



Domestic allergens and endotoxin in three hospitals offering in-patient rehabilitation for allergic diseases in the alpine mountain climate of Bavaria – The AURA study[☆]

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Abstract

Endotoxins and allergens represent the major relevant contents of the atmospheric bioaerosol with regard to the triggering and exacerbation of allergic diseases. In this study, mattress concentrations of endotoxin and indoor allergens were measured in three hospitals in the alpine climate of Bavaria and in adjacent homes. Dust was collected from each of 10 mattresses according to a standardized protocol, and endotoxin was analyzed with the Limulus Amebocyte Lysate (LAL) test, indoor allergens Der p 1, Der f 1 and Fel d 1 were analyzed by ELISAs.

The concentration of endotoxin in the mattresses did not differ significantly between different cities. The percentiles of endotoxin were significantly higher in hospitals than in homes. The concentrations of mite allergens (Der p 1 and Der f 1) in the dust were significantly lower in all hospitals than in homes. There was no significant difference of mite allergens between different time points.

The concentrations of Fel d 1 were significantly higher in the autumn than in the summer (median: 1376 vs. 478 ng/g). No significant differences of Fel d 1 were found between hospitals and homes or between different hospitals.

As Fel d 1 concentrations reached levels at which cat allergic patients can experience symptoms, efforts had to be made to reduce the concentrations of Fel d 1 especially in hospitals. In contrast, mite allergens were low in hospitals, which can be clinically beneficial for patients with mite allergies.

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Keywords: Endotoxin; Der p 1; Der f 1; Fel d 1; Indoor allergens

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Introduction

Exposure to indoor allergens may be a primary cause of asthma in sensitized patients (Sporik et al., 1992). Also continued exposure to endotoxins may be pro-inflammatory and increase the expression of asthma (Michel et al., 1997). For patients with atopic eczema it has been proven by history and the atopy patch test that indoor allergen exposure can contribute to an exacerbation of eczematous skin lesions (Darsow and Ring, 2002; Ring et al., 2001). In severe cases of asthma and atopic eczema, a hospital rehabilitation can lead to a significant improvement of symptoms and disease-related parameters (Baur et al., 2002; Schultz et al., 2002; Simon et al., 1999; Weinstein et al., 1996). Some of these hospitals specialized in this kind of treatment are situated in special climatic conditions (high altitude, alpine climate, North sea), which might influence the allergen levels (Vervloet et al., 1982). The aim of this study was to investigate the mattress concentrations of domestic allergens (mite-Der p 1, mite-Der f 1, cat-Fel d 1) and endotoxin in three rehabilitation hospitals in the Bavarian mountain climate. In order to identify regional or local distinctions, the hospital values were compared to the values of regular houses in the same geographical area.

Methods

AURA study

The “Allergien und Umweltkrankheiten in der Rehabilitation im Alpenraum – Allergies and Environmental Diseases in Rehabilitation in the Alps” (AURA) study on the effects of Bavarian mountain climate therapy on allergic diseases was initiated in 2002. The goal of the AURA study was to explore the conditions for rehabilitation procedures in allergy treatment. The levels of indoor allergens and bacterial endotoxin were assessed in the participating hospitals and adjacent homes. Of the participating hospitals one was situated in Oberjoch (1200 m alt., on a high plateau at the Jochpass), one in Pfronten (853 m alt., in southeast Allgäu) and one in Berchtesgaden (1000 m alt., in southeast Germany). All of these hospitals are specialized in rehabilitation of allergic and inflammatory diseases of skin and airways (especially asthma and atopic eczema). The hospitals provide adult (Berchtesgaden, Pfronten) and pediatric care (Berchtesgaden, Oberjoch).

Dust collection and extraction

Endotoxin, house dust mite and cat allergen levels were measured in the dust collected from each 10 mattresses in each of the three hospitals and from

adjacent homes. The type and the age of the mattresses as well as the presence of domestic animals were recorded. All samples were collected in November 2003 and August 2004 (before and after the winter when the heating system is turned on).

Dust samples were collected on fiber filters using vacuum cleaners with sampling nozzles (ALK, Horsholm, Denmark) according to a standardized protocol. The filters were supported in a plastic dust trap located behind the cleaner attachment (nozzle). The whole area of the mattress was sampled for 5 min. Dust mite-proof covers which were used in the hospitals of Pfronten and Berchtesgaden were not removed. One hundred milligrams of fine dust was extracted with 2 mL PBS-T (0.05% Tween 20 in phosphate buffered saline, pH 7.4). The dust was resuspended using a vortex mixer. Samples were mixed end-over-end on an orbital rotator for 2 h at room temperature and then centrifuged for 20 min at 2500 RPM at 4 °C. Supernatants were removed and stored at –20° for future analysis of allergen content.

Analysis of endotoxin

Endotoxin was analyzed with the kinetic chromogenic Limulus Amebocyte Lysate (LAL) test, using the same batch of LAL reagents and standards for all analyses (Cambrex; Walkersville, Maryland, USA).

ELISA measurement of indoor allergens

Der p 1, Der f 1 and Fel d 1 were assayed using a two-site monoclonal antibody ELISA (INDOOR Biotechnologies, Cardiff, UK).

