

Perinatal exposure to endotoxin and the development of eczema during the first 6 years of life

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Summary

Background. Previous studies have reported a protective association between high levels of exposure to endotoxin during infancy and the development of subsequent eczema within the first 6 months of life.

Aim. To investigate the association between exposure in infancy to endotoxin from mattress dust and the development of eczema up to age of 6 years in 2166 children participating in the German Influences of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood (LISA) study, an ongoing population-based birth-cohort study.

Methods. Endotoxin levels in house dust samples collected at 3 months after birth were quantified using the kinetic *Limulus* amoebocyte lysate assay. Specific IgE antibodies to common food and aeroallergens were measured using radioallergen sorbent test, fluorenszyme immunoassay (Pharmacia CAP system) when children were 2 and 6 years old. Information on eczema symptoms and physician-diagnosed eczema were collected at each follow-up using a questionnaire.

Results. No association was found between endotoxin exposure from mattresses (the mattresses of each child and their parents were examined) during infancy and the development of eczema symptoms or doctor-diagnosed eczema by 6 years of age (OR = 1.1, 95% CI 0.5–2.3, and OR = 1.1, 95% CI 0.4–3.3, respectively). No association was found when children with only atopic eczema.

Conclusion. Endotoxin exposure during infancy is unlikely to have a large long-term effect on the development of eczema, especially the atopic form.

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Introduction

According to the hygiene hypothesis, lack of early life exposure to infections and microbes increases the risk of allergic diseases.^{1,2} In support of the hygiene hypothesis, previous studies have shown that children growing up on farms have a lower prevalence of hay fever and

atopic sensitization.^{3–5} Studies that have investigated microbial exposure from the farm environment reported that levels of endotoxin, a constituent of the outer membrane of Gram-negative bacteria, is significantly high.⁶ Other studies have shown that exposure to higher levels of indoor endotoxin is associated with a decreased risk of allergic sensitization and disorders in children of preschool and school age.^{7–9} Two cohort studies from the USA have reported a negative association between indoor endotoxin exposure during infancy and the development of eczema in the first year of life.^{10,11} In keeping with these studies, the German Influences of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood (LISA) study also reported that exposure to higher levels of endotoxin during infancy might protect against the development of 'eczema' [parental report of a diagnosis of 'eczema' (unspecified) by a doctor] within the first 6 months of life (OR = 0.50; 95% CI 0.28–0.88), yet no association was found between endotoxin exposure and parental report of a diagnosis of atopic eczema by a doctor (OR, 1.15; 95% CI 0.61–2.14).¹²

One of the reasons for the found inconsistency in the association between endotoxins and eczema in the LISA study was that the classification of 'eczema' was based on parental report of physician diagnosis, and that all eczema outcomes were measured at 6 months, when diagnosis may be difficult and confused with irritant contact dermatitis and other transient forms of eczematous inflammation. We sought to overcome these limitations by exploring the association between exposure to endotoxins in infancy with development of eczema up to the age of 6 years. We used standardized reported eczema symptoms to minimize variations between different doctors' diagnoses and further categorized eczema into atopic and nonatopic eczema on the basis of allergen-specific IgE sensitization.

Methods

Study population

The LISA study is an ongoing population-based birth-cohort study. A detailed description of screening and recruitment has been described previously.¹³ Briefly, parents of neonates admitted to maternity hospitals in Munich, Leipzig, Wesel, and Bad Honnef, Germany were contacted. In total, 3097 neonates were recruited to the study between December 1997 and January 1999, and the participants were not preselected based on family history of allergic disease. House dust samples were collected from the Munich and Leipzig populations

($n = 1467$ and $n = 976$, respectively), and these are the focus of this study. The detailed description of the Munich and Leipzig subcohort has been provided in previous publications.^{9,14,15} The study was approved by the ethics committees of the Bavarian General Medical Council and the University of Leipzig, and parents gave informed consent for their children's participation.

