

Breast-feeding does not protect against allergic sensitization in early childhood and allergy-associated disease at age 7 years

Ea Jelding-Dannemand, MSc, Ann-Marie Malby Schoos, MD, and Hans Bisgaard, MD, DMSc *Gentofte, Denmark*

Background: Extended breast-feeding is recommended for newborn children at risk of allergy-associated diseases, but the evidence of a protective effect on sensitization and these diseases remains elusive.

Objective: The aim of this study was to investigate the effects of the duration of exclusive breast-feeding on the development of sensitization in preschool children.

Methods: Information on breast-feeding was gathered by interviews involving 335 children aged 1, 6, and 12 months from the Copenhagen Prospective Study on Asthma in Childhood₂₀₀₀ birth cohort born to mothers with a history of asthma. Skin prick test responses and specific IgE levels against 12 common inhalant and 10 food allergens were assessed longitudinally at ages ½ year, 1½ years, 4 years, and 6 years. Eczema, wheeze/asthma, and allergic rhinitis were diagnosed at the Copenhagen Prospective Studies on Asthma in Childhood clinic at 7 years of age, strictly adhering to predefined algorithms. Associations between duration of exclusive breast-feeding and outcomes were analyzed by logistic regression.

Results: We found no significant association between duration of exclusive breast-feeding and development of sensitization in the first 6 years of life (odds ratio [OR]: ½ year, 1.10 [95% CI, 0.90-1.36]; 1½ years, 1.15 [95% CI, 0.97-1.36]; 4 years, 1.08 [95% CI, 0.93-1.25]; and 6 years, 0.96 [95% CI, 0.84-1.10]) or with current eczema, wheeze/asthma, and allergic rhinitis at age 7 years (OR, 1.07 [95% CI, 0.92-1.24]; OR, 0.97 [95% CI, 0.82-1.14]; and OR, 1.02 [95% CI, 0.84-1.23], respectively).

Adjusting for reverse causation by excluding children with

eczema, wheeze, or a positive skin prick test response before ending exclusive breast-feeding did not alter the results.

Conclusion: Exclusive breast-feeding does not affect sensitization in early childhood or associated diseases at 7 years of age in at-risk children. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: Breast-feeding, sensitization, asthma, eczema, skin prick test, specific IgE, children

The beneficial effects of breast-feeding on allergy-associated diseases have been debated since an American study of 20,061 children from 1936¹ reported a protective effect of breast-feeding on the development of eczema. In particular, it is recommended that high-risk children should be offered a prolonged duration of exclusive breast-feeding or hydrolyzed substitution.²⁻⁴

It appears intuitively sensible to avoid allergens in early life to avoid sensitization, allergy, and maybe even allergy-related disorders. However, the evidence on such associations has been conflicting, with some reporting a protective effect on the development of allergy-associated diseases,⁵⁻⁷ others reporting no effect,^{8,9} and some even reporting an increase in risk.¹⁰⁻¹³

Reverse causation might explain the conflicting results. This can lead to misinterpretation of the results, showing an increased risk of allergy-associated diseases with prolonged breast-feeding, when in fact it is the development of allergy-associated disease that leads mothers to extend breast-feeding.¹⁴ We have previously reported that exclusive breast-feeding reduced the risk of wheezy disorders, but, surprisingly, increased the risk of eczema at the age of 2 years.¹⁰

This study aimed to analyze the effect of duration of breast-feeding on the development of sensitization during the first 6 years of life. In addition, we investigated the long-term effect of breast-feeding on eczema, wheeze/asthma, and allergic rhinitis at 7 years of age in the longitudinal high-risk birth cohort Copenhagen Prospective Studies on Asthma in Childhood₂₀₀₀ (COPSAC₂₀₀₀).

METHODS

Design

The COPSAC₂₀₀₀ birth cohort is a single-center prospective clinical birth cohort study of 411 children born between August 1998 and December 2001 to mothers with a history of asthma. The children were enrolled at 1 month of age and closely monitored by scheduled 6-month visits at the clinical research unit until the age of 7 years. At each visit, the children were followed with comprehensive clinical investigations according to standard operating procedures, and clinical outcomes were diagnosed and monitored by the COPSAC physicians. The families also used the clinical research unit instead of the family practitioner for diagnosis and treatment of any acute episodes of lung, skin, or allergy-related symptoms. The recruitment and baseline characteristics of the participants have been described in detail.¹⁵⁻¹⁷

From Copenhagen Prospective Studies on Asthma in Childhood, Faculty of Health and Medical Sciences, University of Copenhagen & Danish Pediatric Asthma Center, Gentofte Hospital, University of Copenhagen.

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Corresponding author: Hans Bisgaard, MD, DMSc, Copenhagen Prospective Studies on Asthma in Childhood, Faculty of Health and Medical Sciences, University of Copenhagen & Danish Pediatric Asthma Center, Gentofte Hospital, University of Copenhagen, Ledreborg Allé 34, 2820 Gentofte, Denmark. E-mail: bisgaard@copsac.com.

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Abbreviations used

COPSAC: Copenhagen Prospective Studies on Asthma in Childhood

IHRP: In-house reference preparation

OR: Odds ratio

sIgE: Specific IgE

SPT: Skin prick test

The Copenhagen Ethics Committee (KF 01-289/96) and the Danish Data Protection Agency (2008-41-2434) approved the study, and informed consent was obtained from both parents at enrollment.

Breast-feeding

Information on breast-feeding was collected prospectively by experienced research personnel who interviewed the mothers at the 1-, 6-, and 12-month clinical visits about the duration of exclusive and total breast-feeding and the use of infant formula. As soon as the child's diet was supplemented by even short periods of supplementation with formula, we considered exclusive breast-feeding to be terminated. It was standard procedure in the maternity ward to supply a hypoallergenic milk supplement until breast-feeding was established. Children receiving hypoallergenic formula for more than 7 days before establishing breast-feeding were excluded from this study because they were registered as never having been fully breast-fed.

