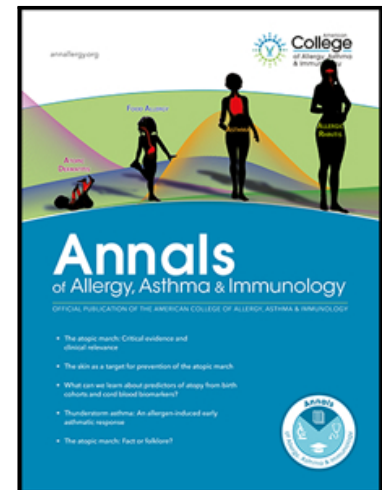


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Inverse associations between food diversity in the 2nd year of life and allergic diseases

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Keywords

Allergy, childhood, food, diet diversity, dairy, allergy prevention

Abbreviations

FDS: food diversity score

LCA: latent class analysis

OR : Odds ratios

aOR: Adjusted odds ratio

PASTURE: Protection against Allergy- Study in Rural Environments

Author Contribution Statements

Martha Stampfli: analysis and interpretation of data, and drafted the article

Remo Frei: analysis and interpretation of data, reviewed the article critically

Amandine Divaret-Chauveau: PI of the French centre, acquisition of data and reviewed the article critically

Elisabeth Schmausser-Hechfellner: acquisition of data and reviewed the article critically

Anne M. Karvonen: acquisition of data and reviewed the article critically

Juha Pekkanen: PI of the Finnish centre, design the study, and reviewed the article critically

Josef Riedler: PI of the Austrian centre, design the study, acquisition of data and reviewed the article critically

Bianca Schaub: design the study, acquisition of data and reviewed the article critically

Erika von Mutius: PI of the German centre, design the study, and reviewed the article critically

Roger Lauener: design the study, and reviewed the article critically

Caroline Roduit: design, acquisition of data, analysis and interpretation of data, and drafted the article

All the authors have given final approval of this submitted version and agree to be accountable for all aspects of the work related to its accuracy or integrity.

Background: The influence of diet in early childhood on later allergic diseases is currently a highly debated research topic. We and others have suggested that an increased diet diversity in the first year of life has a protective effect on the development of allergic diseases.

Objective: The aim of this follow-up study was to investigate associations between diet in the 2nd year of life and later allergic diseases.

Methods: 1014 children from rural areas in 5 European countries (PASTURE birth cohort) were included. Information on feeding practices in their 2nd year of life and allergic diseases were collected up to age 6 years. Multivariate logistic regressions were performed, with different models considering reverse causality, such as excluding children with a positive sensitization to egg and/or cow's milk at the age of 1 year.

Results: An increased food diversity score during the 2nd year of life, was negatively associated with the development of asthma. Consumption of dairy products and eggs in the 2nd year of life showed an inverse association with reported allergic outcomes. Consumption of butter was strongly associated with protection against asthma and food sensitization. Egg was inversely associated to atopic dermatitis (OR 0.17, 95% CI 0.04-0.77). Yogurt and cow's milk were inversely associated against food allergy (OR for yogurt 0.05, 95% CI 0.01-0.55; OR for cow's milk 0.31, 95% CI 0.11-0.89).

Conclusion: Increased food diversity in the 2nd year of life is inversely associated with the development of asthma, and consumption of dairy products might have a protective effect on allergic diseases.

Introduction

It is well documented that environmental factors in early life play an important role in the development of allergy in childhood. However, contributing factors, such as nutrition, still require further understanding. As an international prospective long-term birth cohort, the Protection Against Allergy: Study in Rural Environments (PASTURE) study contributes to this goal.¹ The PASTURE study was designed to investigate rural factors associated with protection against allergies and to determine at which period in early life they may be most effective. A more diverse diet in the first year of life has already been shown to be protective against allergies in European countries.²⁻⁵

Numerous prospective studies and reviews have demonstrated that, despite potential risks of bacterial infection, early introduction of unprocessed cow's milk has a preventive association with the development of allergic diseases.^{6,7,8,9,10} However, none of these studies were randomized clinical trials. The protective effect of unprocessed farm milk intake on asthma at school age was suggested to be related to a higher intake of Omega-3 PUFAs.¹¹ With regard to other dairy products, consumption of cheese and yogurt in early life were shown to be protective against the development of allergic diseases.^{4,5,12} Conversely, a French study found that butter intake was associated with an increased prevalence of asthma.¹³

In a discussion on the effect of diet on the development of allergic conditions, a pediatric review highlighted that a healthy diet rich in antioxidants and omega-3 fatty acids might significantly reduce the occurrence of allergic diseases.¹⁴ Furthermore, Grimshaw et al. showed that a dietary pattern with high intake of fruits, vegetables and home-made foods in the first year of life was associated with less food allergy at the age of two years.¹⁵ In a meta-analysis from 2017 on the effects of fruit and vegetable intake on the risk of developing asthma, wheezing and immune responses, most of the examined studies confirmed a protective effect against asthma.¹⁶

The interaction between diet and the gut microbiome with its metabolites has been described as one underpinning mechanism mediating the protective effect of nutrition on allergic diseases. Among these metabolites are SCFAs (short chain fatty acids), such as acetate, propionate and butyrate, produced by the gut microbiome by fermentation of fiber. SCFAs have broad anti-inflammatory effects which are associated with protection against allergies and inflammatory bowel disease.^{17,18}

We previously found a protective association between intake of cow's milk products, especially yogurt, and an increased food diversity within the first year of life with later allergic disease.^{4,5} The aim of this present study was to follow up these children and to document any association between diet in the 2nd year of life and later allergic diseases up to age 6 years. Currently, dietary interventions for primary allergy prevention have focused on the first year of life. However, the introduction of solid foods to an infant's diet can be challenging for parents, especially to implement the recommendation of a more diverse diet in early life. Therefore, nutrition in the 2nd year of life might be of considerable interest as a continuous strategy for allergy prevention. To our knowledge, so far there have been no studies analysing the diet diversity beyond the first year of life, which makes this prospective study unique.