Questionnaire data and definitions of eczema

Information on parental educational level, family history of allergic disease, physician-diagnosed eczema and eczema symptoms were collected using self-administered questionnaires after birth and at follow-up. The questions relating to eczema included two referring to a history of doctor-diagnosed eczema: 'Has your child been diagnosed with allergic or atopic eczema by a doctor in the past 6/12 months?' and 'Has your child been diagnosed with eczema (without any specification) by a doctor in the past 6/12 months?'. These two questions were designated as physician-diagnosed allergic or nonspecific eczema, respectively, at each follow-up. Questions on eczema symptoms that were free from the diagnostic label of the term 'eczema' included: 'Did your child have itchy skin rash?', 'Did the itchy skin rash last at least 2 weeks?', and 'Is there a flexural involvement of the itchy skin rash?'. We regarded a child as positive for eczema symptoms at each follow-up if the parents responded 'Yes' to all three questions (i.e. having itchy skin rash > 2 weeks with flexural involvement in the last 6–12 months).

Both the reported physician-diagnosed eczema and the reported eczema symptoms were used as health outcomes in this study. Furthermore, in a subgroup of the study population for whom blood samples were taken at the ages of 2 or 6 years for serum-specific IgE antibody testing against common allergens (77%), the reported eczema symptoms and physician-diagnosed eczema (of any type) at each follow-up after the age of 1 year were further divided into atopic and nonatopic on the basis of IgE-specific antibodies present or absent in the blood test at 2 or 6 years of age (Table 1).

Collection and analysis of blood samples

Blood samples were collected from 1591 children (73%) and 857 children (40%) at 2 and 6 years of age, respectively. Specific IgE antibodies to common food and aeroallergens were measured using a commercial test (radioallergosorbent test, fluorezyme immunoassay, CAP system; Pharmacia, Freiburg, Germany). At 2 years of age, specific IgE antibodies against inhalant

Table 1 The main definitions of outcomes.

Outcomes	Definition	Information based on
Physician-diagnosed allergic eczema	Parental report of a diagnosis of atopic eczema by a doctor	Questionnaires
Physician-diagnosed eczema without specification	Parental report of a diagnosis of 'eczema' (unspecified) by a doctor	Questionnaires
Eczema symptoms	Itchy skin rash of > 2 weeks with flexural involvement in the last 6/12 months	Questionnaires
Physician-diagnosed eczema with atopy	The child was reported to be diagnosed as having eczema of any form and was found to be sensitized to common inhalant or food allergens in one or both of the blood tests	Questionnaires and blood tests
Physician-diagnosed eczema without atopy	The child was reported to be diagnosed as having eczema of any form and was not found to be sensitized to common inhalant or food allergens in one or both of the blood tests	Questionnaires and blood tests
Eczema symptoms with atopy	The child was reported to have itchy rash of > 2 weeks with flexural involvement and was found to be sensitized to any common inhalant or food allergens in one or both of the blood tests	Questionnaires and blood tests
Eczema symptoms without atopy	The child was reported to have itchy rash of > 2 weeks with flexural involvement and was not found to be sensitized to any common inhalant or food allergens in one or both of the blood tests	Questionnaires and blood tests

Table 2 Baseline information.

Follow-ups	Age of child					
	18 months	2 years	3 years	4 years	5 years	6 years
Physician-diagnosed eczema*	339/1986 (17.1)	342/1959 (17.5)	323/1744 (18.5)	326/1728 (18.9)	272/1600 (17.0)	271/1596 (17.0)
Physician-diagnosed eczema with atopy†‡	109/1672 (6.5)	99/1672 (6.3)	106/1672 (6.3)	96/1672 (5.7)	84/1672 (5.7)	83/1672 (5.0)
Having eczema symptoms§	91/755 (12.1)	111/821 (13.5)	–	97/608 (16.0)	–	79/466 (17.0)
Reported eczema symptoms with atopy¶**	42/1672 (2.5)	37/1672 (2.2)	–	32/1672 (1.9)	–	24/1672 (1.4)
Munich study centre	1271/2004 (63.4)	1262/1977 (63.8)	–	1181/1788 (66.1)	–	1110/1621 (68.5)
Parents have asthma, eczema, or hay fever	1102/1872 (58.9)	1089/1848 (58.9)	–	999/1677 (60.0)	–	909/1523 (60.0)
Parental educational level						
High	1410/1984 (71.1)	1395/1959 (71.2)	–	1290/1771 (72.8)	–	1192/1605 (74.3)
Medium	506/1984 (25.5)	497/1959 (25.4)	–	424/1771 (23.9)	–	361/1605 (22.5)
Low	68/1984 (3.4)	67/1959 (3.4)	–	57/1771 (3.2)	–	52/1605 (3.2)
Male gender	1030/2004 (51.4)	1011/1977 (51.1)	–	918/1788 (51.3)	–	823/1621 (50.8)
Sensitization to common allergens	–	190/1591 (11.9)	–	–	–	265/860 (30.8)

