

Increased risk of eczema but reduced risk of early wheezy disorder from exclusive breast-feeding in high-risk infants

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Background: Breast-feeding is recommended for the prevention of eczema, asthma, and allergy, particularly in high-risk families, but recent studies have raised concern that this may not protect children and may even increase the risk. However, disease risk, disease manifestation, lifestyle, and the choice to breast-feed are interrelated, and therefore, analyzing true causal effects presents a number of methodologic challenges. **Objective:** *First*, to assess the effect from duration of exclusive breast-feeding on the development of eczema and wheezy disorders during the first 2 years of life in a high-risk clinical birth cohort. *Second*, to assess any influence from the fatty acid composition of mother's milk on the risk from breast-feeding. **Methods:** We studied disease development during the first two years of life of the 411 infants from the Copenhagen Study on Asthma in Childhood (COPSAC) birth cohort, born to mothers with a history of asthma. We analyzed the effect from duration of breast-feeding before disease onset on the disease risk, avoiding the effect from disease-related modification of exposure (inverse causation). Polyunsaturated fatty acids were measured in breast milk.

Results: Breast-feeding significantly increased the risk of eczema adjusted for demographics, filaggrin variants, parents' eczema, and pets at home (N = 306; relative risk, 2.09; 95% CI 1.15-3.80; $P = .016$) but reduced the risk of wheezy episodes (relative risk, 0.67; 95% CI 0.48-0.96; $P = .021$) and of severe wheezy exacerbation (relative risk, 0.16; 95% CI 0.03-1.01;

$P = .051$). There was no association between the fatty acid composition of mother's milk and the risk of eczema or wheeze. **Conclusion:** The risk of eczema was increased in infants with increasing duration of breast-feeding. In contrast, the risk of wheezy disorder and severe wheezy exacerbations was reduced. There were no significant effects from the fatty acid composition of the breast milk on risk of eczema or wheezy disorders. (J Allergy Clin Immunol 2010;■■■:■■■-■■■.)

Key words: Breast-feeding, eczema, wheezy disorder, infant

Breast-feeding is widely advocated to reduce risk of eczema, sensitization, and wheezy disorders, particularly in high-risk families. This aligns with the understanding that the causes of these diseases should be sought in pregnancy or early infancy because the diseases typically debut in the first months of life. The early diet is one of very few environmental exposures of the young infant, and avoiding nonhuman protein in the diet seems sensible. However, the evidence on such protective effect is ambiguous.¹⁻⁹

We have performed a clinical study to assess the effect from environmental exposures on the development of eczema and wheezy disorders in a high-risk birth cohort. Our previous conventional cross-sectional analysis at 3 years of age raised suspicion that duration of exclusive breast-feeding increased risk of eczema with an odds ratio of 2.80 (95% CI 0.87-9.03), but this was not statistically significant ($P = .10$).¹⁰ Furthermore, as in other previous studies, this observed trend could be biased from inverse causality—that is, disease-related modification of exposure—as well as a number of other methodologic issues that often confound such complicated interrelations. Typically, mothers may prolong breast-feeding after debut of eczema or wheeze in an infant because of the common perception of a protective effect from breast-feeding. This could erroneously bias the conclusion toward prolonged breast-feeding as a cause of disease. Therefore, we developed a novel statistical strategy for such analysis, taking advantage of the longitudinal information on symptom debut and duration of breast-feeding.

Atopic dermatitis has been related to a disturbed metabolism of polyunsaturated fatty acids,¹¹⁻¹³ and we have previously shown that the breast milk from the atopic mothers of this cohort had significantly higher levels of Ω -6 and lower levels of Ω -3 fatty acids than nonatopic mothers.¹⁴ We therefore studied the potential influence of Ω -3 and other fatty acids in breast milk on the risk of eczema and wheezy disorders.