Methods

Study design

As a prospective birth cohort study, the PASTURE study included 1133 children from rural areas in Austria, Finland, France, Germany and Switzerland. Initially, between August 2002 and March 2005, pregnant women were recruited in their third trimester of pregnancy. Those living on a farm were allocated to the farm group, the rest was held as reference group. In all five countries the study went through the local ethics committee, written informed consent was gained from all parents. Detailed study design is available elsewhere.¹

Study population

1014 children of the PASTURE birth cohort with data available on allergic diseases up to age 6 years, nutrition within the 2nd year of age, parental allergic history, number of siblings, pets, mode of delivery, pregnancy smoking status, as well as breastfeeding during the first year of life, were included in these analyses.

Exposures definition

Data on nutrition in the 2nd year of life were collected in questionnaires at 1.5 and 2 years of age. The following food items were reported in those questionnaires and therefore included in our analyses: yogurt, butter (only butter, no margarines), cow's milk, cheese, egg, meat, fish, nuts, vegetables, fruits, and cereals. A food diversity score (FDS), which included these items, was calculated as the number of different food items included in the child's diet within the 2nd year of life, resulting in a score from 0 to 11. The FDS item numbers differed between the 1st and 2nd year (15 and 11 items, respectively), due to differences in food items reported at these periods of time. For the categorization of the FDS, we performed latent class analysis (LCA). Doing LCA, the best model was with 2 classes, ≤ 8 or > 8 . Therefore, we decided to

categorize the FDS with one class ≤ 8 , but still keeping 2 more categories for FDS > 8 (FDS 9-10, and FDS 11), in order to include more information.

Outcomes definition

Atopic dermatitis and food allergy were defined as a doctor-diagnosis reported in the year 4, 5- and 6 year questionnaires. Children were defined as having asthma when parents reported that the child had either a doctor-diagnosis of asthma or at least two episodes of doctor-diagnosis of spastic, obstructive or asthmatic bronchitis in the 4, 5 or 6 year-questionnaire, independently of a history of asthma in the first 3 years.

Atopic sensitization at the age of 6 years, was defined as the presence of specific IgE to foods (hen's egg, cow's milk, peanut, hazelnut, carrot and wheat flour) or inhalants (*Dermatophagoides pteronyssinus*, *D. farinae*, alder, birch, hazel, grass pollen, rye, mugwort, plantain, cat, horse, dog, alternaria) with levels of ≥ 0.35 kU/l.

Statistical analysis

Logistic regressions were performed to test for associations between food exposure and atopic dermatitis, food allergy, asthma and atopic sensitization. Odds ratios (OR), adjusted for the following potential confounders: gender, center, farming status, parental allergic status, mode of delivery, number of siblings, smoking during pregnancy, contact with pets (cats and dogs) in the first year of life, breastfeeding and food diversity score at 12 months as previously described, were calculated.⁵ Confounders were selected depending on their effect on the OR (change of more than 10%). However, some variables such as farmer, gender, breastfeeding are well known as potential confounders, and were therefore still kept in the adjusted model. For all the analyses, we excluded children with a diagnosis of atopic dermatitis up to the age of 1 year (n=147) and/or children with sensitization to egg and/or cow's milk at the age of one year (specific IgE ≥ 0.35 kU/l) (n=60) in order to consider reverse causality. Those two

allergens were chosen as they represent the two most frequent food allergies in early life, other sensitizations were much less prevalent and therefore not considered for exclusion. Moreover, a sensitivity analysis in a subgroup of children with no specific exclusion of cow's milk products in the 1st year of life was performed.

Statistical analysis was performed using STATA (version 12.1, Stata Corp LP, College Station, TX, USA) and SAS software, version 9.4 (SAS Institute, Inc., Cary, NC). Statistical significance was accepted at $p < 0.05$.

Results

We included 1014 children, out of the 1133 at baseline, in the analyses and divided them in subgroups according to their food diversity score (FDS) within the 2nd year of life (Table 1). Three food diversity patterns were selected: consumption of 8 or less food items, consumption of 9 to 10 items and consumption of all 11 food items. Within these groups, gender, farmer status, having at least one sibling, exposure to pets and smoking status in pregnancy were equally scattered. However, atopic parents were more frequent in the low food diversity group ($FDS \leq 8$) and breastfeeding was least prevalent in this group. Differences between centers were observed, French participants having the highest percentage of participants in the $FDS \leq 8$. Correlation with the FDS from the 1st year of life showed that infants who had been in the low and medium FDS mostly remained in the low FDS in the 2nd year, but children from the 6-15 items score progressed into the higher FDS. The most discriminating differences between the three groups were seen in butter, egg, fish, nuts, cow's milk, cheese and cereals consumption whereas the other items seemed to be consumed by the majority in the 3 groups.

The outcomes atopic dermatitis, asthma and food allergy were less prevalent in the medium and high FDS groups.

Food diversity in the 2nd year of life and allergic diseases

Among children with no sensitization to cow's milk and/or egg, and no atopic dermatitis in the 1st year of life, an inverse and linear relationship was observed between an increased diet diversity in the 2nd year of life, reflected by an increasing score (FDS as continuous variable), and atopic dermatitis and asthma, (Figure 1, A-B). We found a significant reduction of 25% for the development of asthma with each additional food item introduced in the 2nd year of life. This pattern was not observed for the relationship between FDS and food allergy (Figure 1, C). Children with a low food diversity score in the 2nd year of life (≤ 8 food items) had an increased risk of developing asthma, among children with no sensitization to cow's milk or egg at 1 year (Table 2). After also excluding children with atopic dermatitis in the 1st year of life, an increased risk for asthma remained with a low food diversity, however, only a tendency was observed for atopic dermatitis. As four of the food items included in the FDS are dairy products, we calculated a FDS without dairy products (0 to 7). The analyses, again among children with no sensitization to cow's milk and/or egg, and no atopic dermatitis in the 1st year of life, showed still a negative association between this new FDS (without dairy products) and atopic dermatitis and asthma (OR for atopic dermatitis: 0.70, 95% CI: 0.50-0.97 and after adjustment: 0.79, 95% CI: 0.54-1.14; for asthma: 0.74, 95% CI: 0.50-1.10, and after adjustment: 0.69, 95% CI: 0.45-1.08).

