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Duration and exclusiveness of breastfeeding and risk of childhood atopic diseases

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CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose..

SHORT TITLE

Breastfeeding and risk of childhood atopic diseases

KEYWORDS

Atopic diseases, birth cohort, breastfeeding, children, exclusiveness.

ABBREVIATIONS

aOR : adjusted odds ratio

BMI : body mass index

CI : confidence interval

ISAAC : International Study of Asthma and Allergies in Childhood

ABSTRACT

Background Breastfeeding may have immune modulatory effects that influence the development of childhood allergic sensitization and atopic diseases. We aimed to examine the associations of breastfeeding with childhood allergic sensitization, inhalant or food allergy and eczema, and whether any association was affected by disease-related modification of the exposure or modified by maternal history of maternal history of allergy, eczema or asthma.

Methods This study among 5,828 children was performed in a population-based prospective cohort from fetal life onwards. We collected information on duration (<2 months, 2-4 months,

4-6 months and ≥ 6 months) and exclusiveness (non-exclusive vs. exclusive for 4 months) of breastfeeding in infancy by postal questionnaires. At age 10 years, inhalant and food allergic sensitization were measured by skin prick tests, and physician-diagnosed inhalant and food allergy by a postal questionnaire. Data on parental-reported eczema were available from birth until age 10 years.

Results We observed no association of breastfeeding with any allergic sensitization, physician-diagnosed allergy, or combination of these outcomes. Shorter breastfeeding duration was associated with an overall increased risk of eczema (p-value for trend < 0.05). Non-exclusively breastfed children had an overall increased risk of eczema (aOR (95% CI): 1.11 (1.01, 1.23)), compared with children exclusively breastfed for 4 months. Risk period-specific sensitivity analyses, additional adjustment for ointment use for eczema at age 2 months, and cross-lagged modeling showed no consistent results for disease-related modification of the exposure. Results were not modified by maternal history of allergy, eczema or asthma (lowest p-value for interaction = 0.13).

Conclusions Shorter duration or non-exclusiveness of breastfeeding is associated with a weak overall increased risk of eczema but not allergic sensitization or physician-diagnosed allergy at age 10 years.

INTRODUCTION

Breastfeeding may affect the development of childhood allergic sensitization and atopic diseases such as allergy and eczema(1). Underlying biological mechanisms are not fully clear but might include various components of human milk that modulate the child's immune system and alter the balance between pro-inflammatory and anti-inflammatory signals(1, 2). Also, human milk oligosaccharides are suggested to influence the development of atopic diseases by modulating gut microbiota diversity(2, 3). Previous birth cohort studies showed that breastfeeding was not consistently associated with allergic sensitization measured by

skin prick tests(4-8). A recent meta-analysis of observational studies showed that longer duration and exclusiveness of breastfeeding were associated with an up to 26% decreased risk of allergic rhinitis and eczema in preschool-aged children, but not with food allergy(9). Furthermore, results from a cluster randomized trial among 13,889 children showed that children of mothers who were promoted to breastfeed longer and more exclusively did not have reduced prevalences of inhalant allergic sensitization, allergic rhinitis and eczema until age 6.5 years, compared to children of mothers who were not promoted(10). However, the effects of breastfeeding on allergic sensitization and atopic diseases at older age are less known(4, 6). Also, the effects of disease-related modification of the exposure, meaning that early symptoms of allergy or eczema in the child may encourage a mother to alter breastfeeding habits(11), and the modifying effects of maternal history of allergy, eczema or asthma on the associations of breastfeeding with atopic diseases in school-aged children are not fully clear(5, 6, 8).

Therefore, we aimed to examine the associations of duration and exclusiveness of breastfeeding with allergic sensitization, inhalant or food allergy at age 10 years, and eczema from birth until age 10 years among 5,828 children and their mothers participating in a population-based prospective cohort study. Additionally, we examined whether any association was affected by altered maternal breastfeeding habits due to early symptoms of allergy or eczema in the child or modified by maternal history of allergy, eczema or asthma.

METHODS

General design This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards(12). The study has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Center Rotterdam, The Netherlands (MEC-2012-165). Written informed consent was obtained from both parents or legal representatives. For the current study, twins (n=208), and children without data on

breastfeeding (n=2,111), and allergic sensitization, allergy and eczema (n=297) were excluded, leaving a total of 5,828 children for the analyses (Supplementary Figure 1).