METHODS

The study is reported in accordance with STROBE (Strengthening the Reporting of Observational Studies in Epidemiology).¹⁵

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Abbreviations used

COPSAC: Copenhagen Prospective Study on Asthma in Childhood
RR: Relative risk

Subjects

Copenhagen Prospective Study on Asthma in Childhood (COPSAC) was approved by the Ethics Committee for Copenhagen (KF 01-289/96) and the Danish Data Protection Agency (2008-41-1754), and both parents consented to participation. COPSAC enrolled 411 newborns at the age of 1 month born to mothers with a history of asthma; the recruitment was previously described in detail.¹⁶⁻¹⁹ The families used the clinical research unit (not the family practitioner) for diagnosis and treatment of any skin-related or lung-related symptoms and attended scheduled visits to the clinical research unit every 6 months in addition to visits for any acute symptoms related to skin or airways.

Clinical end points

Eczema was diagnosed by the doctors at the COPSAC research unit according to criteria given by Hanifin and Rajka.²⁰ We previously described the development of skin lesions in detail.^{10,21,22}

Wheezy episode

Wheeze was recorded by the parents in daily diaries since the first month of life. The term *wheeze* was explained to the parents as wheeze or whistling sounds, breathlessness, or recurrent troublesome cough severely affecting the well being of the infant. Wheezy episode was defined from the diaries as an episode of 3 consecutive days with recorded wheeze. This end point definitions was described and validated in previous studies.¹⁷⁻¹⁹

Wheezy exacerbations

Exacerbations were defined as wheezy symptoms treated with high-dose inhaled steroid or oral steroid or leading to hospitalization as previously described.¹⁷⁻¹⁹

Risk assessments

Mothers were asked about breast-feeding cessation at every scheduled and acute visit at the clinical research unit. In the database, breast-feeding was recorded as the duration of exclusive breast-feeding. Mother's milk was collected at the visit 1 month after birth. Milk samples (2-5 mL) were added to 0.01% butylated hydroxytoluene (Sigma-Aldrich Denmark A/S, Brøndby, Denmark) and frozen at -80°C. All milk samples were analyzed within 1 year after they had been sampled. Lipids were extracted and separated by gas-liquid chromatography as previously described.¹⁴ The fatty acid composition of all breast milk samples was determined in duplicate, and all analyses were successful.

Information about the potential confounders sex, race, length and body mass index at birth, eczema history in father and mother, cat and dog at home at birth, mother's smoking in third trimester, and age at the infant's start in day care was collected prospectively, and filaggrin variants R501X and 2282del4 were analyzed as previously described.²³

Statistical analysis

The effect of exclusive breast-feeding (yes/no at any specific age) and duration of exclusive breast-feeding on risk of eczema and wheezy disorder was analyzed by Poisson regression with log person-months as offset. The incidence of first eczema diagnosis and first wheezy episode was estimated as cases per person-month.

To avoid inverse causation, only information on exposures before disease onset was used. Person-months at risk were calculated for the children from birth to age of diagnosis, 2 years of age, or first age of dropout, whichever came first. To avoid conditioning on the future, we evaluated the effect of breast-feeding duration in individuals for whom exclusive breast-feeding ended before disease onset. This was done by analyzing the effect of time since end

of breast-feeding, adjusting for age. The relative risk (RR) of exclusive breast-feeding and time since end of exclusive breast-feeding was assessed and interpreted as a comparison with a child of similar age still breast-fed. The *P* values associated with RR corresponded to Wald tests. All other *P* values corresponded to likelihood ratio tests.

The time variables (age and time since breast-feeding) entered the analysis in 3-month intervals. Robustness of the results was evaluated by redoing the analysis with different groupings of time variables.

In the analysis of eczema we adjusted for sex, birth weight, body mass index, filaggrin, father's and mother's eczema, and cat and dog at home at birth. In the analysis of wheezing episodes, we adjusted for day care and mother's smoking during the third trimester. The confounders were placed in the regression models simultaneously. Effect modification was assessed by likelihood ratio tests comparing models with and without interaction terms.

