Sleeping on animal fur is related to asthma outcomes in later childhood

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ABSTRACT   Animal furs might represent a "proxy" for high and diverse microbial exposures within a critical time window of immune development. We assessed whether sleeping on animal fur shortly after birth is associated with asthma and atopy up to the age of 10 years.

LISAplus participants (n=2441) from Munich and Leipzig, Germany, were included in the analysis. Animal fur exposure, cofactors and health outcomes were obtained periodically up to 10 years of age by parental questionnaires. Information on specific IgE to aeroallergens was available at 10 years. Cytokine-producing peripheral T-cells were assessed in a subgroup of children at 2 and 3 years. Confounder-adjusted associations were evaluated using logistic regression analyses.

Sleeping on animal fur was very common (55%). In adjusted logistic regression analyses, sleeping on animal fur was inversely associated with recurrent early wheezing at 4 years (adjusted OR 0.75, 95% CI 0.61–0.93) and current asthma at 6 years (adjusted OR 0.56, 95% CI 0.31–1.01). Furthermore, sleeping on animal fur during the first 3 months of life was significantly associated with a persistently stimulated interferon-γ response until the age of 3 years.

Animal fur could be an effective measure of creating environments associated with higher microbial exposure.

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Use of animal fur could be effective for creating environments associated with possibly higher microbial exposure http://ow.ly/IWhTp

Introduction

Over the past 20 years, studies in rural environments have consistently documented inverse relationships of exposure to traditional farm environments with the development of asthma and allergies in children. It has been suggested that ongoing contact with livestock, contact with animal feed, and the consumption of unprocessed cow's milk during pregnancy and infancy might be particularly decisive for the observed protective effects [1]. Subsequently, the "farm environment" has been more specifically described by measuring microbial agents in settled house dust samples [2–4]. In this context, there is a common consensus that the time-window comprising pregnancy and early infancy seems to be crucial, where the biological diversity might have the potential to shape the child's immune response by upregulating the innate immunity receptors [1]. Following these observations, some birth cohort studies have investigated whether the findings from farm and rural environments can be transferred to urban environments. Thus far, not only inverse [5–8] but also positive associations, or no associations at all [9–13], between exposure to measured biocontaminants in settled dust and respiratory and allergic health outcomes have been reported. The reasons for the inconclusiveness are not entirely clear; however, it has been suggested that the microbial profiles in urban environments might differ considerably from those in rural areas in composition and diversity [14].

Nevertheless, the remarkable observations made by the so-called farm studies have all started with a simple "proxy" measure ("growing up on farms") characterising a specific microbial environment. Very recently, within the German Multizentrische Allergie Studie (MAS) birth cohort study of early-life determinants of asthma, sleeping on animal fur at 3 months of age was observed to be (not significantly) protective against asthma up to the age of 20 years [15]. We therefore hypothesise that animal furs (*e.g.* sheepskins) might have the potential to represent a proxy for high and diverse microbial exposures [16], with the exposure occurring within a critical time-window of immunity. Bacterial endotoxin in mattress dust sampled at 3 months of age was additionally considered as a further source of exposure to biocontaminants. We aimed to investigate whether sleeping on animal fur early in infancy is associated with immune system development and asthma outcomes as well as atopic sensitisation up to 10 years of age.

Methods

Study design and participants

LISAplus (The Influence of Life-Style Factors on the Development of the Immune System and Allergies in East and West Germany plus the Influence of Traffic Emissions and Genetics Study) is an ongoing, population-based birth cohort study with four centres in Germany (Munich, Leipzig, Bad Honnef and Wesel). Screening, recruitment and exclusion criteria have been described elsewhere in detail [17, 18]. In short, a total of 3094 healthy, full-term neonates were recruited between December 1997 and January 1999. Only healthy, full-term neonates with a gestational age ⩾37 weeks were included in the study. For the current investigation, only children from Munich and Leipzig for whom information on animal fur and house dust samples at 3 months of age was available were included (n=2441). In the final models, the sample size varied between 719 and 1800 children, depending on the included variables.