Additionally, we combined the FDS in the 1st and 2nd year of life in a new variable with 4 categories (children with a low or high FDS at 1 year and a low or high FDS at 2 years) (Table 3). For these analyses, only children with a sensitization to egg and/or cow's milk at 1 year were excluded. Additional exclusion of children with AD in the 1st year enabled us to perform the analyses due to the small numbers. The strongest positive association with atopic dermatitis, asthma and food allergy was observed in children with low FDS at both time

points. Nevertheless, a low FDS in the 2nd year of life was positively associated with allergic diseases, independently of the FDS in the 1st year.

Single food items in the 2nd year of life and allergic diseases

The subsequent analyses were performed excluding children with atopic dermatitis within the first year of life and excluding children with sensitization to egg and/or cow's milk (IgE $\geq$ 0.35 kU/l). A negative association between egg and cereals consumption in the 2nd year of life and atopic dermatitis was observed (Figure 2, A). Butter demonstrated a tendency towards a negative association with atopic dermatitis.

Regarding asthma, we found an inverse association between butter towards asthma (OR 0.42, 95% CI: 0.20-0.86) (Figure 2, B). Nuts and cow's milk showed protective tendencies.

Yogurt and cow's milk were inversely associated with food allergy (OR for yogurt 0.05, 95% CI: 0.01-0.55; OR for cow's milk 0.31, 95% CI: 0.11-0.89) (Figure 2, C). Concerning food sensitization, butter was protectively associated, nuts showed a protective tendency and cow's milk and cereals were positively associated (Figure 2, D).

Analyses among children with no exclusion of cow's milk products in the 1st year of life

Cow's milk allergy is the most frequent food allergy in early life and is associated with other allergic diseases. Therefore, we performed analyses in a subgroup of children, for whom no specific exclusion of cow's milk was reported in the 1st year of life. This subgroup encompassed a total of 885 children (78%) and was done to reduce the potential effect of reverse causation. The calculated adjusted odds ratio showed an inverse association between egg and atopic dermatitis (Table 4). Egg also appeared to have a protective tendency against food sensitization, however numbers were small. Yoghurt consumed in the 2nd year of life was negatively associated with food allergy. Butter showed an evident protective association

with food sensitization (aOR 0.51, 95% CI 0.28-0.92). No associations were observed with the outcome asthma.

Discussion

In this study, we show that an increased food diversity during the 2nd year of life is negatively associated with the development of asthma, suggesting a protective effect. Also a trend for a negative association between an increasing food diversity during the 2nd year of life and atopic dermatitis was observed. Secondly, our findings highlight the fact that dairy products, as well as egg, consumed within the 2nd year of life might have preventive effects against the development of allergic diseases by 6 years of age.

Our results are in line with previous studies showing that consumption of dairy products in early life have a protective association with the development of allergies.^{6,7,8,10,12} A discrepancy between the effect of cow's milk on food allergy (negative association) and food sensitization (positive association) was found, which might be due to a high sensitization rate without clinically evident food allergy. Allergen sensitization itself is a complex host-immune factor interaction and, sensitization alone does not implicate allergy.¹⁹ Moreover, in the LEAP study, it was shown that an early introduction to peanut led to better peanut allergy prevention than avoidance, and this also among sensitized children.²⁰ To date, most of these studies have investigated the benefits of unprocessed cow's milk. Here, we propose that dairy products in general have a beneficial correlation with allergic diseases. Further investigation in relation to processing methods and their impact on milk components will be needed. As early as 2003, Wijga et al. stated that asthma was less prevalent in 3 year old children receiving full cream milk and butter daily.²¹ A previous Finnish longitudinal study, analyzing food habits in children aged 3-18 years, has shown that atopic children consumed more margarine than nonatopic subjects.²² Our overall cheese item showed an inverse tendency with atopic

dermatitis and asthma, thus supporting the results of Nicklaus et al. who, among PASTURE children, found negative associations between cheese consumption from 12-18 months and atopic dermatitis and food allergy.¹² Additionally, results from the PASTURE group revealed that raw farm milk consumption was associated with an increased expression of innate immunity receptors at 1 year of age.²³

Our results are consistent with the continued beneficial effect of an increased diet diversity on allergic diseases, as we previously showed for the first year of life.⁵ After adjustment with the FDS from the first year of life and combined analysis of FDS at 1 and 2 years of age, we can state that an increased diet diversity in the 2nd year of life might be considered as an independent protective factor on the development of allergic diseases, especially on asthma.

A limitation of this study consists in the selected rural study population. It thus remains unclear if these results can be extrapolated to urban children, since allergies are provenly a multifactorial disease. The strengths of the study are the prospective and international study design, the detailed data on nutrition and an accurate follow-up. Although reverse causality cannot be completely excluded, this potential bias, present in all analyses of associations between diet and allergic diseases, was minimized by excluding children with egg and cow's milk sensitization and/or those having atopic dermatitis at age 1 year. We also performed additional analyses, excluding children with specific food exclusions. By performing the analyses in those subgroups of children, we better take into account the reverse causality, but it results in a loss of statistical power.

In terms of the links between gut microbiome, short chain fatty acids and allergy, Arrieta et al., analyzing participants of the CHILD cohort in Canada, have identified harmful gut microbiota by deciphering microbes who were present in children at risk for asthma, showing a gut dysbiosis in their first 100 days of life.²⁴ Von Mutius highlighted the strengths of the microbiome as counteractor of asthma as illustrated by farm and Amish children.²⁵ Our

results from PASTURE data showed that high levels of SCFAs in feces of children at 1 year were associated with less atopic sensitization.¹⁷ Interestingly, this is the same population that we included in this study and results are consistent, in the sense that dairy products, which contain SCFAs, especially butyrate, have a protective effect against allergy. Moreover, we recently showed that diverse feeding patterns, as well as consumption of cow's milk and egg directly from a farm, are related to the maturation of the gut microbiome.²⁶ In a more international comparison, de Filippo et al. revealed noteworthy differences in the gut microbiota of Italian and African children.²⁷ A clearly higher amount of SCFAs was found in the African cohort, whose diet was low in fat and animal protein, but rich in fiber. SCFAs derived from skimmed milk have been found to have regulatory effects on cytokines and of being anti-inflammatory.²⁸ Moreover, in a mouse model, high fiber feeding was shown to be protective against food allergy.²⁹ Hence, the books are still open over SCFAs and their potential for modifying multiple diseases through regulation of host metabolism, cell proliferation and the immune system.^{30,31}

In conclusion, our results from this cohort study show inverse associations between an increased food diversity in the 2nd year of life and asthma. Also, dairy product consumption in the 2nd year of life was associated with a reduced risk of allergic diseases. However, to completely exclude reverse causality, randomized clinical trials need to be conducted. This study emphasizes the potential protective association of diet diversity against asthma, and likely against other allergic diseases, beyond the first year of life. As the first year of life holds challenges with diverse food introduction, the second year of life might still be a window of opportunity concerning diet diversity or diet recommendations for allergy prevention. We would like to underscore the importance of a continuous feeding practice of all types of food even in atopic families.