The correlation between the composition of fatty acids in mother's milk and eczema or wheezy disease was examined by survival analysis. To take into account the duration of fatty acids transmission, we only considered the children as long as they were exclusively breast-fed. Survival analysis was performed with the use of Cox proportional hazards regression. The dependent variable was the time to a first eczema/wheeze diagnosis. The fatty acids were included as the quantity by which they were measured in the breast milk. For this analysis, a child was censored at the end of breast-feeding or when the child was no longer participating in the study, whichever came first. The analyses were adjusted for the same confounders as the analyses of the effects of breast-feeding. Controls for functional form and proportional hazards were performed by using plots and *P* values based on martingales.²⁴

The analysis was done by using PROC GENMOD and PROC PHREG in SAS version 9.1 (SAS Institute Inc, Cary, NC) as well as R version 2.6.1 (www.r-project.org). The overall significance level used was .05.

RESULTS**Breast-feeding**

The COPSAC birth cohort included 411 newborns, of whom 58 were missing information on duration of exclusive breast-feeding. We excluded 11 nonwhite subjects because we adjusted for filaggrin. Twenty-one were never breast-fed and could not be analyzed in the chosen model. The remaining 321 infants (153 boys) were exclusively breast-fed for a mean duration of 121 days (range, 1-274). Sixty-nine infants were exclusively breast-fed less than 3 months, 203 for 3 to 6 months, and 49 for more than 6 months.

Eczema

Eczema was diagnosed in 122 (38%) of the 321 infants before age 2 years. The proportions of children who developed eczema were 29% (20), 37% (75), and 55% (27) in children who were exclusively breast-fed less than 3 months, 3 to 6 months, or more than 6 months, respectively.

The multivariate analyses of eczema only including breast-feeding information before disease onset included 306 infants (6 were missing information on filaggrin variants R501X and 2282del4, 8 were missing information on father's atopic history, and 1 was missing information on pets at home at birth). Eczema was diagnosed in 116 of those infants before they either left the study or turned 2 years of age.

The effect of exclusive breast-feeding on the risk of development of eczema was significant after adjustment for demographics, filaggrin variants R501X and 2282del4 status, parents' eczema, and pets at home (RR, 2.09; 95% CI 1.15-3.80; *P* = .016). In addition, there was a significant effect of duration of exclusive breast-feeding (*P* = .0430), because the RR of eczema was increasing with increasing duration of breast-feeding (Fig 1). Fig 1 illustrates that children who were still breast-fed at a given age had RRs

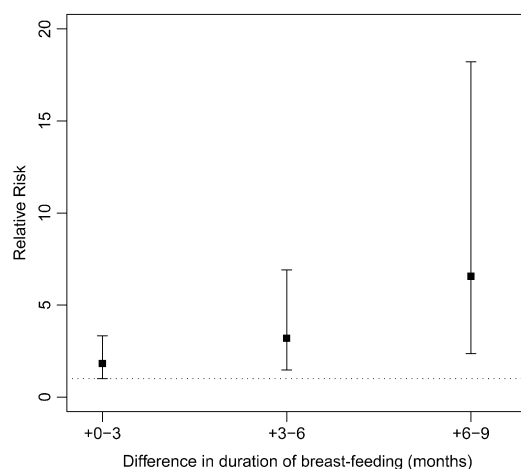


FIG 1. RR of eczema in exclusively breast-fed children compared with children for whom breast-feeding ended earlier. Children of similar age are compared by adjustment for age. The figure illustrates that children who are still breast-fed at a given age have RRs of eczema of 1.82, 3.22, and 6.67 compared with children for whom breast-feeding ended 0 to 3, 3 to 6, and 6 to 9 months earlier—that is, the risk of eczema is increased with longer duration of breast-feeding.

of eczema of 1.82, 3.22, and 6.67 compared with children for whom breast-feeding ended 0 to 3, 3 to 6, and 6 to 9 months earlier, respectively.