Breastfeeding duration and exclusiveness Detailed information on breastfeeding initiation and continuation was obtained from parental postal questionnaires at the ages of 2, 6 and 12 months after birth. Mothers were asked whether they ever breastfed their child (no; yes) and at what age (in months) they stopped breastfeeding. Of mothers that started breastfeeding, duration of breastfeeding was categorized into four groups: <2 months, 2-4 months, 4-6 months, and ≥ 6 months(13). Exclusiveness of breastfeeding was defined using information on the introduction of milk or solids, and categorized into non-exclusive breastfeeding for 4 months and exclusive breastfeeding for 4 months. Analyses that focused on breastfeeding duration and exclusiveness were performed among children that were ever breastfed.

Allergic sensitization, allergy and eczema Children visited the research center at a median age of 9.7 years (2.5-97.5th percentile range 9.3-10.6). Inhalant and food allergic sensitization (no; yes) to house dust mite, 5-grass mixture, birch, cat, and dog (ALK-Abelló B.V., Almere, The Netherlands), and hazelnut, cashew nut, peanut and peach were measured by skin prick tests using the scanned area method(14). We used a positive control (histamine dihydrochloride 10 mg/mL) in duplicate and a saline solution (NaCl 0.9%) as a negative control. Skin responses were considered positive if the area of the wheal was $\geq 40\%$ of the histamine response (i.e., histamine equivalent prick index area ≥ 0.40)(14). The scanned area method does not require adjustment for the negative control. However, children without a negative control or with any skin response to the saline solution were omitted from the analyses. Questions adapted from the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaires provided information on physician-diagnosed inhalant ("Was your child ever diagnosed by a physician with an allergy to pollen

(hay fever)/house dust mite/cat/dog?" (no; yes)) and food ("Was your child ever diagnosed by a physician with an allergy to cashew nut/peanut?" (no; yes)) allergy at age 10 years(15).

We further combined allergic sensitization and physician-diagnosed allergy into groups of 'no allergic sensitization and no allergy', 'any allergic sensitization, but no allergy', 'no allergic sensitization, but any allergy', and 'any allergic sensitization and any allergy'. Physician-diagnosed eczema was parental-reported at ages 6 months and 1, 2, 3, 4 and 10 years ("Was your child diagnosed by a physician with eczema in the last 6 months/last year?") (no; yes).

Covariates Information on maternal education (primary or secondary; higher), history of allergy, eczema or asthma (no; yes), parity (nulliparous; multiparous), pet keeping (no; yes) and body mass index (BMI) was obtained by postal questionnaires completed by the mother at enrollment. Information on maternal smoking was obtained by questionnaires multiple times during pregnancy and combined (no; yes). We assessed maternal psychiatric symptoms in the second trimester of pregnancy using the Global Severity Index of the Brief Symptom Inventory(16), denoting overall psychiatric symptoms. Information on child's sex, gestational age at birth and birth weight was obtained from obstetric and midwife records at birth. We based ethnic origin (European; non-European) of the child on the country of birth of the parents(17). We obtained information on ointment use for eczema (no; yes) and day care attendance (no; yes) by questionnaires at ages 2 months and 1 year, respectively.

Statistical analysis We compared characteristics of children included and not included using independent samples T-tests, Mann-Whitney U tests, and Pearson's Chi-square tests. We used logistic regression or multinomial logistic regression models to examine the associations of duration and exclusiveness of breastfeeding with the risk of allergic sensitization and physician-diagnosed allergy or combined allergic sensitization and allergy