Data are *n*/total *n* (%). *Parental report of a diagnosis of eczema (of any form) by a doctor in the questionnaires; †(the child was reported to be diagnosed as having eczema of any form and was found to be sensitized to common inhalant or food allergens in one or both of the blood tests)/(physician-diagnosed eczema with atopy and participated in one or both of the blood tests but did not report physician-diagnosed eczema); §parental report of their child having an itchy skin rash of > 2 weeks with flexural involvement in the last 6–12 months in the questionnaires; ¶the child was reported to have eczema symptoms and was found to be sensitized to any common inhalant or food allergens in one or both of the blood tests; **eczema symptoms with atopy and participated in one or both of the blood tests but did not report eczema symptoms.

and food allergens including RX1, HX2, MX1, and Mix fx5[®] were tested. At 6 years of age, specific IgE antibodies against inhalant (SX1) and food allergens

(Mix fx5[®]) were tested. Sensitization was defined as having a specific serum IgE titre > 0.35 kU/L to any common allergens.

Collection and analysis of house dust

The house dust samples were collected when the children were 3 months old. In total, 2166 families (89%) recruited from the Munich and Leipzig centre participated. Field workers carried out dust sampling based on a standardized operating procedure.^{9,14} Dust samples were taken from the mattresses on which each child and their parents slept.

The samples were collected using vacuum cleaners equipped with special nozzles (allergen mouthpiece; ALK-Abelló Hørsholm, Denmark). To prevent bacterial growth, the samples were stored at -20°C until extraction. The dust samples plus filter paper were extracted with 0.125 mol/L ammonium bicarbonate plus 0.05% Tween-20 (v/v) for 2 h at room temperature with constant shaking, with an extraction ratio of 1 : 10–1 : 100 (w/v), depending on the amount of sampled dust. House dust endotoxin levels from the dust extracts were quantified using the kinetic *Limulus* amebocyte lysate assay. The endotoxin levels were expressed as surface load (the amount of endotoxin per m^2 of sample surface).

Statistical analysis

To describe the associations between endotoxin exposure in infancy and the development of eczema at follow-up, longitudinal analysis with random effects logistic regression models was used. This approach takes into account the dependence of repeated outcome measures within each subject. The confounders adjusted for in the model were selected based on previous literature and included gender, parental educational level, parental allergy, and study centre. The results were presented as odds ratios with 95% CI for every interquartile range increase in endotoxin exposure. The analyses were performed using SAS software (version 9.1; PROC GLIMMIX procedure; SAS Institute Inc., Cary, NC, USA).

Results

Eczema prevalence

The prevalence of reported physician diagnosed eczema and eczema symptoms are listed in Table 2.

We found a significantly higher prevalence of physician-diagnosed eczema in the Leipzig study population in comparison with the Munich cohort when we looked at children aged > 3 years (22% and 17%, respectively, at 3–4 years; 20% and 15% at 5 years; 20% and 16% at 6 years). However, there was no difference in

the prevalence of reported eczema symptoms between the two study centres (17% and 16%, respectively, at 4 years; 19% and 16% at 6 years).

Endotoxin exposure

Dust samples from 2118 (87%) parents' mattresses and 2098 (86%) children mattresses were available. All the dust samples contained endotoxin above the detection limit of 50 EU/g. The median of the load and concentration of endotoxin in mothers' mattress-dust samples were 2071 EU/ m^2 (25th–75th percentile 595–6919) and 3008 EU/g (1046–7913), respectively, and 1015 EU/ m^2 (330–3022) and 5866 EU/g (2336–14 669) in the children's mattress-dust samples. The correlation between the amount of endotoxin in mothers' and children's mattress-dust samples was 0.4 for both concentration and surface load. The 25% of subjects lost during follow-up up to 6 years of age did not substantially change the distribution of the endotoxin exposure (data not shown).