Wheezy disorders

Confounder-adjusted multivariate analyses of wheezy episodes included 313 infants (8 were missing information on start in day care). The analysis of severe wheezy exacerbations was not adjusted because of small number of cases and therefore included 321 infants. Wheezy episodes were diagnosed in 262 and severe wheezy exacerbation in 36 infants before they either left the study or turned 2 years of age.

Exclusive breast-feeding reduced the risk of wheezy episodes in the multivariate analysis adjusted for mothers smoking and age at start in day care (RR, 0.67; 95% CI 0.48-0.96; $P = .021$) without any further effect from duration of breast-feeding ($P = .637$). Furthermore, exclusive breast-feeding reduced the risk of severe wheezy exacerbation (RR, 0.16; 95% CI 0.03-1.01; $P = .051$) without any further effect from duration of breast-feeding ($P = .819$).

FATTY ACID COMPOSITION OF MOTHER'S MILK

Breast milk samples were collected from 314 women 25.2 ± 0.9 ($\pm$ SEM) days after birth. The confounder-adjusted multivariate analyses of eczema included 249 infants. Of the 321 infants described, the analyses of fatty acid composition were not possible because of missing samples or technical errors for 62 children, 4 had missing information on filaggrin variants R501X and 2282del4, 5 had missing information on the father's atopic history, and 1 lacked information on pets at home at birth. Eczema was diagnosed in 23 of the infants while they were still exclusively breast-fed. There was a nominal but nonsignificant protective effect from the total Ω -3 fatty acid content in the mother's milk sample (hazard ratio, 0.625; $P > .1$) but no effect of the content of Ω -6 fatty acid (hazard ratio, 0.958; $P > .1$) and trans-fatty

acid (hazard ratio, 1.00; $P > .1$; hazard ratios adjusted for the covariates described).

There were no effects from the fatty acid composition of the breast milk on wheezy disorders.

DISCUSSION

Principal findings

Exclusive breast-feeding was a significant risk factor for development of eczema during the first 2 years of life and the risk of eczema increased in infants with increased duration of breast-feeding. This dose-response relation strengthens the validity of the conclusion. In contrast, the risk of wheezy disorder and severe wheezy exacerbations was reduced during the time the infant was exclusively breast-fed. There were no significant effects from the fatty acid composition of the breast milk on risk of eczema or wheezy disorders, although we found a nominal but nonsignificant protective effect on eczema from the total Ω -3 fatty acid content in the mother's milk sample.

Strength and limitations of the study

We have tried to address the most important of the many intricate methodologic issues that may bias analyses of the association between breast-feeding and disease development.

Disease-related modification of exposure (inverse causality) is a likely interaction between breast-feeding and atopic disease. Debut of symptoms of eczema or wheezy disorder tends to prolong the duration of exclusive breast-feeding because of the general belief in its protective effect.^{25,26} Such inverse causation could be misinterpreted as duration of breast-feeding leading to eczema or wheezy disorder when, in fact, the disorders lead to longer breast-feeding. Cross-sectional studies with retrospective data collection cannot reliably account for the temporal relationship between disease and exposure and are therefore prone to bias because of such inverse causation.²⁵ Indeed, it has been suggested that observational studies may not be able to control effectively for selection bias and inverse causation,⁸ and conclusions from studies unable to take this into account may not be valid.^{4,5,25,27} Risk-specific analyses have been used to attempt avoiding such inverse causality—that is, analyzing the risk of eczema after 3 months of age.²⁸ However, selecting the cases with a late debut after the age of 3 months leads to a major loss of cases for analysis and a biased phenotype excluding infants with early onset. Similarly, another study excluded children with symptoms during the period of breast-feeding.²⁹ The median length of breast-feeding was 5 months, during which period a major proportion of eczema had its debut.²¹ This highly selected analysis excluding children with early symptoms limits the validity of the conclusions from such studies. A recent study avoided the problem of inverse causality by analyzing the risk rate at the time of eczema debut. The effect of length of breast-feeding was subsequently analyzed as the effect from 4-month exclusive breast-feeding on subsequent development of eczema, which is also prone to the bias discussed.³⁰