Allergic sensitization

Skin prick tests (SPTs) and specific IgE (sIgE) measurements were performed at ½, 1½, 4, and 6 years of age against 10 food and 12 inhalant allergens (see Table E1 in this article's Online Repository at www.jacionline.org).

Serum sIgE levels were determined by using a screening method (ImmunoCAP Phadiatop Infant and ImmunoCAP Phadiatop; Thermo Fisher Scientific, Waltham, Mass),¹⁸ followed by analyses of sIgE levels against single allergens. sIgE values of 0.35 kU_A/L or greater were considered indicative of sensitization.¹⁷

SPTs were performed on the children's volar forearm with standard allergen extracts (Soluprick SQ; ALK, Hørsholm, Denmark) and ALK lancets. ALK standardizes every lot of allergen extract by using an in-house reference preparation (IHRP). This IHRP is thoroughly characterized, including tests on patients, and each new batch is compared with the IHRP by using appropriate laboratory tests. These include tests for complexity, major allergen content, and IgE-binding capacity. All SPTs were done by the same experienced research personnel. The positive control was read after 10 minutes, and the negative control and reactions to allergen extracts were read after 15 minutes. The average of 2 perpendicular wheal diameters was noted, and a test result was defined as positive with a wheal size at least 2 mm greater than that elicited by the negative control at ages ½ and 1½ years and at least 3 mm greater than that elicited by the negative control at age 4 and 6 years.

Children did not use antihistamines within 72 hours of testing (a positive control was used as screening for use of antihistamines with longer half-life), mild steroid creams (group 1-2) on the arms 24 hours before testing or stronger steroid creams (group 3-4) 14 days before testing.

We defined allergic sensitization as a positive SPT response, a positive sIgE level, or both.

Asthma and recurrent wheeze

The diagnosis of asthma by age 7 years was based on 4 mandatory criteria. The first was a history of recurrent wheeze, as defined by our strict algorithms. Daily symptoms were recorded from birth by parents and discussed with the accompanying parent by the COPSAC physicians at planned and acute visits. A wheezy episode was defined from the diary card as 3 consecutive days of troublesome lung symptoms that seriously hampered the well-being of the

child, including cough, wheeze, or breathlessness, which was thoroughly explained to the parents at the regular clinic visits, as well as described in written material. Recurrent wheeze was defined as 5 wheezy episodes within 6 months, daily symptoms for 4 successive weeks, or acute severe asthma symptoms resulting in hospitalization or the need for systemic corticosteroid treatment.^{16,17}

The second criterion was the presence of symptoms judged by the COPSAC physicians to be typical of asthma (eg, exercise-induced symptoms, prolonged nocturnal cough, recurrent cough outside common cold, and symptoms causing waking at night).

The third criterion was need for intermittent rescue use of inhaled β_2 -agonists.

The fourth and final criterion was response to a 3-month program of daily inhaled corticosteroids and relapse after treatment's end.

Allergic rhinitis

The diagnosis of allergic rhinitis in the seventh year of life was based on 2 criteria. The first was a history of symptoms based on parental interviews. Symptoms were defined as significant problems with sneezing or blocked or runny nose in the past 12 months in periods outside the common cold or flu. The second criterion was sensitization to allergens associated with relevant symptoms on exposure.¹⁹ Symptoms without association to documented sensitization were diagnosed as nonallergic rhinitis.

Eczema

Eczema was diagnosed according to the Hanifin and Rajka criteria,²⁰ which are based on the presence of 3 of 4 major criteria and at least 3 of 23 minor signs. The major criteria are (1) pruritus, (2) typical morphologic features and distribution, (3) chronic dermatitis, and (4) atopic history. The diagnosis was made by the physicians at the clinical research unit. Of the minor signs in the criteria, the following 6 were excluded: sensitization verified by SPT responses or sIgE levels, keratoconus and anterior subcapsular cataracts (because they required identification by an ophthalmologist), delayed blanch (because it required an injection of methacholine), and impaired cell-mediated immunity.

Eczema was assessed longitudinally from birth to age 7 years, defining both age of debut and end.²¹

Statistical methods

Dropout analyses were performed by using the χ^2 , Student *t*, and Wilcoxon rank sum tests. Associations between duration of exclusive breast-feeding and sensitization, eczema, wheeze/asthma, and allergic rhinitis were analyzed by logistic regression. For power analyses, see the Methods section in this article's Online Repository at www.jacionline.org.

Exclusive breast-feeding was classified as months of breast-feeding and investigated as a continuous trait, and each health outcome was dichotomized as yes or no. Sensitization was investigated at ages ½, 1½, 4, and 6 years.

Eczema, wheeze/asthma, and allergic rhinitis were investigated at age 7 years. Data on recurrent wheeze and asthma were combined and analyzed as one outcome.

Children with incomplete data (missing values on either health outcome or breast-feeding) and those who were never exclusively breast-fed (*n* = 22) were excluded from the analyses.

The results are presented as odds ratios (ORs) and 95% CIs by each additional month of exclusive breast-feeding. All corresponding *P* values were estimated by using Wald tests.

The robustness of the results was evaluated by redoing the analyses with breast-feeding as a categorical variable, dichotomizing duration of breast-feeding into categories of more or less than 4 months, and more or less than 6 months.

Subgroup analyses were done by evaluating the effect of breast-feeding in children with a double disposition to allergy-associated diseases, by only analyzing children with both parents with allergy-associated disease. Analyses were additionally done including only children born to mothers with positive sIgE levels.

To avoid reverse causation affecting our results, we also performed analyses by excluding children who had eczema, recurrent wheeze, or a positive SPT response during the period of exclusive breast-feeding. Children with positive sIgE levels were not excluded because the families were not informed of these findings. The samples were not analyzed until years later, and therefore could not be a cause of reverse causation.

All statistical analyses were performed with SAS version 9.3 software (SAS Institute, Cary, NC). *P* values of less than .05 were considered statistically significant.