Exposure and health outcome information

Exposure, health outcome and risk factor information was obtained periodically (at 6, 12, 18 and 24 months, and 4, 6 and 10 years, of age) by parent-completed questionnaires. Exposure to animal fur at 3 months of age was reported by the parents using the question: "Did your child lie on animal fur during the first 3 months of life?". "Recurrent early wheeze" up to the age of 4 years, as a risk factor for later asthma (definition according to LYNCH *et al.* [19]), was based on at least three wheezing episodes, with at least one episode occurring in the fourth year. "Current asthma" at the age of 6 and 10 years was defined as fulfilling at least two out of three parent-reported conditions: 1) physician-diagnosed asthma ever; 2) reported wheezing symptoms in the past 12 months, both based on the ISAAC (International Study of Asthma and Allergy in Childhood) core questions [20]); and 3) reported asthma treatment in the past 12 months [21, 22]. Controls were defined as "complete" controls and had information available for all time-points. In addition, lifetime prevalence of asthma was defined as parent-reported, doctor-diagnosed asthma until the age of 10 years. Sensitisation to aeroallergens at 10 years was defined as positive if specific IgE to the sx1 inhalant mixture (timothy, rye, mugwort, mite, cat, dog and mould mixture) was ⩾0.35 $kU \cdot L^{-1}$.

Measurement of cytokines

In a subgroup of 276 2-year-old children from Munich and Leipzig of the LISA cohort, we had the possibility to investigate the association of sleeping on animal fur with cytokine-producing peripheral T-cells at the age of 2 years and for 376 3-year-old children from the Leipzig subgroup at the age of 3 years. Cytokine-producing peripheral T-cells were analysed by intracellular cytokine staining, as described previously [23]. Within 6 h after blood was drawn, whole blood samples were stimulated with phorbol ester 10 $ng \cdot mL^{-1}$ (Sigma Aldrich, Deisenhofen, Germany) and ionomycin 1 µM (Calbiochem, Bad

Soden, Germany) in the presence of monensin 2.5 µM (Sigma Aldrich) for 5 h at 37°C and 5% CO_2. After fixation for 10 min at 4°C with 4% paraformaldehyde and permeabilisation with 0.1% saponin, cells were stained with fluorescence-labelled monoclonal antibodies against the T-cell surface marker CD3 and the human cytokines interferon (IFN)-γ, tumour necrosis factor (TNF)-α, interleukin (IL)-2 and IL-4 (all antibodies from Beckmann-Coulter, Krefeld, Germany). Fluorescence-labelled cells were analysed by flow cytometry (FACSCalibur; Becton Dickinson, Heidelberg, Germany) using the CELLQUEST software. Data from 10 000 cells were collected. Results are presented as percentages of cytokine-producing T-cells of the respective cell population. For comparability, all cytokine samples were finally analysed in the same laboratory (Leipzig); blood samples of the Munich children were stimulated and fixed in Munich, and then shipped to the cytokine detection laboratory overnight.

Endotoxin in settled dust from mothers' and children's mattresses at 3 months of age

Bacterial endotoxin was measured in settled dust from mothers' and children's mattresses when the children were 3 months old. A detailed description of the dust sampling and analysis procedures has been given elsewhere [17, 24]. Endotoxin content was quantified by using a quantitative chromogenic kinetic *Limulus* amoebocyte lysate test (Kinetic-QCL; Bio Whittaker, Darmstadt, Germany) at 37°C. The limit of detection was 3.5 ng·g^{-1} dust.

Statistical analysis

The distribution of bacterial endotoxin in settled dust (loads and concentrations) was best described by a log-normal distribution. Linear regression models were used to describe associations between sleeping on animal fur and endotoxin levels in mothers' and children's mattress dust. Results were expressed as mean ratios with 95% confidence intervals.