groups, respectively, at age 10 years. We used generalized estimating equation models to examine the associations of duration and exclusiveness of breastfeeding with the longitudinal odds of eczema at ages 6 months, and 1, 2, 3, 4 and 10 years independently and overall, taking into account correlations between repeated measurements of eczema within the same child. Confounders were included in the models based on literature, if they were associated with both the determinant and the outcome, or if they changed the effect estimates with $\geq 10\%$ in bivariate analyses. Analyses with inhalant or food allergic sensitization or allergy as the outcomes were mutually adjusted for each other. Tests for trends were performed by including the breastfeeding categories as continuous variables in the models. We performed risk period-specific sensitivity analyses by excluding children who developed eczema during the period of breastfeeding until age 6 months ($n=962$) and additional adjustment for ointment use for eczema at age 2 months to account for possible altered maternal breastfeeding habits due to early eczema in the child. We applied an adjusted cross-lagged model to examine bidirectional associations of breastfeeding with eczema(18, 19). More detailed information on cross-lagged modeling is provided in the Supplementary Methods. For these analyses, ointment use for eczema at age 2 months was used as a proxy for the presence of eczema at age 2 months. The modifying effect of maternal history of allergy, eczema or asthma and the time-varying effect of age at eczema measurement were tested by adding them as product terms with the breastfeeding variables in the models. Missing data of covariates and eczema were multiple-imputed to reduce potential bias and improve efficiency (Supplementary Table 1). We assumed that missing data were missing at random(20). The best indicator for the presence or absence of eczema is an eczema measurement at a different age. Therefore, at least one eczema measurement was available in our population for analysis to predict other eczema measurements. Because we lacked repeated measurements on allergic sensitization and physician-diagnosed allergy, we did not impute missing data of these outcomes. The size or direction of the effect estimates did not materially differ between analyses with imputed data and complete cases only (data not shown). Therefore, we present results based on imputed

analyses only. Measures of association are presented as adjusted odds ratios (aOR) with their 95% confidence intervals (CI). Statistical analyses were performed using SPSS 21.0.0.1 (IBM Corp., Armonk, NY, USA), SAS 9.3 (SAS Institute Inc., Cary, NC, USA), and Mplus 7.11 for Windows (Muthén & Muthén).

RESULTS

General Maternal and child characteristics are presented in Table 1 and Supplementary Table 2. Of the children, 92.1% (n=5,365) was ever breastfed, 25.6% (n=1,182) was breastfed for <2 months, 21.2% (n=980) for 2-4 months, 11.8% (n=547) for 4-6 months, 31.4% (n=1,449) for ≥6 months, and 25.2% (n=1,201) exclusively breastfed for 4 months. Inhalant or food allergic sensitization was present in 32.1% (n=1,085) and 6.8% (n=230) of the children at age 10 years, respectively. Physician-diagnosed inhalant or food allergy was present in 12.1% (n=467) and 2.3% (n=87) of the children at age 10 years, respectively. The prevalence of eczema declined from 16.5% (n=962) at age 6 months to 7.9% (n=460) at age 10 years. Participants without follow-up data had younger, lower educated mothers who had higher parity and BMI at enrollment, smoked more during pregnancy, and had more psychiatric symptoms during pregnancy. Children were more often of non-European origin and attended day care more often than those included in the study (Supplementary Table 3).

Breastfeeding and childhood atopic diseases Never breastfeeding, duration and exclusiveness of breastfeeding were not associated with allergic sensitization, physician-diagnosed allergy or combined allergic sensitization and allergy groups (Table 2 and Supplementary Table 4). Never breastfeeding was not associated with eczema per year or overall, compared with ever breastfeeding (Supplementary Table 5). A shorter breastfeeding duration was associated with a higher overall risk of eczema (p-value for trend <0.05) (Figure A and Supplementary Table 5). Specifically, children breastfed <2 months had an

overall increased risk of eczema (aOR (95% CI) 1.16 (1.02, 1.32)), compared with children breastfed ≥ 6 months. Children non-exclusively breastfed for 4 months had an overall increased risk of eczema (1.11 (1.01, 1.23), compared with children exclusively breastfed for 4 months. (Figure B and Supplementary Table 5). Sensitivity analyses excluding children who developed eczema during the period of breastfeeding until age 6 months showed that effect estimates for the associations of breastfeeding < 2 months and non-exclusive breastfeeding for 4 months with eczema overall attenuated to non-significant (1.10 (0.92, 1.32) and 0.99 (0.86, 1.14), respectively). Effect estimates did not materially change when we additionally adjusted for ointment use for eczema at age 2 months (data not shown). Cross-lagged modeling showed that effect estimates for the associations of eczema or ointment use for eczema with breastfeeding, which were considered as associations in the opposite direction, were non-significant (Supplementary Figure 2). We observed no modifying effect of maternal history of allergy, eczema or asthma, and no time-varying effect of age at eczema measurement on the associations of breastfeeding with child's allergic sensitization, physician-diagnosed allergy, combined allergic sensitization and allergy groups or eczema (lowest p-value for interaction = 0.13).