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Table 1: Patient characteristics, classified by food diversity score groups

			Food diversity score in the 2nd year of life						
	Total		FDS ≤ 8		FDS 9-10		FDS 11		
Total	N=1014	%	N=7	%	N=44	%	N=32	%	p-value
			3	%	0	%	9	%	
Gender: female	495/101	48.9	32/7	43.8	218/4	49.6	162/3		
	2	%	3	%	40	%	28	49.4%	0.653
Farmer: yes	486/101	47.9	33/7	45.2	226/4	51.4	178/3		
Atopic parents* (at least one parent)	4	%	3	%	40	%	29	54.1%	0.368
	536/100	53.1	49/7	67.1	231/4	52.7	153/3		
	9	%	3	%	38	%	29	46.5%	0.005
Siblings: yes (at least one)	641/101	63.2	48/7	65.8	287/4	65.2	208/3		
	4	%	3	%	40	%	29	63.2%	0.928
C-Section: yes	176/100	17.5	12/7	16.7	71/43	16.3	58/32		
Pets (dogs and/or cats) in 1st year: yes	5	%	2	%	6	%	6	17.8%	0.859
	570/997	57.2	34/7	47.2	263/4	60.3	196/3		
		%	2	%	36	%	26	60.1%	0.100
Breastfeeding: yes	900/996	90.4	59/7	81.9	401/4	92.0	304/3		<0.001
		%	2	%	36	%	25	93.5%	<0.001
Center									
Austria	206/101	20.3		11.0	64/44	14.5	97/32		
	4	%	8/73	%	0	%	9	29.5%	
Switzerland	213/101	21.0	11/7	15.1	90/44	20.5	92/32		
	4	%	3	%	0	%	9	28.0%	
France	187/101	18.4	29/7	39.7	83/44	18.9	18/32		
	4	%	3	%	0	%	9	5.5%	
Germany	231/101	22.8	14/7	19.2	96/44	21.8	95/32		
	4	%	3	%	0	%	9	28.9%	
Finland	177/101	17.5	11/7	15.1	107/4	24.3	27/32		
	4	%	3	%	40	%	9	8.2%	
Smoking pregnancy: yes	133/101	13.1		11.0	60/44	13.6	33/32		
	4	%	8/73	%	0	%	9	10.0%	0.301
Food diversity score in 1st year of life									0.002
0-1 items	18/995	1.8%	2/72	2.8%	7/433	1.6%	3/325	0.9%	
			11/7	15.3	20/43		13/32		
2-5 items	61/995	6.1%	2	%	3	4.6%	5	4.0%	
		92.1	59/7	81.9	406/4	93.8	309/3		
6-15 items	916/995	%	2	%	33	%	25	95.1%	
Food items									
Yoghurt	998/100	99.0	65/7	89.0	438/4	99.6	329/3	100.00	<0.001
	8	%	3	%	40	%	29	%	1
Butter		88.8	32/7	43.8	377/4	85.7	329/3	100.00	<0.001
	860/969	%	3	%	40	%	29	%	1
Egg		98.0	56/7	76.7	438/4	99.6	329/3	100.00	<0.001
	973/993	%	3	%	40	%	29	%	1
Meat	1010/10	99.9	72/7	98.6	440/4	100.0	329/3	100.00	
	11	%	3	%	40	%	29	%	0.005
Fish	919/101	90.6	50/7	68.5	384/4	87.3	329/3	100.00	<0.001
	4	%	3	%	40	%	29	%	1
Nuts		57.4			150/4	34.1	329/3	100.00	<0.001
	515/897	%	7/73	9.6%	40	%	29	%	1
Vegetables	1008/10	99.5	69/7	94.5	439/4	99.8	329/3	100.00	<0.001
	13	%	3	%	40	%	29	%	1

Fruits	1012/1014	99.8 %	71/73	97.3 %	440/440	100.0 %	329/329	100.00 %	<0.001
Cereals	913/984	92.8 %	48/73	65.8 %	405/440	92.1 %	329/329	100.00 %	<0.001
Cow's milk	801/980	81.7 %	31/73	42.5 %	339/440	77.1 %	329/329	100.00 %	<0.001
Cheese	918/1013	90.6 %	40/73	54.8 %	403/440	91.6 %	329/329	100.00 %	<0.001
Reported allergic outcomes									
Atopic dermatitis 3-6years	133/853	15.6 %	18/58	31.0 %	52/382	13.6 %	31/278	11.2 %	<0.001
Asthma 3-6 years	77/866	8.9 %	15/60	25.0 %	35/392	8.9 %	18/279	6.5 %	<0.001
Food allergy 3-6 years	36/824	4.4 %	8/52	15.4 %	0/1037	2.7 %	9/279	3.2 %	<0.001
Food sensitization 6 years	280/724	38.7 %	17/44	38.6 %	137/388	40.5 %	90/226	39.8 %	0.964
Inhalant sensitization 6 years	274/724	37.8 %	18/44	40.9 %	117/388	34.6 %	89/226	39.4 %	0.436
Any sensitization 6 years	387/724	53.5 %	19/44	56.8 %	179/388	53.0 %	122/226	54.0 %	0.882

FDS= Food Diversity Score

*at least one parent (mother or father) with history of asthma, hay fever or atopic dermatitis