Ordinal logistic regression was used to analyse associations between animal fur exposure in the first 3 months of life and recurrent early wheeze up to the age of 4 years. Binomial logistic regression was performed for associations between the exposure to animal fur and the remaining health outcomes until the age of 10 years. The results from the logistic regression are presented as adjusted odds ratios (aORs) with corresponding 95% confidence intervals. Model 1 was adjusted for sex and study region only; model 2 was additionally adjusted for parental allergy (mother or father: yes to "ever asthma", "ever hay fever" or "ever eczema"), parental education (high *versus* low/medium), pets at birth and the interquartile range (IQR) increase of bacterial endotoxin loads (EU·m^{-2}) in children's mattresses. Multiple linear regression models, adjusted for the confounders included in model 2 were used to describe associations between sleeping on animal fur and the number of cytokine-producing T-cells in peripheral blood. Results were expressed as mean ratios with 95% confidence intervals.

All statistical analyses were performed using the statistical software R, version 2.14.1 (R Foundation for Statistical Computing, Vienna).

Results

The population characteristics as well as the median concentrations of bacterial endotoxin in settled dust from mattresses are described in table 1. In total, 2441 children were initially included in the analysis, 1375 (56%) of whom were followed up to 10 years of age. Children included in this study differed significantly from those who were not followed up until the age of 10 years with regard to high parental education (included, 75%; not followed up, 57%), exposure to animal fur (included, 58%; not followed up, 49%) and pets at home at the time of birth (included, 29%; not followed up, 35%). More than half of the children included in the study were born in Munich (60%), the remainder being born in Leipzig (40%). The prevalence of parental allergy was 57% and high parental education was reported for 66% (low/ medium, 34%) of subjects. At the time of birth, 27% and 29% of participants were exposed to mould and pets in the home environment, respectively. Sleeping on animal fur during the first 3 months of life, as reported by the parents, was very common (55%). Median loads and concentrations of bacterial endotoxin in settled dust were higher in children's compared with mothers' mattresses. Of the children included, 3% were affected by recurrent wheeze up to the age of 4 years. Current asthma at 6 years was reported for 3% and was slightly increased at 10 years, to 5%. The lifetime prevalence of asthma until the age of 10 years was 9%. In total, 42% of the children were sensitised to at least one aeroallergen at 10 years. As reported in table 2, sleeping on animal fur was more prominent among participants from Munich compared to those from Leipzig (64% and 40%, respectively). Furthermore, the use of animal fur was more frequently reported among highly educated parents (62%) and parents with allergies (57%). The results in table 3 indicate that children who slept on animal fur in the first 3 months of life had significantly higher endotoxin loads (EU·m^{-2}), measured in settled dust from children's mattresses at the age of 3 months, compared to those children who were not sleeping on animal fur (mean ratio 1.18, 95% CI 1.03–1.36; p=0.02). No associations were observed for endotoxin levels in dust from mothers' mattresses. Therefore,

TABLE 1 Characteristics of the LISAplus study population	
Study population at birth n	2441
Females	1187/2441 (49)
Study region	
Munich	1465/2441 (60)
Leipzig	976/2441 (40)
Parental allergy[#]	1294/2253 (57)
Parental education	
Low/medium	815/2413 (34)
High	1598/2413 (66)
Mould at home, at birth	637/2318 (27)
Pets at home, at birth	677/2135 (29)
Animal fur exposure during first 3 months	1265/2320 (55)
Median endotoxin in settled dust at 3 months of age[¶]	
Child's mattress $EU \cdot m^{-2}$	1030
Child's mattress $EU \cdot mg^{-1}$	5850
Mother's mattress $EU \cdot m^{-2}$	2068
Mother's mattress $EU \cdot mg^{-1}$	3002
Health outcomes	
Recurrent early wheeze 4 years[+]	55/1735 (3)
Transient	602/1801 (33)
Recurrent	59/1801 (3)
Current asthma	
6 years of age	54/1733 (3)
10 years of age	64/1338 (5)
Ever asthma at 10 years of age	102/1187 (9)
Specific IgE to aeroallergens at 10 years of age[§]	344/827 (42)

Data are presented as n/N (%) unless otherwise stated. [#]: asthma, hay fever, eczema (father or mother, asked at birth of child); [¶]: n=2155; [+]: three or more episodes; [§]: specific IgE ($\geqslant$0.35 $kU \cdot L^{-1}$) to sx1 (timothy, rye, mugwort, mite, car, dog and mould mixture).

endotoxin loads in settled dust from children's mattresses, expressed per IQR, were considered a potential confounder or effect modifier in the adjusted association analyses.