DISCUSSION

In this large prospective population-based study, we observed that shorter duration or non-exclusiveness of breastfeeding was associated with a weak overall increased risk of eczema, but not allergic sensitization or physician-diagnosed allergy at age 10 years.

Comparison of main findings with other studies Previous birth cohort studies showed conflicting results on the association of breastfeeding with the risk of allergic inhalant or food sensitization(4-8, 21). Recently, observational studies that examined the associations of breastfeeding with allergic rhinitis, food allergy and eczema were meta-analyzed(9). Longer

duration of breastfeeding was associated with a 21% decreased risk of allergic rhinitis, and exclusive breastfeeding for 3-4 months with a 26% decreased risk of eczema in children until age 5 and 2 years, respectively, but not at older ages and effect estimates generally had high heterogeneity(9). Our results suggest that shorter duration or non-exclusiveness of breastfeeding is associated with an overall increased risk of eczema until age 10 years. In line with the results of the meta-analysis, we found no association of breastfeeding with food allergy, and no modifying effect of maternal history of allergy, eczema or asthma on the associations of breastfeeding with allergic sensitization or atopic diseases. Differences in results might be explained by recall bias of feeding history, different study populations (general population vs. full-term born children only vs. high-risk children), definitions of breastfeeding (never vs. ever, duration, degree of exclusiveness), types of inhalant or food allergens that were measured, age at which measurements were performed (preschool age vs. school age), and measurement and adjustment for potential confounders. Further studies are needed to examine if early introduction of allergenic foods might prevent the development of food allergy(22-24).

Interpretation of results Our results showed that duration or exclusiveness of breastfeeding was not associated with allergic sensitization or physician-diagnosed allergy at age 10 years. Children breastfed during a shorter period or non-exclusively had an up to 1.16-fold increased overall risk of eczema until age 10 years. For exclusiveness of breastfeeding, aOR's translate to an estimated number of 17-122 children that would need to be breastfed exclusively for 4 months to prevent one case of eczema at age 6 months to 10 years(25). Our findings partly support current breastfeeding guidelines of the European Academy of Allergy and Clinical Immunology and the Dutch Youth Health Centre(26, 27). We observed associations of duration or exclusiveness of breastfeeding with eczema overall but not per year. This might be explained by increased statistical power when using overall eczema rather than a chance finding. Differences in observed associations of breastfeeding

with eczema and allergic sensitizations or physician-diagnosed allergies might be due to differences in timing of these outcome measurements. Eczema was assessed longitudinally, while allergic sensitizations and physician-diagnosed allergy were measured at one time point only. Future studies focusing on associations of breastfeeding with potential shifts in allergic sensitization and allergy patterns using skin prick tests and measurements of physician-diagnosed allergy longitudinally from early childhood onwards are needed(28). Underlying biological mechanisms for the associations of breastfeeding with allergic sensitization, allergy and eczema might involve complex interactions of secretory immunoglobulin A, cytokines, chemokines, polyamines, sensitizing and tolerance promoting allergens, and eosinophil-derived granular proteins in human milk, which modulate the infant's developing immune system(1). N-3 polyunsaturated fatty acids in breast milk are suggested to play a mediating role in the development of allergic sensitization, allergy and eczema, but evidence on this topic is rather conflicting(29). Also, human milk oligosaccharides with prebiotic properties are suggested to influence the development of atopic diseases by modulating gut microbiota diversity(3). The impact of human milk on the diversity of the infant's gut microbiota in relation to allergic sensitization and atopic diseases requires further investigation in population-based cohorts.

Risk period-specific sensitivity analyses, additional adjustment for ointment use for eczema at age 2 months and cross-lagged modeling were used to examine potential disease-related modification of the exposure(11, 18, 30). Failure to account for disease-related modification of the exposure could erroneously suggest that shorter or non-exclusive breastfeeding leads to eczema, while it could have been that the development of early-onset eczema encouraged mothers to extend breastfeeding. For risk period-specific sensitivity analyses, the effect estimates attenuated to non-significant, but this might have mainly been due to power issues and less likely due to differences in etiology, severity and further development of atopic diseases between children with early and later onset of eczema(31, 32). The size and direction of the effect estimates did not materially change. Therefore, we

consider our results most probably not affected by disease-related modification of the exposure.