RESULTS

Baseline

Twenty-two subjects were excluded from the analyses because they were never exclusively breast-fed. Information on duration of exclusive breast-feeding was available for 335 (82%) subjects from the total birth cohort of 411 newborns (see Fig E1 in this article's Online Repository at www.jacionline.org). The baseline characteristics of the 330 children with data on both exclusive breast-feeding and sensitization and the 59 children with missing data are outlined in Table I.

Children with missing data came from families with significantly lower household income ($P = .001$), and significantly more of these children had mothers who smoked during the third trimester of pregnancy (25% vs 14%, $P = .026$), had fathers with a positive sIgE level (67% vs 34%, $P = .044$), had a trend of fewer siblings ($P = .054$), or had a significantly shorter period of total breast-feeding (6.3 vs 7.7 months, $P = .033$) compared with children with full data. They did not differ with respect to the remaining baseline characteristics.

Breast-feeding and sensitization

The number of children with available data on both breast-feeding and sensitization were 275 (67%) at ½ year, 284 (69%) at 1½ years, and 266 (65%) at 4 and 6 years of age.

We found no significant association between duration of breast-feeding and sensitization based on SPT responses and/or sIgE levels at any time point (OR: ½ year, 1.10 [95% CI, 0.90-1.36]; 1½ years, 1.15 [95% CI, 0.97-1.36]; 4 years, 1.08 [95% CI, 0.93-1.25]; and 6 years, 0.96 [95% CI, 0.84-1.10]). Results are shown in Fig 1 and Table II. Adjusting for mother's smoking during the third trimester, mother's age at childbirth, older siblings, mother's education, household income, and father's allergic disease did not alter the results significantly (see Table E2 in this article's Online Repository at www.jacionline.org). We found similar conclusions when grouping information on breast-feeding in categorical variables (all $P \geq .341$, see Table E3 in this article's Online Repository at www.jacionline.org).

We analyzed the tendency for reverse causation and found that children who had eczema, wheeze, or a positive SPT response during exclusive breast-feeding had a significantly prolonged period of exclusive breast-feeding compared with children who had none of these symptoms during exclusive breast-feeding (5.8 vs 4.1 months, $P < .0001$, see Table E4 in this article's Online Repository at www.jacionline.org). Therefore, we corrected for such possible reverse causation by excluding children with a positive SPT response and children who received a diagnosis of recurrent wheeze or eczema during the breast-feeding period, which did not change the estimates noteworthy (OR: ½ year, 1.08 [95% CI, 0.86-1.36]; 1½ years, 1.11 [95% CI, 0.92-1.33];

4 years, 1.05 [95% CI, 0.90-1.23]; and 6 years, 0.96 [95% CI, 0.83-1.10]; Table II). Including the 22 children who were never exclusively breast-fed did not change the results either (see Table E5 in this article's Online Repository at www.jacionline.org).

Subanalyses of association between exclusive breast-feeding and sensitization based on sensitization assessment method and type of allergen did not reveal any significant results either (Table III).

Subanalyses at 6 years on the effect of breast-feeding on sensitization in monosensitized or polysensitized children showed no difference in the effect of breast-feeding (see Table E6 in this article's Online Repository at www.jacionline.org).

Adjusting for confounders (see Table E7 in this article's Online Repository at www.jacionline.org) and reverse causation (see Table E8 in this article's Online Repository at www.jacionline.org) in these analyses showed similar results, except the confounder analyses, which showed a significant protective effect of duration of breast-feeding on sensitization to milk at age 6 years (OR, 0.79 [95% CI, 0.64-0.96]).

Additional analyses of children with double heritage of allergic disease (ie, both mother and father with history of allergic disease) and of children born to mothers with positive sIgE levels to any of the most common inhalant allergens similarly showed no effect of duration of breast-feeding on the development of sensitization (see Table E9 in this article's Online Repository at www.jacionline.org).

All analyses mentioned above were repeated with total breast-feeding as the exposure variable, which had no effect on the results (see Table E10 in this article's Online Repository at www.jacionline.org), supporting our findings that duration of breast-feeding, either total or exclusive, is not associated with sensitization in childhood.

Breast-feeding and clinical outcomes

At age 7 years, complete data on exclusive breast-feeding and eczema, wheeze/asthma, and allergic rhinitis were available in 304 (74%), 310 (75%), and 270 (66%) children, respectively. We found no significant association between duration of exclusive breast-feeding and any of the clinical outcomes at 7 years of age (OR: eczema, 1.07 [95% CI, 0.92-1.24]; wheeze/asthma, 0.97 [95% CI, 0.82-1.14]; and allergic rhinitis, 1.02 [95% CI, 0.84-1.23]; Table IV).

In these analyses, the robustness of the results was also evaluated by redoing the analyses with breast-feeding as a categorical variable, as defined above, which did not alter the results (see Table E11 in this article's Online Repository at www.jacionline.org). Finally, analyses were repeated controlling for reverse causation, again with no significant effect on the results (OR: eczema, 0.99 [95% CI, 0.84-1.17]; wheeze/asthma, 0.98 [95% CI, 0.82-1.18]; allergic rhinitis, 1.00 [95% CI, 0.81-1.24]; Table IV).

