0.80 (0.39-1.63)
>12 mo, reference	1	1	1
Nuts			
3-12 mo	0.80 (0.45-1.42)	0.69 (0.36-1.34)	0.80 (0.36-1.80)
>12 mo, reference	1	1	1
Eggs			
3-12 mo	**0.46 (0.28-0.74)**	**0.47 (0.28-0.80)**	0.65 (0.33-1.31)
>12 mo, reference	1	1	1
Meat			
<9 mo	1.28 (0.75-2.18)	1.47 (0.80-2.70)	1.44 (0.67-3.12)
≥9 mo, reference	1	1	1
Cereals			
<9 mo	1.34 (0.78-2.28)	1.13 (0.62-2.05)	1.05 (0.64-2.17)
≥9 mo, reference	1	1	1
Bread			
<9 mo	1.10 (0.67-1.80)	1.06 (0.62-1.80)	1.17 (0.58-2.35)
≥9 mo, reference	1	1	1
Soja			
3-12 mo	1.42 (0.54-3.72)	1.27 (0.46-3.46)	1.45 (0.40-5.21)
>12 mo, reference	1	1	1
Margarine			
3-12 mo	1.44 (0.86-2.41)	1.16 (0.65-2.09)	1.17 (0.55-2.48)
>12 mo, reference	1	1	1
Butter			
3-12 mo	**0.49 (0.30-0.79)**	**0.45 (0.26-0.77)**	**0.43 (0.21-0.86)**
>12 mo, reference	1	1	1
Cake			
<9 mo	0.72 (0.44-1.19)	0.71 (0.40-1.26)	0.64 (0.30-1.34)
≥9 mo, reference	1	1	1
Chocolate			
3-12 mo	**0.47 (0.28-0.78)**	**0.45 (0.25-0.79)**	**0.41 (0.19-0.88)**
>12 mo, reference	1	1	1

Boldface values are significant ($P < .05$).

Model 1, Crude; *Model 2*, model 1 plus adjusted for center, farmer, parents with allergy, maternal education, sex, breast-feeding, siblings, atopic dermatitis up to 6 years, and maternal education; *Model 3*, model 2 plus exclusion of food allergy within the first year (n = 17) and of asthma within the first year (n = 102).

TABLE E5. Association between introduction of single food items in the first year of life and food allergy

	Food allergy		
	Model 1	Model 2	Model 3
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Cow's milk			
3-12 mo	0.71 (0.42-1.21)	0.64 (0.35-1.16)	0.98 (0.49-1.96)
>12 mo, reference	1	1	1
Shop milk			
3-12 mo	0.71 (0.36-1.42)	0.56 (0.26-1.20)	1.01 (0.44-2.31)
>12 mo, reference (reference = no cow's milk)	1	1	1
Farm milk			
3-12 mo	0.71 (0.38-1.33)	0.82 (0.35-1.91)	1.16 (0.44-3.08)
>12 mo, reference (reference = no cow's milk)	1	1	1
Farm milk, unboiled			
3-12 mo	1.46 (0.69-3.07)	1.30 (0.52-3.26)	1.46 (0.50-4.24)
>12 mo, reference	1	1	1
Yogurt			
3-12 mo	**0.39 (0.23-0.69)**	**0.39 (0.20-0.75)**	0.69 (0.31-1.60)
>12 mo, reference	1	1	1
Other milk products			
3-12 mo	**0.46 (0.27-0.79)**	**0.36 (0.19-0.67)**	0.54 (0.26-1.14)
>12 mo, reference	1	1	1
Vegetables or fruits			
<6 mo	1.39 (0.82-2.36)	1.08 (0.53-2.21)	1.01 (0.45-2.29)
≥6 mo, reference	1	1	1
Fish			
3-12 mo	**0.54 (0.32-0.91)**	**0.34 (0.18-0.66)**	**0.41 (0.19-0.88)**
>12 mo, reference	1	1	1
Nuts			
3-12 mo	0.55 (0.27-1.10)	0.55 (0.24-1.25)	0.71 (0.30-1.66)
>12 mo, reference	1	1	1
Eggs			
3-12 mo	**0.55 (0.32-0.-93)**	**0.50 (0.28-0.91)**	0.82 (0.40-1.69)
>12 mo, reference	1	1	1
Meat			
<9 mo	0.80 (0.46-1.38)	0.53 (0.27-1.05)	**0.43 (0.20-0.93)**
≥9 mo, reference	1	1	1
Cereals			
<9 mo	1.41 (0.78-2.55)	1.03 (0.51-2.10)	1.11 (0.50-2.46)
≥9 mo, reference	1	1	1
Bread			
<9 mo	0.69 (0.41-1.17)	0.77 (0.43-1.40)	0.70 (0.35-1.40)
≥9 mo, reference	1	1	1
Soja			
3-12 mo	2.27 (0.92-5.63)	1.80 (0.66-4.87)	1.92 (0.60-6.13)
>12 mo, reference	1	1	1
Margarine			
3-12 mo	1.09 (0.63-1.88)	0.78 (0.41-1.50)	0.87 (0.41-1.82)
>12 mo, reference	1	1	1
Butter			
3-12 mo	**0.51 (0.30-0.87)**	0.60 (0.32-1.11)	0.83 (0.39-1.75)
>12 mo, reference	1	1	1
Cake			
<9 mo	0.62 (0.35-1.08)	0.77 (0.40-1.49)	0.89 (0.43-1.85)
≥9 mo, reference	1	1	1
Chocolate			
3-12 mo	**0.56 (0.33-0.98)**	0.60 (0.32-1.12)	0.69 (0.34-1.42)
>12 mo, reference	1	1	1