The results of the adjusted logistic regression analyses for the associations between animal fur exposure during the first 3 months of life and health outcomes up to 10 years of age are shown in table 4. In the fully adjusted model (model 2), animal fur exposure during the first 3 months of life was significantly inversely associated with the prevalence of recurrent early wheeze and borderline significantly associated with current asthma at the age of 6 years (aOR 0.75 (95% CI 0.61–0.93) and aOR 0.56 (95% CI 0.31–1.01), respectively). The observed effects were independent from exposure to bacterial endotoxin in children's mattresses, and there was in addition no statistical evidence for an interaction between exposure to animal fur, bacterial endotoxin and the assessed health outcomes (data not shown). The negative relationship between early exposure to animal fur and health outcomes persisted when analysing the subgroup of

TABLE 2 Determinants of animal fur use in LISAplus	
Exposure to animal fur at 3 months of age	1265/2320 (54)
Study region	
Munich	910/1422 (64)
Leipzig	355/898 (40)
Sex	
Female	628/1124 (53)
Male	637/1196 (56)
Parental education	
Low/medium	282/733 (38)
High	971/1562 (62)
Parental allergy	
Yes	708/1245 (57)
No	486/906 (54)

Data are presented as n/N (%).

TABLE 3 Associations between animal fur use and endotoxin levels in settled dust from mattresses

	Endotoxin in child's mattress dust		Endotoxin in mother's mattress dust	
	$EU \cdot m^{-2}$	$EU \cdot mg^{-1}$	$EU \cdot m^{-2}$	$EU \cdot mg^{-1}$
Use of animal fur yes *versus* no	1.18 (1.03–1.36)[#]	0.98 (0.87–1.10)[¶]	1.05 (0.91–1.22)[+]	1.07 (0.94–1.21)[§]

Data are presented as mean ratio (95% CI). n=2155. [#]: p=0.02; [¶]: p=0.70; [+]: p=0.50; [§]: p=0.30.

children with allergic parents. All investigated relationships between sleeping on animal fur with health outcomes became significant, except the association with current asthma at 10 years of age (early recurrent wheeze: aOR 0.74, 95% CI 0.56–0.96); current asthma at 6 years of age: aOR 0.39, 95% CI 0.19–0.80; current asthma at 10 years of age: aOR 0.52, 95% CI 0.27–1.01); ever asthma at 10 years of age: aOR 0.56, 95% CI 0.33–0.97; sensitisation to inhalant allergens at 10 years of age: aOR 0.65, 95% CI 0.44–0.97). Further, we additionally tested whether there was an association between early exposure to bacterial endotoxin alone and adjusted for the confounders used in model 2; however, we did not observe any statistically significant association with any of the health outcomes later in childhood (data not shown).

Within the subgroup of Munich and Leipzig children with cytokine measurements, the multiple linear regression models showed that sleeping on animal fur in the first 3 months of life was associated with a significant increased number of IFN-γ-producing T-cells in peripheral blood at the age of 2 years (adjusted mean ratio (aMR) 1.21, 95% CI 1.00–1.47; p=0.05). At the age of 3 years, only blood samples and cytokine data were available for the subcohort from Leipzig. In these children, a persistent stimulation of IFN-γ-producing peripheral T-cells was observed (aMR 1.17, 95% CI 1.06–1.29). For TNF-α-, IL-2- and IL-4-producing T-cells, no significant effects of sleeping on animal fur in the first 3 months of life was found.