DISCUSSION

Main findings

We found no association between duration of breast-feeding and sensitization in the first 6 years of life in this prospective high-risk birth cohort study. The results were similar in subanalyses of different assessment methods (SPT responses or

TABLE I. Baseline characteristics

Characteristics	No. (total) 389*	Participants 330	Dropouts 59	Participants vs dropouts, <i>P</i> value (χ^2 test)
Sex				
Male, no. (%)	389	160 (48)	29 (49)	.925
Socioeconomic factors				
Household income†	369			.001
High, no. (%)		81 (25)	3 (7)	
Average, no. (%)		157 (48)	17 (40)	
Low, no. (%)		88 (27)	23 (53)	
Mother's education	356			.919
University, no. (%)		47 (15)	4 (13)	
Medium, no. (%)		86 (27)	8 (25)	
At most high school, no. (%)		191 (59)	47 (63)	
Smoking during third trimester				
Yes, no. (%)	389	46 (14)	15 (25)	.026
Older siblings				
None, no. (%)	364	200 (62)	23 (56)	.054
1, no. (%)		89 (28)	10 (25)	
2, no. (%)		24 (7)	3 (7)	
≥3, no. (%)		10 (3)	5 (12)	
Maternal allergic disposition				
Asthma: yes, no. (%)	389	330 (100)	59 (100)	NA
Allergic rhinitis: yes, no. (%)	388	258 (78)	46 (79)	.847
Eczema: yes, no. (%)	389	155 (47)	29 (49)	.757
Total IgE (kU _A /L), median (IQR)§	320	52.8 (19.0-153.0)	42.3 (11.5-80.7)	.195
sIgE‡: yes, no. (%)	327	198 (63)	5 (42)	.138
Paternal allergic disposition				
Asthma: yes, no. (%)	388	59 (18)	5 (9)	.071
Allergic rhinitis: yes, no. (%)	386	112 (34)	13 (22)	.065
Eczema: yes, no. (%)	386	46 (14)	3 (5)	.056
Total IgE (kU _A /L), median (IQR)§	289	33.7 (13.3-76.6)	24.2 (22.8-44.7)	.932
sIgE‡: yes, no. (%)	293	97 (34)	6 (67)	.044
Maternal age at birth				
Maternal age at childbirth (y), mean (SD)	389	29.6 (4.5)	29.5 (5.0)	.878
Breast-feeding				
Exclusive breast-feeding (mo), median (IQR)§	335	4.1 (3.1-5.5)	5.4 (5.2-6.0)	.155
Total breast-feeding (mo), median (IQR)§	373	7.7 (5.6-10.9)	6.3 (3.5-9.2)	.033

P values in boldface indicate statistical significance.

NA, Not applicable.

*Twenty-two of the 411 children were excluded from the analysis because they were never exclusively breast-fed.

†High is defined as a total income of greater than €80,000, average is defined as a total income of €53,333 to €80,000, and low is defined as a total income of less than €53,333.

‡Positive sIgE level of 0.35 kU_A/L or greater for any allergen.

§Wilcoxon rank sum test.

|| Student *t* test.

sIgE levels) and allergen types (food, inhalant, and milk and egg allergens). Adjusting for reverse causation did not change these conclusions, nor did analyzing subgroups of children with single or double predisposition to sensitization.

We did not see any association between the duration of breast-feeding and allergy-associated diseases such as eczema, wheeze/asthma, or allergic rhinitis, at the age of 7 years.

Strengths and limitations of the study

This is the first prospective, clinical, high-risk birth cohort study of the effect of breast-feeding on the development of allergic sensitization diagnosed based on both sIgE levels and SPT responses measured longitudinally during the first 6 years of life.

It is a strength of this study that the information on breast-feeding was obtained by means of repeated interviews at 1 month and successively at 6 and 12 months by the research personnel (not questionnaires), minimizing the risk of misclassification and recall bias.

Another strength is the comprehensive data on sensitization measured thoroughly at 4 time points: ½, 1½, 4, and 6 years of age. Both SPT responses and sIgE levels were measured in all children, ensuring a higher detection level than any of the methods alone. All SPTs were performed by a small group of trained research personnel, double checked, and locked in a database.

Sensitization was assessed based on both SPT responses and sIgE levels because these approaches can show very conflicting results in the same child.²²⁻²⁴ Likewise, monosensitized and polysensitized patients with allergic rhinitis have been suggested to constitute 2 different categories,²⁵ which is why we also chose to perform subanalyses based on whether the child was monosensitized or polysensitized.

We also had information on sIgE measurements in the parents, which allowed us to perform subanalyses on the association between the duration of breast-feeding and sensitization in children with double heredity to sensitization.

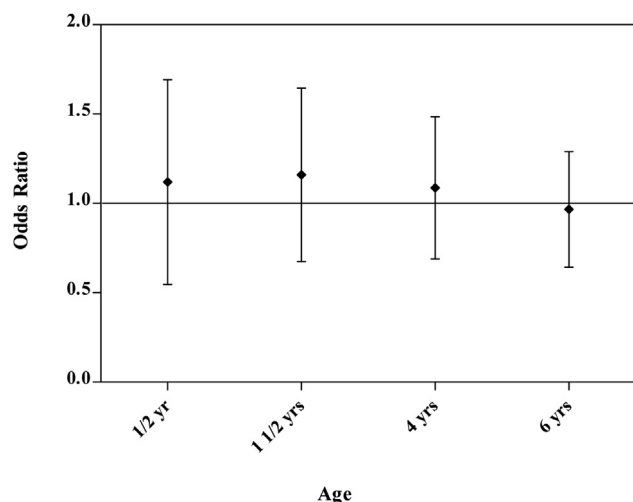


FIG 1. Association between any sensitization and exclusive breast-feeding.

An additional strength is the assessment of the clinical diagnoses that were completed and validated by the same COPSAC physicians based on a well-defined algorithm of criteria, limiting the risk of assessment bias.^{16,19-21} Allergic rhinitis is poorly recognized and can be underreported in young children.^{26,27} By conducting thorough interviews with the parents regarding symptoms of allergic rhinitis, we sought to minimize this issue.

Reverse causation (ie, presentation of symptoms assumed to be associated with sensitization) might lead to extended breast-feeding, as demonstrated in our data. Therefore, we performed subanalyses, excluding subjects with debut of sensitization, eczema, or wheezy symptoms before the end of exclusive breast-feeding.

Power analyses for logistic regression showed that the size of the study excluded a protective effect on the development of sensitization of 35% at 1/2 year of age ($\beta = 0.8$, $\alpha = 0.05$) and 25% at 6 years of age. Similarly, our study power excluded 25% reduction in eczema, 30% reduction in asthma, and 35% reduction in allergic rhinitis at 7 years of age (see the [Methods](#) section in this article's Online Repository).