We found no modifying effect of maternal history of allergy, eczema or asthma on the associations of breastfeeding with allergic sensitization or atopic diseases. Genetic studies are needed to fully explore the role of maternal history of atopic diseases.

Strengths and limitations Strengths of this study are the use of a population-based prospective study design from fetal life onwards with a large number of participants, detailed information on breastfeeding status, allergic sensitization, allergy and eczema, and longitudinal measurements of eczema over time. However, some methodological limitations should be considered. First, selection bias in longitudinal studies mainly arises due to lost to follow-up. Characteristics of non-included subjects differed from those included in the study. This might have led to bias if the associations of breastfeeding with allergic sensitization, allergy and eczema were different between those included and not included in the study. Response rates of questionnaires ranged from 71% to 82%. The main reason for this was that not all children received each questionnaire due to logistical constraints and delayed implementation of some questionnaires after children reached the target age for those questionnaires. Second, we used an advanced software tool to calculate the histamine equivalent prick index. The scanned area method corrects for interobserver variability and ethnic differences in skin response to histamine and is recommended in research settings(14). The histamine equivalent prick index area cut-off value was based on skin response to food allergens but assumed similar for inhalant allergens. We evaluated inhalant and food allergens that we considered most relevant to children of age 10 years at the population level. Other allergens, such as milk and egg, were not taken into account because of low sensitization rates at this age(33). This may have resulted in non-differential misclassification and, subsequently, a dilution of the effect. It might be of interest to further

explore the effects of breastfeeding on allergic sensitization to specific inhalant and food allergens with low sensitization in high-risk populations. Third, we used parental questionnaires to obtain data on child's allergy and eczema, which may have led to non-differential misclassification of these outcomes, specifically food allergy. This most probably led to an underestimation of the effect estimates. Also, breastfeeding mothers are likely to be more health-conscious and might, therefore, be more likely to have allergic symptoms or skin rashes in their children presented for medical review, thus increasing the probability of diagnosing allergy or eczema. This may have resulted in differential misclassification and, subsequently, also an underestimation of the true effect. To reduce potential underestimation of the effect estimates, we opted for a self-reported physician's diagnosis of eczema to maximize the number of children without eczema correctly classified (i.e. specificity) at the expense of misclassifying children with stable disease not requiring medical attention as non-cases (i.e. sensitivity)(34). We used widely accepted, validated questions adapted from the ISAAC core questionnaire that reliably reflect the prevalence of eczema in young children at the population level(15, 35). Self-reported diagnosis of eczema in the last year based on a single question seems sufficiently valid for studying current childhood eczema in epidemiologic studies when compared with a physician's diagnosis(36). Fourth, we aimed to reduce potential bias due to missing data by using multiple imputation methods assuming that missing data were missing at random. However, it remains difficult to quantify the effect of missing data that were missing not at random, partly due to computational limitations and current statistical software availability. Fifth, as in any observational study, residual confounding due to insufficiently or unmeasured confounders might be an issue.

In conclusion, we observed that shorter duration or non-exclusiveness of breastfeeding was associated with a weak overall increased risk of eczema, but not allergic sensitization or physician-diagnosed allergy at age 10 years. Results seemed not affected by disease-related modification of the exposure, and not modified by maternal atopic disease history.

Despite its small protective effect, breastfeeding is encouraged because of its nutritional, immunological and psychosocial benefits. Further studies using detailed information on human milk components are needed to explore the specific underlying pathophysiological mechanisms.

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Table 1. Characteristics of mothers and their children.