Table 2: Food diversity in the second year of life and allergic outcomes

	Model 1:							Model 2:							Model 3:												
	N	%	OR	95% CI	aOR*	95% CI		N	%	OR	95% CI	aOR*	95% CI		N	%	OR	95% CI	aOR*	95% CI							
Eczema 3-6y																											
DS category																											
≤8 items	15/51	29.4	3.21	1.57	6.54	2.05	0.9	4.6	8/41	19.5	2.2	0.9	5.4	1.69	0.6	5	0	4.4	7/36	19.4	2.1	0.8	5.51	1.57	0.5	7	4.33
9-10 items	51/36	14.0	1.2	0.7	2.03	1.04	0.6	1.7	34/32	10.8	1.0	0.6	1.8	0.90	0.5	0	1.6	34/31	10.2	1.1	0.6	1.93	0.88	0.4	8	1.60	
11 ref.	30/26	11.5	1		1.00				24/24	9.8	1.0			1.00				23/23	10.0	1.0							
DS continuous			0.77	0.65	0.93	0.89	0.73	1.0			0.84	0.67	1.0	0.92	0.71	1.1			0.84	0.66	1.07	0.94	0.72	1.22			
Food allergy 3-6y																											
DS category																											
≤8 items	13/54	24.1	4.57	2.07	10.11	3.37	1.37	8.27	7/41	17.1	3.10	1.18	8.16	3.27	1.15	9.36		6/36	16.7	3.02	1.08	8.44	3.01	1.01	9.41		
9-10 items	34/37	9.9	1.4	0.7	2.64	1.34	0.7	2.5	30/33	89.4	1.4	0.7	2.8	1.43	0.7	2.8		29/31	9.2	1.5	0.7	2.94	1.50	0.7	4	3.04	
11 ref.	17/26	6.5	1.0		1.00				15/24	6.2	1.0			1.00				14/22	6.2	1.0							
DS continuous			0.67	0.54	0.83	0.73	0.57	0.92			0.76	0.59	0.97	0.76	0.57	0.99			0.75	0.58	0.98	0.76	0.57	1.01			
Atopic dermatitis 3-																											
DS category																											
≤8 items	6/46	13.0	4.22	1.42	12.49	2.89	0.8	9.9	2/37	5.4	1.9	0.3	9.7	1.81	0.3	10.9		2/33	6.1	2.0	0.4	10.3	1.87	0.3	0	11.6	
9-10 items	8/35	2.3	0.6	0.2	1.71	0.49	0.1	1.3	6/31	1.9	0.6	0.2	1.9	0.58	0.1	1.9		5/30	1.7	0.5	0.1	1.70	0.44	0.1	2	1.56	
11 ref.	9/26	3.4	1		1.00				7/24	2.9	1.0			1.00				7/22	3.1	1.0							
DS continuous			0.67	0.54	0.90	0.75	0.5	1.0			1.0	0.6	1.7					1.0	0.6	1.85	1.20	0.6	2.31				

7 0 3 5 5 1 9 5 0 2

FDS=Food
Diversity
Score, 11 food

items: yogurt, butter, cow's milk, cheese, egg, meat, fish, nuts, vegetables, fruits and cereals
Model 1: exclusion of children with pos. IgE to cow's milk and/or to egg at the age of 1 year (≥ 0.35 kU/l); Model 2: exclusion of children with AD in the 1st year of life
Model 3: exclusion of children with both pos. IgE to cow's milk and/or to egg at the age of 1 year (≥ 0.35 kU/l) AND children with AD in the 1st year of life
*adjusted for gender, center, farmer status, atopic parents, contact to pets in the 1st year of life, breastfeeding, smoking during pregnancy, FDS in 1st of life

Table 3: Food diversity scores in the 1st and 2nd year of life combined and allergic diseases

	N	%	OR	95%CI
Atopic dermatitis 3-6y				
<i>FDS category</i>				
- FDS 1y low & FDS 2y low	3/6	50.0	6.82	(1.35-34.39)
- FDS 1y low & FDS 2y high	3/21	14.3	1.14	(0.33-3.95)
- FDS 1y high & FDS 2y low	11/44	25.0	2.27	(1.10-4.68)
- FDS 1y high & FDS 2y high, ref	77/602	12.8	1.00	
Asthma 3-6y				
<i>FDS category</i>				
- FDS 1y low & FDS 2y low	2/6	33.3	6.00	(1.07-33.62)
- FDS 1y low & FDS 2y high	3/21	14.3	2.00	(0.57-7.04)
- FDS 1y high & FDS 2y low	10/47	21.3	3.24	(1.52-6.93)
- FDS 1y high & FDS 2y high, ref	47/611	75.8	1.00	
Food allergy 3-6y				
<i>FDS category</i>				
- FDS 1y low & FDS 2y low	2/4	50.0	33.88	(4.50-255.02)
- FDS 1y low & FDS 2y high	0/19	0	NA	
- FDS 1y high & FDS 2y low	4/41	9.8	3.66	(1.17-11.44)
- FDS 1y high & FDS 2y high, ref	17/593	2.9	1.00	

FDS 1 yr and 2 yr combined:

FDS 1y low = ≤ 3 food items, FDS 1y high = > 3 food items

FDS 2y low = ≤ 8 , FDS 2y high = > 8

Children with both pos. IgE to cow's milk and/or to egg at the age of 1 year (≥ 0.35 kU/l) were excluded.

Table 4: Associations between food items and allergic diseases among children with no exclusion of cow's milk products in the 1st year of life