The findings of this study represent solely children at increased risk of asthma. Subanalyses of children with allergy-associated diseases in both parents showed no protection either.

Interpretation

Many studies have investigated the health effects of breast-feeding, but the results remain ambiguous. There seems to be convincing evidence that breast-feeding protects against both gastrointestinal and respiratory tract infections, as well as being an ideal source of nutrition for healthy growth and development of infants.^{2,28} It contains all the nourishment and nearly all the vitamins and minerals infants need² and provides important mother-infant bonding.²

Study results on breast-feeding and sensitization have been contradictory. A Swedish unselected questionnaire-based cohort study on 3825 children reported more than 4 months of breast-feeding to result in a protective effect on the development of sensitization assessed by SPT response at age 8 years⁶ but no

TABLE II. Association between exclusive breast-feeding and any sensitization

Analysis	OR (95% CI)*	P value	No. sensitization/total†
1/2 y	1.10 (0.90-1.36)	.346	31/275
1 1/2 y	1.15 (0.97-1.36)	.104	54/284
4 y	1.08 (0.93-1.25)	.302	84/266
6 y	0.96 (0.84-1.10)	.550	101/266
Adjusted for reverse causation			
1/2 y	1.08 (0.86-1.36)	.496	26/253
1 1/2 y	1.11 (0.92-1.33)	.290	43/258
4 y	1.05 (0.90-1.23)	.515	69/240
6 y	0.96 (0.83-1.10)	.544	88/242

Results were adjusted for reverse causation by excluding children who had eczema, recurrent wheeze, or a positive SPT response during the period of exclusive breast-feeding.

*Logistic regression was used in the analyses calculating the OR of sensitization for each day of prolonged breast-feeding.

†Total of children with complete data on sensitization and breast-feeding at a given age.

effect at 4 years of age.²⁹ In contrast, an Australian high-risk cohort study found an increased risk of sensitization with prolonged breast-feeding,³⁰ and similar results were shown in a unselected cohort study from New Zealand¹¹; however, in the latter group no adjustment for reverse causation was made. A cluster-randomized clinical trial in 17,046 mother-child pairs found no effect of breast-feeding on the development of sensitization diagnosed based on SPT responses at the age of 6 1/2 years.⁹

A number of methodological problems can explain the many conflicting results on the subject. These include indiscriminate use of SPTs and sIgE measurements (although these tests are highly incongruent),^{23,24} assessment of exposure and outcome by retrospective questionnaires or by interview with a risk of recall bias, failure to distinguish between exclusive and total breast-feeding, and choice of control groups when assessing outcomes, all of which complicate the comparison of results from different research groups and might explain the conflicting results.

Failure to adjust for reverse causation¹⁴ is a particular concern. The effect of reverse causation could erroneously lead to false conclusions of an increased risk of allergy-associated disease with prolonged breast-feeding when it could be the presence of an allergy-associated disease that leads to prolonged breast-feeding. In the COPSAC₂₀₀₀ cohort, we found a significantly prolonged period of exclusive breast-feeding in children with eczema or wheeze or who had a positive SPT response during exclusive breast-feeding compared with that seen in children who had none of these symptoms during exclusive breast-feeding, supporting the risk of reverse causation. We mitigated against this by excluding all subjects in whom the proposed end point (disease manifestation) came before completing the proposed prophylaxis (breast-feeding). This confirmed our main findings, strengthening the validity of our conclusions.

It is the general recommendation to use hypoallergenic formula before the age of 4 months in case supplementation or replacement is needed. This is likely to limit the contrast between breast-feeding and not breast-feeding.

We also chose to exclude the 22 children who were never exclusively breast-fed from our analyses because the mothers distinguish themselves by smoking more and having a lower level

TABLE III. Subanalyses of association between exclusive breast-feeding and sensitization based on sensitization assessment method and type of allergen

Analysis	OR (95% CI)*	P value	No. sensitization/total†
Any sensitization			
SPT ½ y	1.14 (0.89-1.48)	.303	20/297
SPT 1½ y	1.17 (0.94-1.44)	.155	32/289
SPT 4 y	1.10 (0.87-1.39)	.421	25/257
SPT 6 y	1.06 (0.88-1.28)	.532	41/264
sIgE ½ y	1.15 (0.90-1.48)	.269	22/302
sIgE 1½ y	1.11 (0.91-1.36)	.286	35/306
sIgE 4 y	1.09 (0.95-1.26)	.231	79/296
sIgE 6 y	0.97 (0.85-1.10)	.608	96/276
SPT and/or sIgE			
Food ½ y	1.17 (0.93-1.47)	.192	26/254
Food 1½ y	1.19 (0.99-1.43)	.070	42/256
Food 4 y	1.06 (0.89-1.25)	.522	53/263
Food 6 y	0.94 (0.80-1.09)	.397	57/261
Inhalant ½ y	0.87 (0.61-1.25)	.448	8/283
Inhalant 1½ y	1.13 (0.89-1.45)	.311	22/285
Inhalant 4 y	1.14 (0.96-1.35)	.130	55/272
Inhalant 6 y	0.97 (0.84-1.12)	.643	77/264
Milk ½ y	1.23 (0.86-1.74)	.253	11/270
Milk 1½ y	1.22 (0.94-1.59)	.144	21/277
Milk 4 y	1.01 (0.82-1.23)	.946	31/262
Milk 6 y	0.85 (0.70-1.01)	.056	37/258
Egg ½ y	1.16 (0.86-1.55)	.337	15/273
Egg 1½ y	1.19 (0.92-1.53)	.190	22/278
Egg 4 y	1.01 (0.79-1.29)	.944	21/257
Egg 6 y	1.06 (0.83-1.34)	.659	23/258

*Logistic regression was used in the analyses calculating the OR of allergic outcome for each month of prolonged breast-feeding.

†Total of children with complete data on sensitization and breast-feeding at a given age.

of education.³¹ Including these 22, however, did not change the conclusions.