Discussion

The results of the present study suggest that sleeping on animal fur during the first 3 months of life may confer protection against recurrent early wheeze and asthma outcomes in later childhood, in particular with persisting effects at early ages. These effects were independent of exposure to higher levels of bacterial endotoxin measured in settled dust from children's mattresses. The inverse effects of sleeping on animal fur became significant for all investigated health outcomes except for current asthma at 10 years when looking at children from allergic parents only. In addition, sleeping on animal fur has been also associated with a significant increase in IFN-γ-producing peripheral T-cells until the age of 3 years in a subgroup of the investigated children.

This study in Germany used animal fur specifically as a proxy for suggested diverse exposure to various biological agents in early infancy during a critical period of immune development. To date, there has been only a few studies in Australia and New Zealand, and one in Germany, which addressed early animal fur exposure in the context of asthma and allergies in childhood as a result of the high asthma rates [20]. Considering this, sleeping on animal fur has been investigated as a possible risk factor for asthma and

TABLE 4 Results of the logistic regression for the associations between animal fur exposure during the first 3 months of life and recurrent early wheeze, (current) asthma outcomes at 6 and 10 years, and sensitisation to aeroallergens 10 years of age

	Recurrent wheeze at 4 years of age[#]	Current asthma		Ever asthma at 10 years of age	IgE to aeroallergens 10 years
		6 years of age	10 years of age		
Animal fur					
Model 1[¶]	0.71 (0.58–0.86) (n=1800)	0.57 (0.33–1.00) (n=1732)	0.64 (0.38–1.08) (n=1338)	0.74 (0.49–1.12) (n=1187)	0.83 (0.62–1.11) (n=827)
Model 2[+]	0.75 (0.61–0.93) (n=1561)	0.56 (0.31–1.01) (n=1497)	0.63 (0.36–1.11) (n=1152)	0.72 (0.46–1.12) (n=1032)	0.83 (0.61–1.14) (n=719)
Subgroup analysis of children of allergic parents at 10 years of age	0.75 (0.61–0.93) (n=940)	0.39 (0.19–0.80) (n=898)	0.52 (0.27–1.01) (n=689)	0.65 (0.33–0.97) (n=627)	0.65 (0.44–0.97) (n=433)

Data are presented as adjusted OR (95% CI). [#]: three or more episodes; [¶]: adjusted for sex and study region; [+]: adjusted for sex, study region, interquartile range increase of endotoxin ($EU \cdot m^{-2}$) from children's mattresses, parental allergy, parental education and pets at home at birth.

atopic sensitisation due to the suggested higher mite exposure. Indeed, Trevillian et al. [25] observed that having sheepskin under the bedding during infancy was a risk factor for sensitisation to house dust mites at age 8 years among Australian children. A case–control study from New Zealand reported that any sheepskin use in the first year of life was associated with an increased risk of asthma between 7 and 9 years of age, especially among atopic children [26]. Conversely, two further studies from New Zealand found no association with later-life asthma and allergic sensitisation [27, 28]. Recently, the German MAS birth cohort studied possible early-life determinants of asthma in young adulthood and, surprisingly, sleeping on animal fur at 3 months of age was associated with a (statistically nonsignificant) decreased risk of asthma at 20 years of age [15].