	n = 5,828
Maternal characteristics	
Education, higher (%)	51.8 (3,017)
History of allergy, eczema or asthma, yes (%)	38.2 (2,228)
Parity, ≥ 1 (%)	43.8 (2,553)
Pet keeping during pregnancy, yes (%)	35.1 (2,047)
Body mass index at enrollment (kg/m^2) [†]	23.7 (18.8-35.7)
Smoking during pregnancy, yes (%)	23.5 (1,372)
Psychiatric symptoms during pregnancy [†]	0.15 (0-1.40)
Child characteristics	
Sex, female (%)	50.1 (2,919)
Gestational age at birth (weeks) [†]	40.1 (36.0-42.3)
Birth weight (grams)*	3,455 (548)
Ethnic origin, European (%)	68.8 (4,012)
Ointment use for eczema at age 2 months, yes (%)	8.0 (464)
Day care attendance until age 1 year, yes (%)	55.9 (3,259)
Breastfed ever, yes (%)	92.1 (5,365)
Breastfeeding duration (%)	
Never	10.0 (463)
<2 months	25.6 (1,182)
2-4 months	21.2 (980)
4-6 months	11.8 (547)
≥ 6 months	31.4 (1,449)
Breastfeeding exclusiveness (%)	
Never	9.7 (463)
Non-exclusive for 4 months	65.0 (3,095)
Exclusive for 4 months	25.2 (1,201)
Allergic sensitization at age 10 years, yes (%)	
Inhalant	32.1 (1,085)
Food	6.8 (230)
Physician-diagnosed allergy at age 10 years, yes (%)	
Inhalant	12.1 (467)
Food	2.3 (87)
Allergic sensitization and allergy combined at age 10 years (%)	
No allergic sensitization and no allergy	66.3 (1,868)
Any allergic sensitization, but no allergy	22.6 (636)
No allergic sensitization, but any allergy	1.3 (36)
Any allergic sensitization and any allergy	9.9 (279)

Eczema, yes (%)

Age 6 months	16.5 (962)
Age 1 year	13.2 (768)
Age 2 years	14.2 (825)
Age 3 years	10.1 (586)
Age 4 years	8.7 (508)
Age 10 years	7.9 (460)

Values are *means (SD), †medians (2.5-97.5th percentile) or percentages (absolute numbers) based on imputed data. Data on breastfeeding duration and exclusiveness, allergic sensitizations and physician-diagnosed allergies are not imputed.

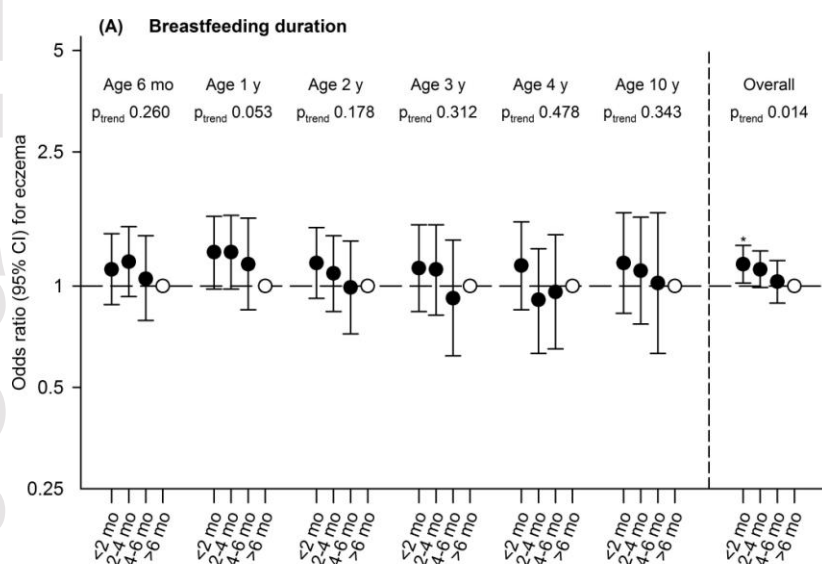
Table 2. Associations of duration and exclusiveness of breastfeeding with allergic sensitization and physician-diagnosed allergies in children at age 10 years.