Finally, subanalyses were done to evaluate the protective allergen-avoidance effect of breast-feeding because it is biologically reasonable to think that breast-feeding would protect against sensitization to food allergens, such as milk and egg, as the child needs to have been exposed to the allergen in question to be sensitized. Subanalyses showed that the duration of breast-feeding had no effect on sensitization to either milk or egg allergens or other food or inhalant allergens, except for a protective effect of breast-feeding on sensitization to milk at age 6 years in the confounder-adjusted analysis but not at any of the 3 previous time points, which we interpret as a spurious finding in view of the repeated amount of tests we performed (we did not correct for multiple analyses). Nor did we find any effect of breast-feeding on sensitization diagnosed based on SPT responses or sIgE levels separately. Our results were similar when using either test method.

In the current study, we investigated the effect of breast-feeding on sensitization in infancy, toddlerhood, and childhood to possibly find alterations in the effect as the children grew older. Even so, we found no protective effect of breast-feeding on sensitization at any age.

Previously, we found that breast-feeding increased the risk of eczema and decreased the risk of recurrent wheeze in the first 2 years of life.¹⁰ The present study shows that these early effects from breast-feeding are temporary because there was no longer

TABLE IV. Association between exclusive breast-feeding and eczema, wheeze/asthma, and allergic rhinitis

Analysis	OR (95% CI)*	P value	No. disease/total†
Eczema 7 y	1.07 (0.92-1.24)	.373	67/304
Wheeze and/or asthma 7 y	0.97 (0.82-1.14)	.689	46/310
Allergic rhinitis 7 y	1.02 (0.84-1.23)	.858	35/270
Adjusted for reverse causation			
Eczema 7 y	0.99 (0.84-1.17)	.932	53/277
Wheeze and/or asthma 7 y	0.98 (0.82-1.18)	.857	38/283
Allergic rhinitis 7 y	1.00 (0.81-1.24)	.996	27/247

Results were adjusted for reverse causation by excluding children who had eczema, recurrent wheeze, or a positive SPT response during the period of exclusive breast-feeding.

*Logistic regression was used in the analyses calculating the OR of sensitization for each month of prolonged breast-feeding.

†Total of children with complete data on sensitization and breast-feeding at a given age.

any association between breast-feeding and eczema or wheeze/asthma at age 7 years.

Our thorough analyses have shown no effect of breast-feeding on the development of sensitization. This is contrary to the general opinion and current health recommendations. This perception of breast-feeding as an important protection against allergy might cause mothers of children who are at high risk of allergy-associated diseases to feel guilty and distressed if they are not able to breast-feed exclusively for the recommended period of time.³² On the basis of our data together with previous literature,^{9,11,28-30} we did not find evidence of a protective effect of breast-feeding on the development of sensitization. This information should be communicated to the public, moderating the general recommendation of breast-feeding based on the lack of evidence for any protective effect against allergy in at-risk children.

Conclusion

We found that the duration of breast-feeding does not affect the development of sensitization in young, high-risk children, and we found no effect on eczema, wheeze/asthma, or allergic rhinitis by age 7 years. Although it is generally acknowledged that breast-feeding provides the most optimal source of food for infants, these findings should be emphasized to prevent distress and guilt in allergy-disposed mothers with breast-feeding difficulties.

We thank the children and families of the COPSAC cohort study for all their support and commitment. We acknowledge and appreciate the unique efforts of the COPSAC research team and Thermo Fisher Scientific Inc for their dedicated work with the IgE analyses.

Clinical implications: Exclusive breast-feeding does not affect the development of sensitization in early childhood or associated diseases at school age in at-risk children.

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METHODS

Power calculations

In the following calculations, power is 0.8 and the α value is 0.05.

Breast-feeding and sensitization

- At age ½ year, 275 children had data on breast-feeding and sensitization, and 11% of the children were sensitized. With a power of 80%, we could show an effect corresponding to an OR of 1.35.
- At age 1½ years, 284 children had data on breast-feeding and sensitization, and 19% of the children were sensitized. With a power of 80%, we could show an effect corresponding to an OR of 1.30.
- At age 4 years, 266 children had data on breast-feeding and sensitization, and 32% of the children were sensitized. With a power of 80%, we could show an effect corresponding to an OR of 1.25.

- At age 6 years, 284 children had data on breast-feeding and sensitization, and 38% of the children were sensitized. With a power of 80%, we could show an effect corresponding to an OR of 1.25.

Breast-feeding and clinical outcomes

- At age 7 years, 304 children had data on breast-feeding and eczema, and 22% of the children had eczema. With a power of 80%, we could show an effect corresponding to an OR of 1.25.
- At age 7 years, 310 children had data on breast-feeding and asthma, wheeze, or both, and 15% of the children had asthma, wheeze, or both. With a power of 80%, we could show an effect corresponding to an OR of 1.30.
- At age 7 years, 270 children had data on breast-feeding and allergic rhinitis, and 13% of the children had allergic rhinitis. With a power of 80%, we could show an effect corresponding to an OR of 1.35.

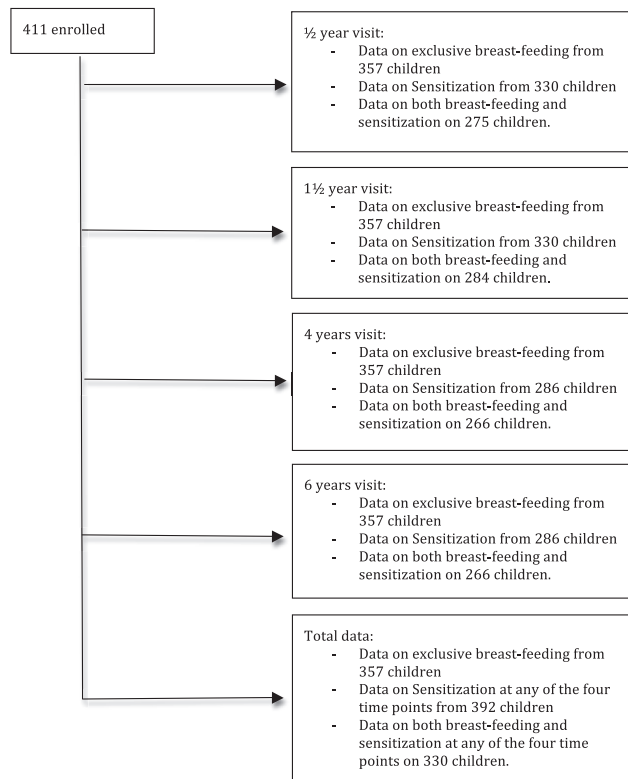
**FIG E1.** Participants and dropout flowchart.