In general, little is known about the true exposures represented by animal fur. Unfortunately, we did not perform any measurements within the animal fur; however, apart from increased loads of bacterial endotoxin in mattresses of those children who were sleeping on animal skin, levels of mite allergens Der p 1 and Der f 1 in dust from children's mattresses were also significantly increased in those exposed to animal skin (Wilcoxon–Mann–Whitney test, $p<0.001$; data not shown). There was one study that determined that house dust mite allergen (Der p 1) levels rapidly increased within new sheepskins on mattresses and on living-room floors. After cleaning with warm water and dry-cleaning, Der p 1 accumulation on sheepskins was even higher after a further 6 weeks [16]. According to what has been found in settled dust or airborne dust in indoor environments, other compounds might also accumulate on animal fur over time. "Bio-aerosols", such as bacteria, fungi, pollen, viruses and their byproducts, as well as fragments from living organisms, such as allergens, occur in communities and are ubiquitous in indoor air [29]. Therefore, the microbial milieu within the animal fur is suggested to be dependent on the home environment, housing and personal characteristics of the inhabitants [30, 31]. In addition to this, the use of synthetic bedding above feather bedding has been considered as a possible risk factor for respiratory symptoms in children in some previous studies due to higher levels of mite allergens [32, 33]. Unfortunately, we were not able to test whether the type of bedding material may confound the association between sleeping on animal fur in the first 3 months of life and later respiratory outcomes as a major extent of the included children used different type of materials for bedding simultaneously. Therefore, we cannot rule out that the type of bedding might have an effect, of unknown impact, on asthma and allergic outcomes later in childhood.

Microbial exposure has been shown to contribute to stimulated immune reactivity in the postnatal period and, in particular, an increased Th1 response [1, 34]. It has been discussed that a reduced capacity to produce IFN-γ is an intrinsic feature of atopy and one possible reason for an enhanced susceptibility to develop allergic disorders. A characteristic feature of atopy is the over-expression of the Th2 cytokines IL-4, IL-5 and IL-13, which are involved in the induction and maintenance of the IgE synthesis. The importance of Th1 cytokines like IFN-γ in atopy development is to antagonise the effect of Th2 cytokines and prevent the development of allergic inflammation [1]. Although in the past decade, further T-cell populations, in particular regulatory T-cells [35, 36] were described to be involved in the regulation of allergic inflammation, the protective role of IFN-γ in atopy is still accepted. Data from animal studies clearly demonstrate that the asthma-protective effect induced by microbial exposure depends on IFN-γ [37, 38]. In our study, we demonstrate that sleeping on animal fur in the first 3 months of life contributes to persistently higher amounts of IFN-γ-producing peripheral T-cells at least until the age of 3 years in a subgroup of the study population. This stimulation of a protective Th1 response could provide a mechanistic explanation for the observed asthma-protective effect of sleeping on animal fur.

Nevertheless, a few limitations must be considered when interpreting our results. First, apart from the type of animal fur, it was not possible to determine the actual exposures in the animal fur or its microbial profile. However, as sheepskin has been the most common used during this time, we can only guess that the infants were most likely sleeping on an animal fur based on sheepskin material. Second, we did not have data on the intensity of exposure. However, during the first 3 months of life, the exposure is probably more intense than later in infancy. Third, only children for whom animal fur exposure and endotoxin level information was available were included. Of these children, only 56% participated until the 10-year follow-up. In addition, we cannot completely rule out that the observed inverse effects require a constant signal of exposure to microbial components and cannot exclusively attributed to the crucial period shortly after birth. That might explain why the inverse effects were attenuated later in childhood. With increasing age, the school environment and activities conducted in different places might start to become more important and the daily individual microbial exposure may change in composition [39, 40]. Lastly, we cannot rule out residual confounding, despite cautiously adjusting for parental education. The use of animal skin might be a lifestyle factor, which is difficult to assess more specifically. Since we observed more common use of animal skin in more educated families and since asthma is more common in better-educated families in Germany [41], the reported protective effect of sleeping on animal skin on asthma is not likely to be confounded by social factors.

In conclusion, the use of animal fur during the first 3 months of life was associated with a reduced risk of asthma outcomes later in childhood and within a subgroup of the study population it was also associated with a stimulated Th1 reactivity. Animal fur exposure could act as a proxy, and might represent a close and more intense contact with diverse and higher bioaerosol concentrations, leading to similar immune-stimulatory and, thereby, protective mechanisms in relation to asthma and allergy as has been observed for farm and rural environments. Therefore, the use of animal fur could be an effective measure of creating environments associated with higher microbial exposure. Further studies are needed to investigate the actual microbial milieu within animal fur in order to validate the findings with objective measurements.

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