Endotoxin and eczema symptoms

We failed to find a significant association between exposure to endotoxin during infancy and the development of eczema symptoms up to 6 years of age (Table 3). The eczema symptoms were further divided into atopic and nonatopic on the basis of IgE specific antibodies that presented in one or both blood tests. As shown in Table 3, the magnitude of the odds ratio did not change substantially when atopic children with eczema symptoms were compared with nonatopic children with eczema symptoms, and there was no significant association found.

Table 3 Longitudinal model for the association between endotoxin exposure during infancy and later development of eczema symptoms*.

	Sampling site for endotoxin levels, EU/ m^2 †	
	Parents' mattress	Children's mattress
Eczema symptoms	0.8 (0.4–1.6)	1.1 (0.5–2.1)
Atopy		
Eczema symptoms		
With atopy‡	1.4 (0.4–4.7)	1.6 (0.5–4.9)
Without atopy‡	0.9 (0.2–4.0)	1.8 (0.5–7.3)

*Defined as having an itchy rash of > 2 weeks with flexural involvement, with every interquartile range increase in endotoxin exposure; †mixed model, adjusted for gender, study centre, parental educational level, and parental allergy; OR (95% CI); ‡on the basis of IgE-specific antibodies to common inhalant and food allergens present in the blood test at 2 or 6 years of age.

Endotoxin and physician-diagnosed eczema

There was no association between endotoxin exposure during infancy and the development of physician-diagnosed eczema of any form in children aged > 1 year (Table 4). Reported diagnosed eczema was further divided into atopic and nonatopic on the basis of IgE-specific antibodies in blood samples taken at 2 or 6 years of age. As shown in Table 4, the magnitude of the odds ratio differed when atopic children with diagnosed eczema were compared with nonatopic children with diagnosed eczema. There is a hint of negative association between endotoxin exposure and non-atopic children with diagnosed eczema, but no significant association was found.

The association between endotoxin exposure during infancy and reported diagnosed atopic eczema and eczema without any specification were analysed (Table 4). No associations were found between endotoxin exposure and both forms of physician-diagnosed eczema. However, when we included the data at age of 6 months and 1 year in the analysis, a negative association between endotoxin exposure during infancy and diagnosed eczema without any specification up to age of 6 years was found (OR = 0.7, 95% CI 0.4–1.0).

Discussion

Although our previous study suggested an association between endotoxin levels in early life and the subsequent development of eczema by the age of 6 months, the current study did not confirm such associations

when better definitions of eczema were used and longer-term analysis was carried out. We believe that the definitions of eczema used in this study are more valid than those used in other studies. There is no suggestion of a protective effect of endotoxin exposure on eczema development by 6 years, regardless of how it is defined.

Comparison of results with other studies

Only a few studies have reported an association between endotoxin exposure and the development of eczema. Two cohort studies conducted in inner-city homes in the New York and Boston metropolitan areas found a negative association between endotoxin exposure during infancy and the development of eczema up to age of 1 year.^{10,11} One cross-sectional study reported that exposure to endotoxin was inversely associated with symptoms of asthma-associated atopic dermatitis in school-age children.¹⁶ In our study, no associations between endotoxin exposure during infancy and the reported diagnosis of eczema and eczema symptoms in children aged > 1 year were found. This result conflicts with our previous publication using data from the same cohort at a younger age, and with the two studies conducted in the USA. One of the reasons may be that previous studies have investigated the short-term effect of endotoxin, whereas in our current study, we assessed the long-term effect of endotoxin. Exposure to endotoxin during infancy may only have a short-term effect on the presentation of more transient forms of eczema. A major limitation of our study is that the amount of endotoxin exposure was only measured once, shortly after birth. It has been shown in previous studies that the repeatability of house dust endotoxin measurements is low.^{17,18} We were unable to investigate the short-term effect of endotoxin exposure on the presence of eczema due to the lack of exposure assessment later in life. If there is a large temporal variation of endotoxin exposure at home, as indicated by the literature, and endotoxin only causes a temporary effect on the presence of eczema, then any association between exposure and outcome would only be found if they were assessed at similar times, which may explain why no associations were found in our study.