TABLE E1. Allergens tested by using SPTs, sIgE measurements, or both in the COPSAC₂₀₀₀ birth cohort

Inhalant	Food
Common silver birch	Milk
Timothy grass	Egg
Mugwort	Wheat flour
Horse	Soybean
Dog	Peanut
Cat	Cod
<i>Dermatophagoides pteronyssinus</i> (house dust mite)	Shrimp
<i>Dermatophagoides farinae</i> (house dust mite)	Rye flour
<i>Aspergillus fumigatus</i> (mold)	Pork
<i>Cladosporium herbarum</i> (mold)	Beef
<i>Penicillium notatum</i> (mold)	
<i>Alternaria alternata</i> (mold)	

TABLE E2. Association between exclusive breast-feeding and any sensitization adjusted for confounders

Analysis	OR (95% CI)*†	P value	No. sensitization/total‡
½ y	1.14 (0.92-1.42)	.216	31/263
1½ y	1.13 (0.95-1.34)	.172	53/277
4 y	1.08 (0.92-1.25)	.347	83/258
6 y	0.97 (0.84-1.12)	.679	97/257

*Logistic regression was used in the analyses calculating the OR of sensitization for each month of prolonged breast-feeding.

†Confounder adjusted for mother smoking during the third trimester, mother's age at childbirth, mother's education, household income, older siblings, and father's allergy.

‡Total of children with complete data on sensitization and breast-feeding at a given age.

TABLE E3. Association between exclusive breast-feeding and any sensitization by using breast-feeding as a categorical variable

Analysis	OR (95% CI)*	P value	No. sensitization/total†
Breast-feeding ≥ 4 vs < 4 mo			
½ y	0.76 (0.35-1.62)	.477	31/275
1½ y	1.22 (0.65-2.27)	.539	54/284
4 y	1.31 (0.75-2.29)	.341	84/266
6 y	0.95 (0.57-1.58)	.836	101/266
Breast-feeding ≥ 6 vs < 6 mo			
½ y	1.26 (0.49-3.28)	.633	31/275
1½ y	1.38 (0.65-2.92)	.402	54/284
4 y	1.24 (0.64-2.39)	.528	84/266
6 y	0.76 (0.38-1.51)	.426	101/266

*Logistic regression was used in the analyses calculating the OR of sensitization for each month of prolonged breast-feeding.

†Total of children with complete data on sensitization and breast-feeding at a given age.

TABLE E4. Reverse causation in the COPSAC cohort

	Children with eczema, wheeze, or positive SPT response before ending exclusive breast-feeding	Children with no eczema, wheeze, or positive SPT response before ending exclusive breast-feeding	<i>P</i> value, Wilcoxon rank sum test
No.	29	306	
Exclusive breast-feeding (mo), median (IQR)	5.8 (6.3-4.6)	4.1 (5.2-3.1)	<.0001

P value in boldface indicates statistical significance.

TABLE E5. Association between exclusive breast-feeding and any sensitization, including the 22 never exclusively breast-fed children

Analysis	OR (95% CI)*	P value	No. sensitization/total†
½ y	1.02 (0.86-1.21)	.847	35/294
1½ y	1.05 (0.92-1.21)	.459	60/302
4 y	1.06 (0.93-1.20)	.397	89/281
6 y	0.92 (0.82-1.03)	.145	112/284

*Logistic regression was used in the analyses calculating the OR of sensitization for each month of prolonged breast-feeding.

†Total of children with complete data on sensitization and breast-feeding at a given age.

TABLE E6. Association between exclusive breast-feeding and monosensitized and polysensitized children at 6 years of age

Analysis	OR (95% CI)*	P value	No. sensitization/total†
Monosensitized	0.95 (0.80-1.13)	.559	45/165
Polysensitized	0.97 (0.83-1.15)	.756	56/163

*Logistic regression was used in the analyses calculating the OR of sensitization for each month of prolonged breast-feeding.

†Total of children with complete data on sensitization and breast-feeding at a given age.

TABLE E7. Subanalyses of association between exclusive breast-feeding and sensitization based on sensitization assessment method and type of allergen adjusted for confounders

Analysis	Adjusted OR (95% CI)*†	P value	No. sensitization/total‡
Any sensitization			
SPT ½ y	1.19 (0.92-1.55)	.192	20/284
SPT 1½ y	1.15 (0.92-1.43)	.221	32/282
SPT 4 y	1.11 (0.85-1.45)	.437	24/249
SPT 6 y	1.05 (0.85-1.28)	.669	40/256
sIgE ½ y	1.18 (0.92-1.53)	.195	22/290
sIgE 1½ y	1.09 (0.89-1.34)	.417	34/296
sIgE 4 y	1.08 (0.93-1.25)	.315	78/287
sIgE 6 y	0.97 (0.84-1.12)	.648	92/267
SPT and/or sIgE			
Food ½ y	1.24 (0.97-1.57)	.082	26/243
Food 1½ y	1.21 (0.98-1.47)	.071	41/249
Food 4 y	1.05 (0.88-1.25)	.574	52/255
Food 6 y	0.89 (0.75-1.06)	.190	57/253
Inhalant ½ y	0.85 (0.9-1.24)	.398	8/270
Inhalant 1½ y	1.10 (0.85-1.41)	.475	22/278
Inhalant 4 y	1.14 (0.95-1.36)	.169	54/264
Inhalant 6 y	0.99 (0.85-1.16)	.912	73/255
Milk ½ y	1.27 (0.88-1.82)	.204	11/258
Milk 1½ y	1.20 (0.91-1.60)	.199	21/270
Milk 4 y	0.97 (0.79-1.20)	.808	31/254
Milk 6 y	0.79 (0.64-0.96)	.019	37/250
Egg ½ y	1.25 (0.93-1.67)	.140	15/261
Egg 1½ y	1.22 (0.92-1.63)	.165	21/271
Egg 4 y	0.98 (0.76-1.27)	.866	21/249
Egg 6 y	1.00 (0.77-1.28)	.978	23/250

P value in boldface indicates statistical significance.