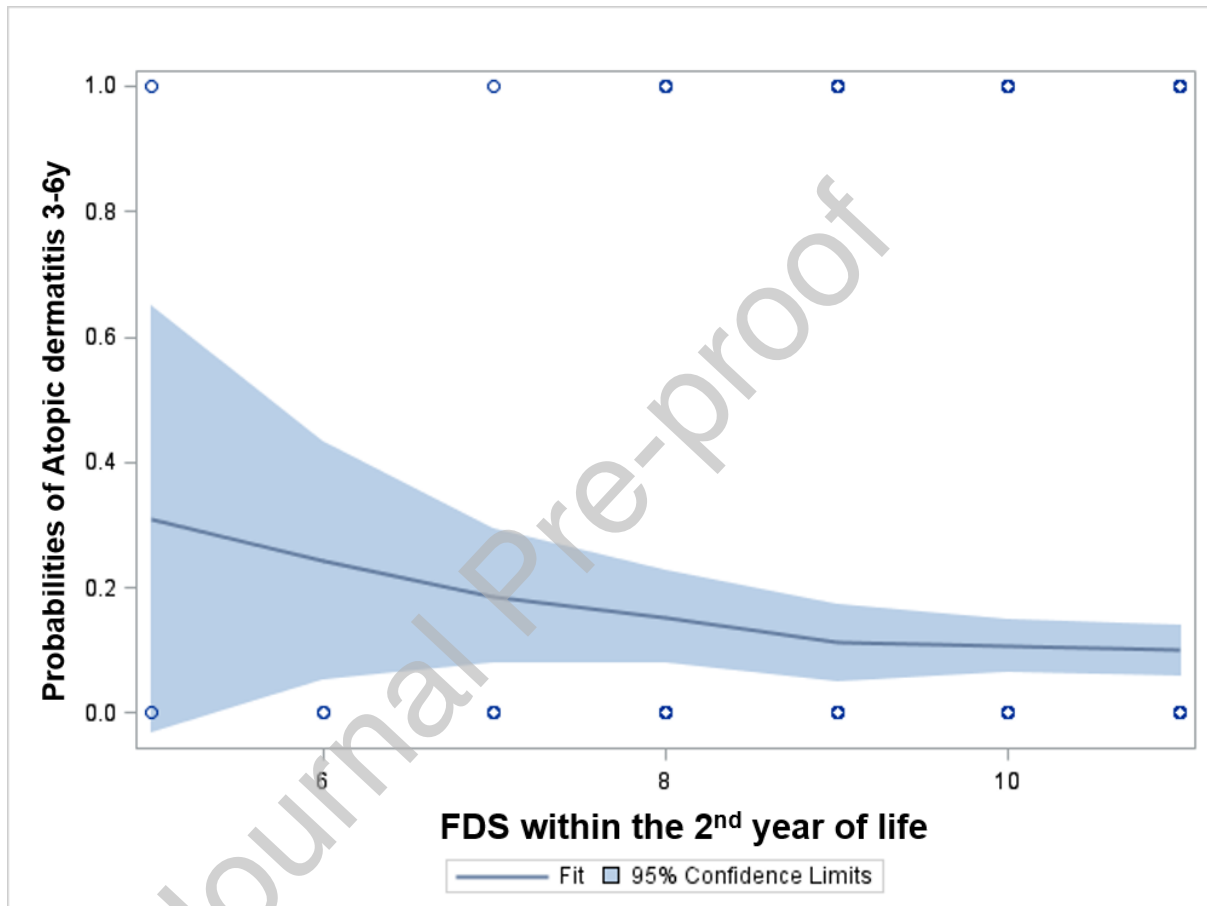
	N	Atopic dermatitis aOR 95% CI	N	Asthma aOR 95% CI	N	Food allergy aOR 95% CI	N	Food sensitization aOR 95% CI
Yoghurt								
Yes	106/652	0.53 (0.05-5.94)	53/657	na	16/626	0.04 (0.002-0.72)	215/557	na
No	1/4	1	0/3	-	1/3	1	2/2	-
Butter								
Yes	83/546	0.99 (0.52-1.89)	45/552	0.62 (0.26-1.50)	16/534	2.44 (0.30-20.16)	166/456	0.51 (0.28-0.92)
No	18/82	1	8/80	1	1/72	1	40/75	1
Cow's milk								
Yes	81/531	1.27 (0.59-2.74)	42/533	1.34 (0.54-3.35)	12/512	0.47 (0.10-2.23)	196/450	1.81 (0.94-3.51)
No	18/99	1	9/102	1	4/94	1	19/84	1
Cheese								
Yes	96/603	0.73 (0.33-1.63)	47/607	0.94 (0.34-2.59)	15/578	1.11 (0.13-9.43)	199/517	0.71 (0.35-1.42)
No	10/54	1	6/54	1	2/52	1	19/44	1
Egg								
Yes	99/636	0.20 (0.05-0.88)	50/638	0.40 (0.07-2.17)	16/611	0.30 (0.03-3.29)	210/539	0.31 (0.08-1.25)
No	6/10	1	2/11	1	1/8	1	6/11	1
Fish								
Yes	102/610	1.13 (0.41-3.09)	48/613	0.83 (0.26-2.62)	15/584	0.48 (0.09-2.45)	206/524	1.75 (0.78-3.89)
No	5/48	1	5/49	1	2/47	1	12/37	1
Nuts								
Yes	39/312	0.91 (0.52-1.59)	23/316	0.64 (0.31-1.31)	8/308	1.004 (0.30-3.34)	102/264	0.88 (0.55-1.41)
No	53/275	1	26/275	1	7/259	1	95/242	1
Cereals								
Yes	90/588	1.03 (0.47-2.23)	47/591	0.94 (0.28-3.17)	15/566	0.32 (0.05-2.04)	202/498	1.15 (0.51-2.61)
No	13/55	1	5/55	1	2/51	1	10/51	1
Vegetables								
Yes	106/655	0.27 (0.02-4.87)	52/658	0.15 (0.01-1.97)	17/627	na	217/558	na
No	1/2	1	1/3	1	0/3	-	0/2	-
Fruits								
Yes	107/658	na	53/662	na	17/631	na	218/61	na
No	0	-	0	-	0	-	0	-

Meat								
	107/6		53/6		17/6		216/5	
Yes	55	na	60	na	29	na	58	na
No	0/1	-	0	-	0/1	-	1/1	-

Adjusted OR for sex, center, farmer, parental allergic status, contact to pets in the first year of life, mode of delivery, breastfeeding, siblings, smoking in pregnancy, FDS at 12 months of age.

Figure 1

A) Association between increasing food diversity score within the 2nd year of life and atopic dermatitis



The solid line represents the predicted probabilities of atopic dermatitis as a function of the food diversity score (FDS). FDS with 11 food items: yogurt, butter, cow's milk, cheese, egg, meat, fish, nuts, vegetables, fruits, cereals.

Children with both pos. IgE to cow's milk and/or to egg at the age of 1 year (≥ 0.35 kU/l) AND children with AD in the 1st year of life were excluded.