We avoided such risk of inverse causality in our analyses because accurate prospective data collection of age of disease onset and end of exclusive breast-feeding allowed us to analyze the age-adjusted risk of eczema only including information on breast-feeding before the time of eczema diagnosis and substituting the duration of breast-feeding with time elapsed since end of breast-feeding for a given age. Time elapsed since end of breast-

feeding is a better measure of duration of breast-feeding because at any given time we know only whether the child is still being breast-fed and how long the child had been breast-fed if the breast-feeding has ended. By this approach, we obtain an evaluation of the effect of breast-feeding while ongoing, as well as an unrestricted evaluation of the effect of breast-feeding duration, thereby avoiding conditioning on the future. A similar statistical approach to analysis of concurrent exposure and end point is known from the literature of, for example, smoking versus asthma.³¹

Differential bias regarding baseline characteristics and recall bias of symptoms and exposure is minimized in our single-center study with close prospective visits to the clinical research unit every 6 months as well as at acute skin and respiratory symptoms and a high follow-up rate. Atopy-related symptoms were recorded prospectively in diaries. Diagnosis and treatment were based on standard operating procedures—that is, less prone to misclassification compared to the general medical community. The risk of misclassification because of interobserver variation is otherwise considerable because the clinical course of eczema and wheezy disorders is capricious and the clinical presentation instrumental to the diagnosis, which is based on a complex algorithm of clinical criteria.³² Differentiation against seborrheic dermatitis is one of several differential diagnoses difficult to distinguish in questionnaires. Likewise, the clinical evaluation and perceptions of the terms associated with wheezy disorders are variable among practitioners and caregivers.^{33–36} Therefore, many studies that have classified eczema and wheeze from symptom questionnaires or history of community doctor's diagnoses are prone to misclassification. In contrast, in the current COPSAC study, eczema was diagnosed clinically by doctors working at the research unit on the basis of standard operating procedures, and wheezy symptoms were recorded prospectively in diary cards by mothers with a personal experience of asthma, minimizing misclassification as previously detailed.^{21,17,18}

Lifestyle factors have a strong influence on both breast-feeding practice and risk of atopic disease, acting as potential confounders.³⁷ Therefore, we prepared the current analysis by analyzing a comprehensive set of risk factors¹⁰ including gene-environmental interaction²² on the development of eczema in the COPSAC birth cohort.²¹ In the current analysis, we included as covariates only those risk factors that we had shown to be relevant within this cohort instead of selecting covariates on the basis of a literature search on studies from different settings.

Maternal and paternal heritage may modify the effect of breast-feeding on disease development.^{25,28,29,38} All mothers in the birth cohort had a history of asthma, but in addition, we included parents' eczema, and more specifically, we also used nonfunctional mutations in the major risk gene *filaggrin*²³ as covariates in our analyses as well as testing for any effect modification.

The comparator chosen is critical for the analysis. Many studies have used infants never breast-fed as the comparator.²⁸ However, there are indications that such infants are distinctly different,³⁹ and we have therefore chosen to analyze the effect from varying duration of breast-feeding excluding infants who were never breast-fed (21 of 411 newborns).

The limitation of the study is mainly its external validity, which is limited by the cohort selection of children born of mothers with a history of asthma and all newborns having a gestational age above 35 weeks with no congenital abnormality, systemic illness,

or history of mechanical ventilation or lower airway infection and of white descent. The risk factors identified may be particular to high-risk populations and need replication in unselected populations.

Interpretation

Our previous risk-analysis in this cohort applied a standard cross-sectional analysis of eczema ever by age 3 years and found a nearly 3-fold increased risk from duration of breast-feeding, which did not reach statistical significance. Therefore, we developed the current novel analytical approach taking the longitudinal information on symptom debut and duration of breast-feeding into account and avoiding the risk of inverse causality.