Boldface values are significant ($P < .05$).

Model 1, Crude; *Model 2*, model 1 plus adjusted for center, farmer, parents with allergy, maternal education, sex, breast-feeding, siblings, and atopic dermatitis up to 6 years; *Model 3*, model 2 plus exclusion of doctor's diagnosis of food allergy within the first year (n = 17).

TABLE E6. Association between introduction of single food items in the first year of life and sensitization to food allergens

	Sensitization to food allergens		
	Model 1	Model 2	Model 3
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Cow's milk			
3-12 mo	0.77 (0.46-1.31)	0.83 (0.46-1.50)	0.90 (0.49-1.67)
>12 mo, reference	1	1	1
Shop milk			
3-12 mo	0.73 (0.35-1.51)	0.73 (0.32-1.64)	0.88 (0.38-2.02)
>12 mo, reference (reference = no cow's milk)	1	1	1
Farm milk			
3-12 mo	0.80 (0.44-1.45)	0.80 (0.38-1.66)	0.83 (0.39-1.77)
>12 mo, reference (reference = no cow's milk)	1	1	1
Farm milk, unboiled			
3-12 mo	0.60 (0.23-1.55)	0.53 (0.17-1.64)	0.55 (0.18-1.71)
>12 mo, reference	1	1	1
Yogurt			
3-12 mo	**0.47 (0.26-0.84)**	0.69 (0.35-1.39)	0.72 (0.35-1.50)
>12 mo, reference	1	1	1
Other milk products			
3-12 mo	**0.50 (0.29-0.88)**	0.67 (0.36-1.27)	0.71 (0.36-1.37)
>12 mo, reference	1	1	1
Vegetables or fruits			
<6 mo	0.94 (0.56-1.61)	1.03 (0.52-2.02)	1.04 (0.52-2.06)
≥6 mo, reference	1	1	1
Fish			
3-12 mo	**0.49 (0.29-0.84)**	0.69 (0.38-1.26)	0.71 (0.38-1.34)
>12 mo, reference	1	1	1
Nuts			
3-12 mo	0.70 (0.35-1.39)	0.48 (0.23-1.01)	0.48 (0.23-1.02)
>12 mo, reference	1	1	1
Eggs			
3-12 mo	0.68 (0.40-1.17)	0.70 (0.38-1.29)	0.70 (0.37-1.32)
>12 mo, reference	1	1	1
Meat			
<9 mo	0.72 (0.41-1.25)	0.97 (0.51-1.84)	1.04 (0.54-2.01)
≥9 mo, reference	1	1	1
Cereals			
<9 mo	0.79 (0.45-1.36)	0.71 (0.38-1.33)	0.68 (0.36-1.29)
≥9 mo, reference	1	1	1
Bread			
<9 mo	1.08 (0.62-1.85)	0.89 (0.48-1.65)	0.93 (0.49-1.77)
≥9 mo, reference	1	1	1
Soja			
3-12 mo	1.05 (0.31-3.58)	1.32 (0.36-4.84)	1.47 (0.39-5.50)
>12 mo, reference	1	1	1
Margarine			
3-12 mo	0.83 (0.49-1.41)	0.71 (0.38-1.32)	0.68 (0.36-1.29)
>12 mo, reference	1	1	1
Butter			
3-12 mo	0.83 (0.48-1.46)	0.73 (0.39-1.39)	0.85 (0.43-1.66)
>12 mo, reference	1	1	1
Cake			
<9 mo	**0.46 (0.26-0.84)**	**0.30 (0.15-0.62)**	**0.31 (0.15-0.64)**
≥9 mo, reference	1	1	1
Chocolate			
3-12 mo	**0.37 (0.21-0.67)**	**0.39 (0.21-0.75)**	**0.36 (0.19-0.70)**
>12 mo, reference	1	1	1

Boldface values are significant ($P < .05$).

Model 1, Crude; *Model 2*, model 1 plus adjusted for center, farmer, parents with allergy, maternal education, sex, breast-feeding, siblings, and atopic dermatitis up to 6 years; *Model 3*, model 2 plus exclusion of doctor's diagnosis of food allergy within the first year (n = 17).