OR for atopic dermatitis with each additional food item introduced in the 2nd year of life: 0.84, 95% CI (0.66-1.07).

B) Association between increasing food diversity score within the 2nd year of life and asthma

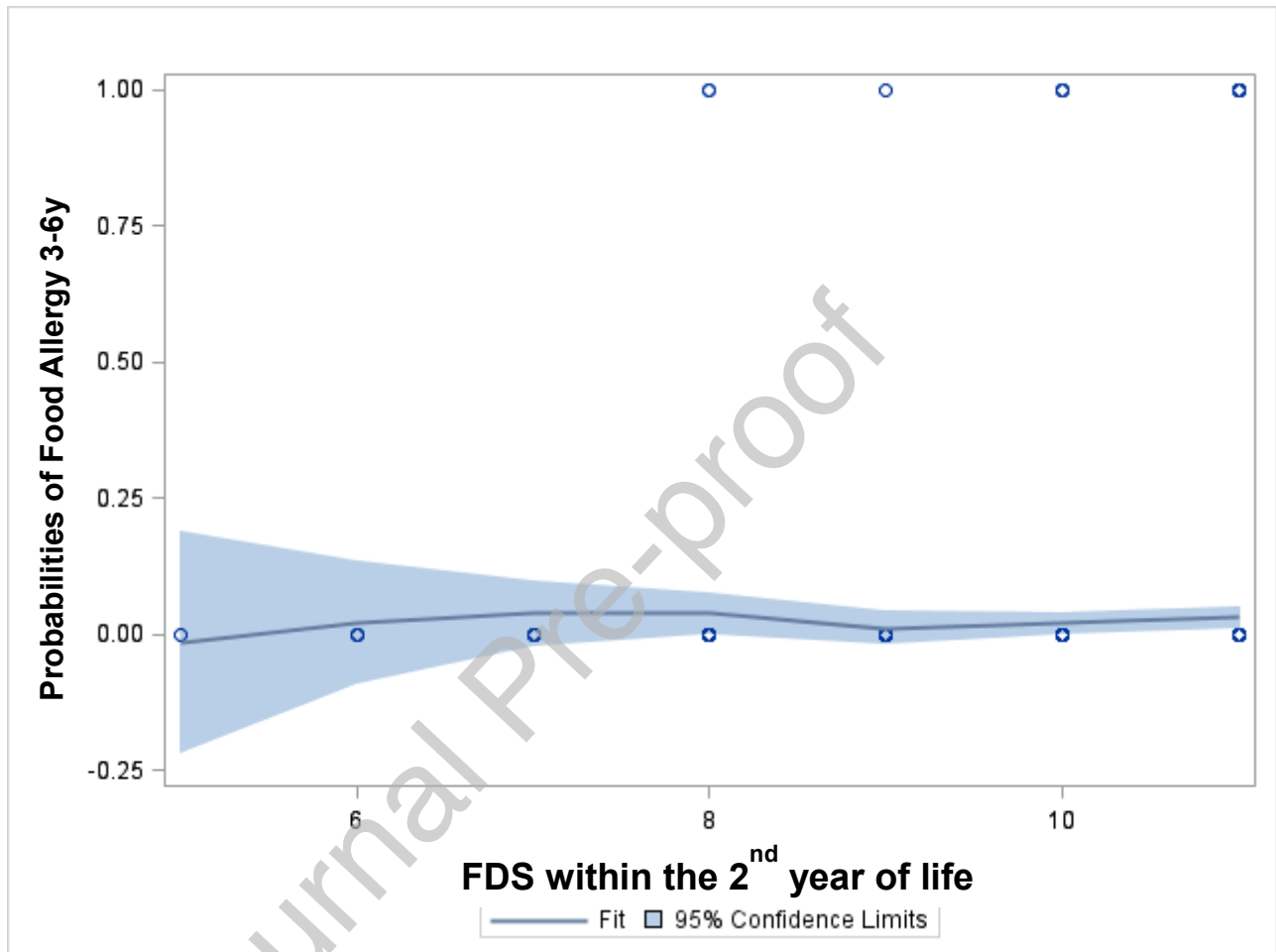


The solid line represents the predicted probabilities of asthma as a function of the food diversity score (FDS). FDS with 11 food items: yogurt, butter, cow's milk, cheese, egg, meat, fish, nuts, vegetables, fruits, cereals.

Children with both pos. IgE to cow's milk and/or to egg at the age of 1 year (≥ 0.35 kU/l) AND children with AD in the 1st year of life were excluded.

OR for asthma with each additional food item introduced in the 2nd year of life: 0.75, 95% CI (0.58-0.98).

C) Association between increasing food diversity score within the 2nd year of life and food allergy