This finding of a significant risk from duration of exclusive breast-feeding is in line with other studies reporting an increased risk of eczema from breast-feeding^{4–7} and a reduced risk of asthma,² but generally the results from previous studies have been contradictory. Systematic reviews with meta-analysis on the risk of eczema¹ and asthma² reported a reduced risk from breast-feeding, particularly in high-risk populations. Since then, a cluster randomized trial in 13,889 infants found no protective effect against asthma or eczema,⁸ and other studies have reported increased risk of eczema^{4–7} and wheezy disorder, asthma, and sensitization³ from breast-feeding in some studies restricted to populations of increased risk.⁹ In contrast, others reported a small protective effect on eczema²⁹ and a dual effect, protecting in high-risk infants but increasing risk in infants without such heredity.³⁰

The indiscriminate use of eczema, sensitization, and asthma as end points has added to the confusion and lack of consensus. Although these diseases are genetically linked, the risk factor profiles are different (as seen in the current study), and extrapolation of evidence on the role of breast-feeding between these diseases is erroneous. Accordingly, our study found a dual effect from exclusive breast-feeding, increasing the risk of eczema while protecting against wheezy disorders in infants with maternal heredity for asthma, suggesting 2 different mechanisms. The increased risk of eczema from breast-feeding was progressively increased with increasing length of the period the child had been exclusively breast-fed. This observation suggests the transmission of a risk factor for eczema in mother's milk, the nature of which may be, for example, cytokines,⁴⁰ immune cells, antibodies, and specific fatty acids, especially the content of Ω -3 fatty acids. In line with this hypothesis, we found a nonsignificant trend suggesting protection against the risk of eczema (but not wheezy disorders) from Ω -3 fatty acid in mother's milk. Previous studies on the role of polyunsaturated fatty acids for the development of eczema and wheezy disorders have been contradictory, some reporting that the Ω -3 to Ω -6 fatty acid ratio was protective for eczema and asthma in at-risk infants,^{13,41,42} whereas others suggested Ω -3 polyunsaturated fatty acids to be a risk factor for allergy in infants of atopic mothers.⁴³ Even though this is the largest cohort to study the effect of fatty acid in mother's milk, the power for these analyses was limited by the number of infants that got eczema while being exclusively breast-fed. Furthermore, the breast milk content of polyunsaturated fatty acids shows large day-to-day variation⁴⁴; it is therefore plausible that the significance of the association would be improved by use of a pool of breast milk samples or a biomarker of the habitual intake in the infants—for example, erythrocyte fatty acid composition—rather than a single breast milk sample. To answer this issue, we are

currently conducting a large-scaled randomized, controlled trial of supplement with Ω -3 polyunsaturated fatty acids during the third trimester in an unselected pregnancy cohort to clarify its potential preventive effect on eczema and wheezy disorder.

Breast-feeding protected infants from wheezy disorders. This is consistent with previous studies reporting a protective effect in cross-sectional populations.⁴⁰ We previously found such an apparent association in an unselected, prospective birth cohort study to be confounded by strong confounders; however, these were adjusted for in the current analysis.³⁷ The mechanism of a possible protection may be speculated to be protection against infections, the main driver of wheezy symptoms in early life. Other studies have found a protective effect from breast-feeding on infant wheeze in the first year of life and associated this with the level of TGF- β 1.⁴²

We have shown an increased risk of eczema but a protective effect on wheezy disorders in infancy from exclusive breast-feeding in a birth cohort born of mothers with asthma followed prospectively in a clinical study. The risk associated with exclusive breast-feeding was not explained by the fatty acid composition of mother's milk, although a trend showed a higher risk of eczema if the mother's milk had low concentrations of Ω -3 fatty acids. Extended breast-feeding for the prevention of eczema should not be recommended to high-risk populations.

We thank the children and parents participating in the COPSAC cohort as well as the COPSAC study team.

Clinical implications: Duration of exclusive breast-feeding increased the infant's risk of eczema, whereas the risk of wheezy disorder was diminished during breast-feeding. This was unrelated to the fatty acid composition of the breast milk.

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