*Logistic regression was used in the analyses calculating the OR of sensitization for each month of prolonged breast-feeding.

†Confounder adjusted for mother smoking during the third trimester, mother's age at childbirth, mother's education, household income, older siblings, and father's allergy.

‡Total of children with complete data on sensitization and breast-feeding at a given age.

TABLE E8. Subanalyses of association between exclusive breast-feeding and sensitization based on sensitization assessment method and type of allergen adjusted for reverse causation

Analysis	Adjusted OR (95% CI)*	P value	No. sensitization/total†
Any sensitization			
SPT ½ y	1.08 (0.82-1.44)	.583	16/272
SPT 1½ y	1.10 (0.30-1.40)	.421	24/263
SPT 4 y	1.11 (0.84-1.46)	.483	18/233
SPT 6 y	1.09 (0.88-1.34)	.452	32/240
sIgE ½ y	1.16 (0.87-1.54)	.320	18/277
sIgE 1½ y	1.13 (0.90-1.41)	.302	28/280
sIgE 4 y	1.06 (0.90-1.23)	.502	64/269
sIgE 6 y	0.96 (0.84-1.11)	.611	85/253
SPT and/or sIgE			
Food ½ y	1.19 (0.92-1.55)	.191	22/233
Food 1½ y	1.11 (0.90-1.37)	.319	32/230
Food 4 y	1.03 (0.86-1.24)	.736	44/237
Food 6 y	0.95 (0.80-1.12)	.533	52/238
Inhalant ½ y	0.77 (0.51-1.17)	.224	6/260
Inhalant 1½ y	1.11 (0.84-1.45)	.472	17/260
Inhalant 4 y	1.09 (0.90-1.32)	.386	42/246
Inhalant 6 y	0.96 (0.82-1.12)	.561	65/240
Milk ½ y	1.33 (0.89-1.99)	.162	10/248
Milk 1½ y	1.18 (0.88-1.59)	.257	17/252
Milk 4 y	1.02 (0.82-1.27)	.881	28/237
Milk 6 y	0.83 (0.68-1.00)	.048	34/235
Egg ½ y	1.13 (0.81-1.58)	.479	12/251
Egg 1½ y	1.13 (0.84-1.52)	.410	16/253
Egg 4 y	0.92 (0.70-1.20)	.527	17/232
Egg 6 y	1.07 (0.83-1.38)	.598	21/235

Results were adjusted for reverse causation by excluding children who had eczema, recurrent wheeze, or a positive SPT response during the period of exclusive breast-feeding. *P* value in boldface indicates statistical significance.

*Logistic regression was used in the analyses calculating the OR of sensitization for each month of prolonged breast-feeding.

†Total of children with complete data on sensitization and breast-feeding at a given age.

TABLE E9. Subanalyses of association between exclusive breast-feeding and sensitization based on parental allergy

Analysis	OR (95% CI)*	P value	No. sensitization/total†
Double disposition			
½ y	1.22 (0.93-1.60)	.159	19/163
1½ y	1.11 (0.91-1.37)	.309	35/170
4 y	1.13 (0.93-1.37)	.218	55/164
6 y	1.04 (0.87-1.24)	.707	61/160
Mother with positive sIgE level			
½ y	1.07 (0.85-1.37)	.559	27/166
1½ y	1.13 (0.92-1.40)	.239	42/172
4 y	1.02 (0.85-1.23)	.806	63/171
6 y	0.97 (0.81-1.16)	.735	74/165

*Logistic regression was used in the analyses calculating the OR of sensitization for each month of prolonged breast-feeding.

†Total of children with complete data on sensitization, breast-feeding, and mother's and father's allergy-related diseases status at a given age.

TABLE E10. Association between total breast-feeding and any sensitization

Analyses	OR (95% CI)*	P value	No. sensitization/total†
½ y	1.02 (0.96-1.08)	.622	36/326
1½ y	1.04 (0.99-1.09)	.130	68/328
4 y	0.99 (0.94-1.04)	.591	90/285
6 y	0.98 (0.93-1.03)	.370	111/284

*Logistic regression was used in the analyses calculating the OR of sensitization for each month of prolonged breast-feeding.

†Total of children with complete data on sensitization and breast-feeding at a given age.

TABLE E11. Association between exclusive breast-feeding and eczema, wheeze, and/or asthma and allergic rhinitis with breast-feeding as a categorical variable

Analysis	OR (95% CI)*	P value	No. sensitization/ total†
Breast-feeding ≥ 4 vs < 4 mo			
Eczema 7 y	1.28 (0.72-2.26)	.406	67/304
Wheeze and/or asthma 7 y	0.60 (0.32-1.13)	.116	46/310
Allergic rhinitis 7 y	1.38 (0.65-2.95)	.408	35/270
Breast-feeding ≥ 6 vs < 6 mo			
Eczema 7 y	1.43 (0.72-2.83)	.308	67/304
Wheeze and/or asthma 7 y	1.08 (0.47-2.48)	.852	46/310
Allergic rhinitis 7 y	0.30 (0.07-1.28)	.103	35/270

*Logistic regression was used in the analyses calculating the OR of sensitization for each month of prolonged breast-feeding.

†Total of children with complete data on health outcome and breast-feeding at a given age.