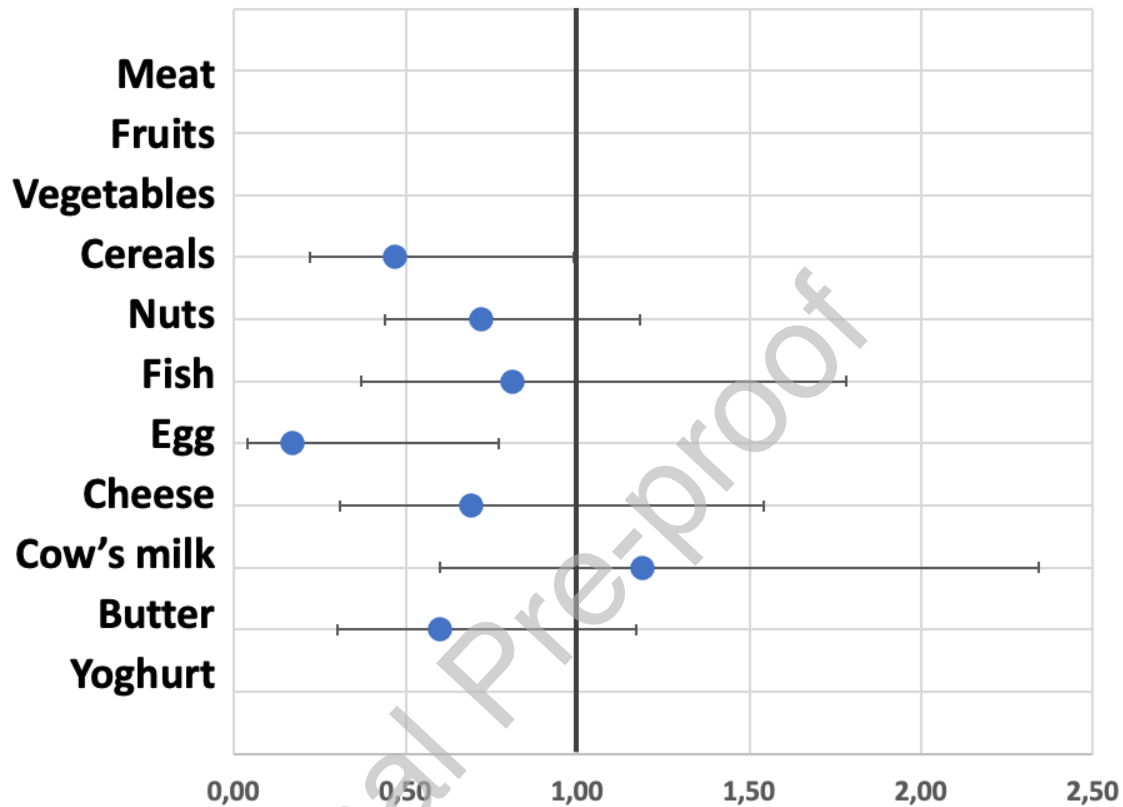
The solid line represents the predicted probabilities of food allergy as a function of the food diversity score (FDS). FDS with 11 food items: yogurt, butter, cow's milk, cheese, egg, meat, fish, nuts, vegetables, fruits, cereals.

Children with both pos. IgE to cow's milk and/or to egg at the age of 1 year (≥ 0.35 kU/l) AND children with AD in the 1st year of life were excluded.

OR for food allerg with each additional food item introduced in the 2nd year of life: 1.05, 95% CI (0.60-1.85).

Figure 2

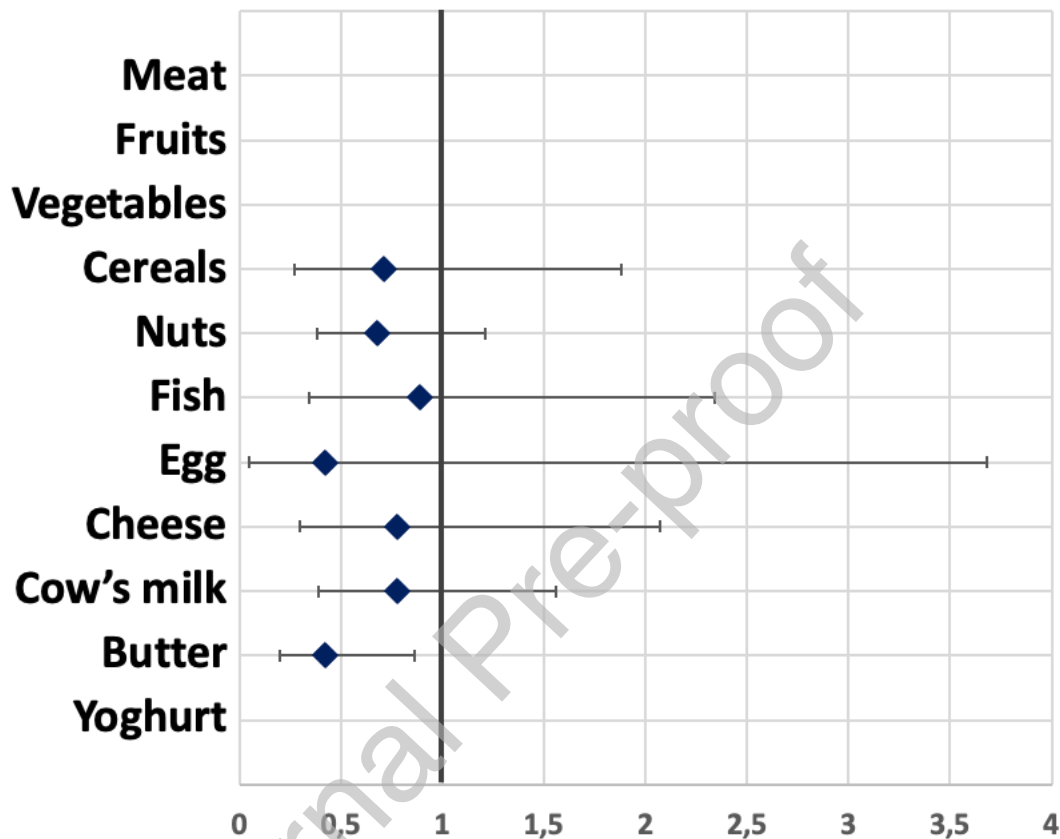
A) Associations between single food items in the 2nd year of life and atopic dermatitis



Odds ratios and 95% CI for atopic dermatitis (from 3-6 years of age) with different food items consumed within the 2nd year of life are shown. For yoghurt, meat, fruits and vegetables: no applicable results due to small numbers.

Children with both pos. IgE to cow's milk and/or to egg at the age of 1 year (≥ 0.35 kU/l) AND children with AD in the 1st year of life were excluded.

B) Associations between single food items in the 2nd year of life and asthma



Odds ratios and 95% CI for asthma (from 3-6 years of age) with different food items consumed within the 2nd year of life are shown. For yoghurt, meat, fruits and vegetables: no applicable results due to small numbers.

Children with both pos. IgE to cow's milk and/or to egg at the age of 1 year (≥ 0.35 kU/l) AND children with AD in the 1st year of life were excluded.

C) Associations between single food items in the 2nd year of life and food allergy



Odds ratios and 95% CI for food allergy (from 3-6 years of age) with different food items consumed within the 2nd year of life are shown. For meat, fruits, vegetables, egg and butter: no applicable results due to small numbers.

Children with both pos. IgE to cow's milk and/or to egg at the age of 1 year (≥ 0.35 kU/l) AND children with AD in the 1st year of life were excluded.

D) Associations between single food items in the 2nd year of life and food sensitization