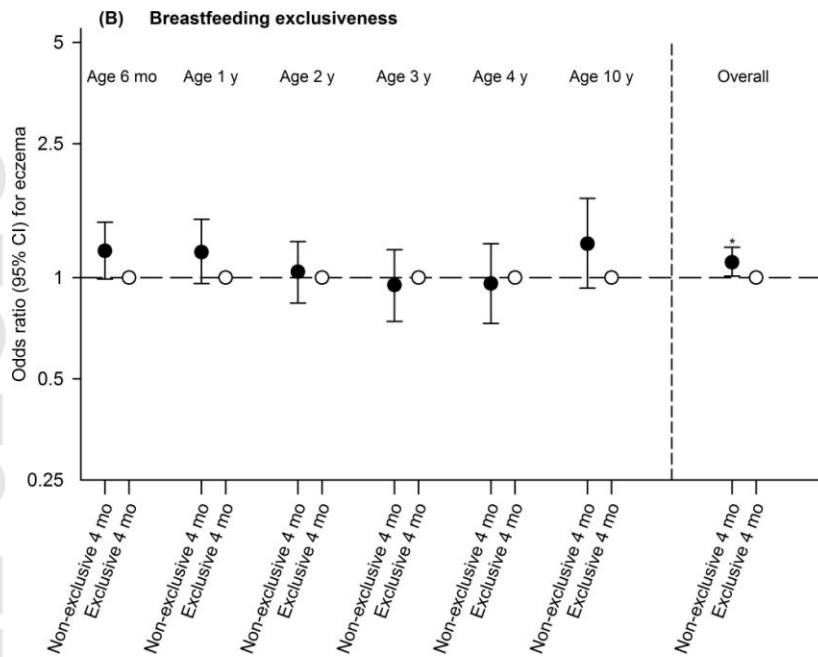
	Odds ratio (95% confidence interval) for allergic sensitization		Odds ratio (95% confidence interval) for physician-diagnosed allergy	
	Inhalant n = 3,381	Food n = 3,369	Inhalant n = 3,771	Food n = 3,846
Breastfeeding				
Never (n = 463)	0.83 (0.60, 1.14)	0.83 (0.42, 1.66)	1.11 (0.73, 1.67)	0.83 (0.30, 2.29)
Ever (n = 5,365)	Reference	Reference	Reference	Reference
Breastfeeding duration				
<2 months (n = 1,182)	1.02 (0.80, 1.30)	1.05 (0.62, 1.76)	1.28 (0.93, 1.77)	1.54 (0.71, 3.32)
2-4 months (n = 980)	1.14 (0.89, 1.46)	1.26 (0.75, 2.12)	0.94 (0.67, 1.33)	1.53 (0.69, 3.39)
4-6 months (n = 547)	1.22 (0.92, 1.63)	0.57 (0.29, 1.13)	0.97 (0.64, 1.46)	1.08 (0.39, 3.01)
≥6 months (n = 1,449)	Reference	Reference	Reference	Reference
P-value for trend	0.79	0.53	0.20	0.22
Breastfeeding exclusiveness				
Non-exclusive for 4 months (n = 3,095)	1.05 (0.86, 1.28)	1.38 (0.88, 2.14)	1.02 (0.78, 1.35)	0.76 (0.41, 1.40)
Exclusive for 4 months (n = 1,201)	Reference	Reference	Reference	Reference

Values are odds ratios (95% confidence interval) from logistic regression models based on imputed data. Models are adjusted for maternal education, history of allergy, eczema or asthma, parity, pet keeping, body mass index at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, ethnic origin and day care attendance, and mutually for inhalant and food allergic sensitization or allergy.

Figure. Associations of (A) duration and (B) exclusiveness of breastfeeding with eczema in children until age 10 years.

Legend: Values are odds ratios (95% confidence interval) from generalized estimating equation models based on imputed data. Reference group is children who were (A) breastfed ≥ 6 months or (B) exclusively breastfed for 4 months. Models are adjusted for maternal education, history of allergy, eczema or asthma, parity, pet keeping, body mass index at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, ethnic origin and day care attendance. *P-value < 0.05 . Mo = months; y = year(s).





Duration and exclusiveness of breastfeeding and risk of childhood atopic diseases

Supplementary material

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Supplementary Methods.

With a cross-lagged model, bidirectional associations of breastfeeding (i.e. exposure) with childhood eczema (i.e. outcome) can be studied within the same model. In this model, we used logistic regression models to study associations of breastfeeding at age 2 months with ointment use for eczema at age 2 months and eczema at ages 6 months to 10 years. Similarly, we studied associations of breastfeeding at age 6 months with eczema at ages 6 months to 10 years, and of breastfeeding at age 12 months with eczema at age 1 to 10 years. In addition, we studied the associations of ointment use for eczema at age 2 months with breastfeeding at ages 6 and 12 months, and of eczema at age 6 months with breastfeeding at age 12 months. Furthermore, the cross-lagged model takes associations of eczema measurements over time into account.