Definitions of eczema

It is suggested by the Nomenclature Review Committee of the World Allergy Organization that the term 'eczema' describes an aggregation of several skin diseases with certain clinical characteristics, and the term 'atopic eczema' is used to describe eczema in which

Table 4 Longitudinal model for the association between endotoxin exposure during infancy and later development of physician-diagnosed eczema.

	Sampling site for endotoxin levels, EU/m ² *	
	Parents' mattress	Children's mattress
Atopy, defined by blood tests†		
Physician-diagnosed eczema		
With atopy‡	1.2 (0.5–2.7)	1.2 (0.5–2.8)
Without atopy‡	0.6 (0.3–1.4)	0.7 (0.3–1.4)
Physician-diagnosed atopy§		
Atopic eczema	0.7 (0.4–1.3)	1.0 (0.6–1.9)
No specification	0.8 (0.5–1.3)	0.8 (0.5–1.3)

*Adjusted mixed model: OR (95% CI), adjusted for gender, study centre, parental educational level, and parental allergy. †Defined as having participated in at least one blood test and never having physician-diagnosed eczema; ‡on the basis of IgE-specific antibodies to common inhalant and food allergens present in the blood test at 2 or 6 years of age; §defined based on questionnaire information (*n* = 2166).

the underlying inflammation is dominated by an IgE antibody-associated reaction.¹⁹ Therefore, it is difficult to interpret the association we found between endotoxin exposure and eczema without further specification. A questionnaire response or parental report of physician-diagnosed eczema is probably not sufficiently reliable to explore such associations. It is difficult to determine whether physician-diagnosed atopic eczema is truly atopic, i.e. whether the children were sensitized to specific allergens as determined by skin-prick tests or blood tests, or whether they were just phenotypically atopic. The category of physician-diagnosed eczema without specification is also not necessarily a good representation of the so-called 'intrinsic' type of 'atopic' eczema, as such a category is likely to contain many other nonspecific rashes. Therefore, we also used reported eczema symptoms 'itchy skin rash of more than 2 weeks with flexural involvement' with blood test results as a better outcome measure. We did not find any association for eczema symptoms with and without elevated IgE antibodies against common allergens or physician-diagnosed eczema with and without atopy.

Publication bias

Only a few published studies have reported finding associations between endotoxin exposure and eczema prevalence, and most have shown a result that supports the hypothesis.^{10,20} There has always been a speculation that high level of endotoxin exposure in a farming environment is the underlying protective factor that leads to lower prevalence of atopic diseases.^{17,21,22} We suspect that there is a publication bias in favour of publishing studies that report 'positive' findings, and that more studies than reported have investigated an association between endotoxin exposure and the development of eczema, but have found negative results.

Our power calculation showed that our sample size had a 80% power to detect an average of a 3% decrease in reported eczema symptoms in children who are exposed to higher levels of endotoxin during infancy (defined as >3 EU/m², the third tertile, from the mother's mattress dust), with $\alpha = 0.1$. However, the results from our current study need to be interpreted with caution, and a replication of our study in a larger study population is needed. It is also possible that the protective effects of endotoxins only occur in specific genetic subgroups of patients with eczema. For example, Simpson *et al.* in 2006 reported significant gene-environment interaction between endotoxin exposure and the *CD14* gene in people with the eczema phenotype in the Manchester Asthma and Allergy Study. In a

cross-sectional study, it was found that increasing endotoxin exposure was associated with a reduced risk of eczema at 5 years of age in children with the CC allele at position -159 of the *CD14* gene. Unfortunately, we did not collect such genotype information in our study and no replication of Simpson's observation has been published to date. However, we cannot exclude the possibility that there is an association between endotoxin exposure and the development of eczema that is modified by certain genetic variants. Future studies are needed to explore such gene-environment interactions, particularly in relation to filaggrin gene mutations, which may be critical for skin-barrier function in early life.²³

Limitations

One difficulty in a longitudinal cohort study is to lose participants during the follow-ups. Our data showed that there is a higher follow-up rate of children who were from the Munich study centre and children whose parents are highly educated. Although sensitivity analysis showed that excluding those who were lost during the follow-ups did not substantially change the effect estimates of allergen exposure on the development of eczema symptoms (data not shown), the results should be interpreted with caution.

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