Statistical analysis

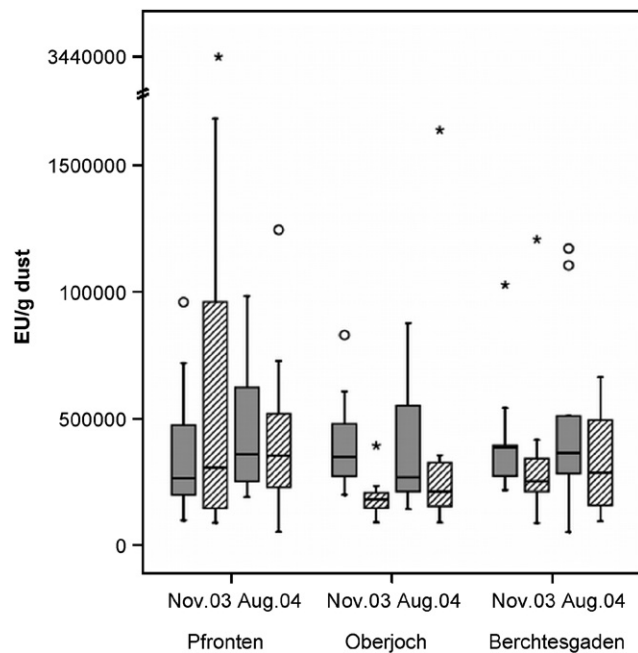
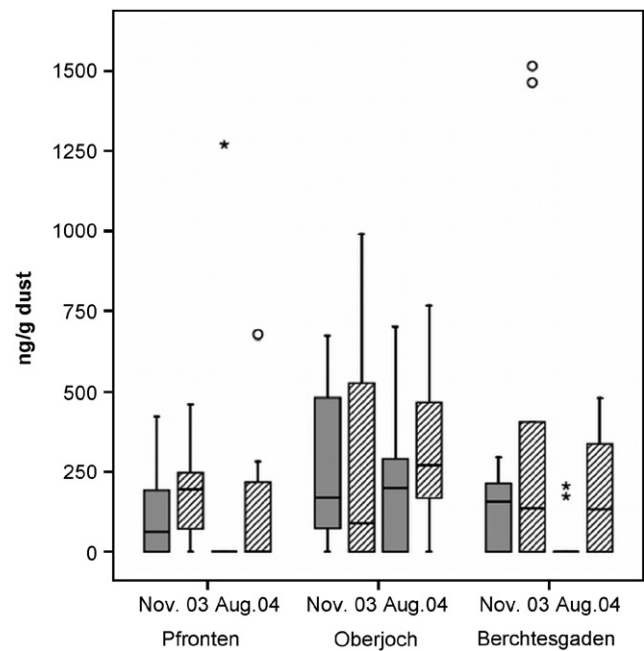
Results are expressed as the median and interquartile range (IQR) as the measurement data did not show normal distribution. The Mann–Whitney *U*-test and the Kruskal–Wallis test were used to compare the levels of allergens and LPS in different sites and at different time points. Adjustment for multiple testing was performed using Bonferroni's correction. Statistical significance was set at a conventional level of <5%. All the tests were done two-tailed. Statistical analysis was performed using SPSS 13.0 for Windows (SPSS, Chicago, IL, USA).

Results

Table 1 shows the number of samples collected, the age of the mattresses, and the presence of domestic animals. Sixty mattresses were sampled at two different time points (120 samples).

Table 1. Age of mattresses (November 2003) as well as presence of domestic animals at the different hospitals and homes

	Pfronten		Oberjoch		Berchtesgaden	
	Hospital	Homes	Hospital	Homes	Hospital	Homes
Number of mattresses	10	10	10	10	10	10
Age of mattress (years; mean $\pm$ SD)	7.2 $\pm$ 4.3	4.6 $\pm$ 4.3	3.6 $\pm$ 1.8	4.8 $\pm$ 2.5	3.7 $\pm$ 3.2	4.6 $\pm$ 2.9
Number of contact to cats	0	3	0	0	0	0

**Fig. 1.** Endotoxin levels in each 10 mattresses in different locations (hospitals (□); homes (▨)) at different time points. Results were expressed as median and interquartile range (IQR).**Fig. 2.** Mite (Der p 1) allergen levels in each 10 mattresses in different locations (hospitals (□); homes (▨)) at different time points. Results were expressed as median and interquartile range (IQR).

Figs. 1–4 show the concentrations of endotoxin, Der p 1, Der f 1 and Fel d 1 (box-and-whisker plots representing quartiles of the distribution) in the dust samples collected at the different hospitals and homes at the different time points. The concentration of endotoxin in the mattresses was not significantly different between the various locations. The concentration of endotoxin was higher in all hospitals than in the homes (321,720 (230,500; 518,700) vs. 243,980 (156,520; 399,020) EU/g; $p < 0.05$). The concentration of mite allergens in the dust was significantly lower in each of the hospitals than in the homes (Der p1: 0 (0; 208) vs. 163 (0; 391) ng/g; $p < 0.05$, Der f 1: 409 (152; 839) vs. 791 (320; 2074) ng/g; $p < 0.01$). There was no significant difference between different time points.

The hospital in Oberjoch showed the highest concentration of Der p 1 (202 (0; 467) ng/g) compared to Pfronten (0 (0; 218) ng/g; $p < 0.01$) and Berchtesgaden

(97 (0; 222) ng/g; $p < 0.05$). Oberjoch had a significantly lower concentration of Der f 1 than Pfronten (435 (126; 854) vs. 839 (536; 1500) ng/g; $p < 0.01$). Fel d 1 had the highest concentration at the first sampling time point in November with a significant decrease to the second sampling time point in August (1376 (375; 3750) vs. 478 (17; 1845) ng/g; $p < 0.05$). No significant differences were found between hospitals and homes, or between different locations.

Discussion

In this study, the concentrations of various indoor air allergens and endotoxin were measured in hospitals offering in-patients rehabilitation for allergic diseases in the alpine mountain climate of Bavaria in order to get informations about allergen and endotoxin exposure of these patients.

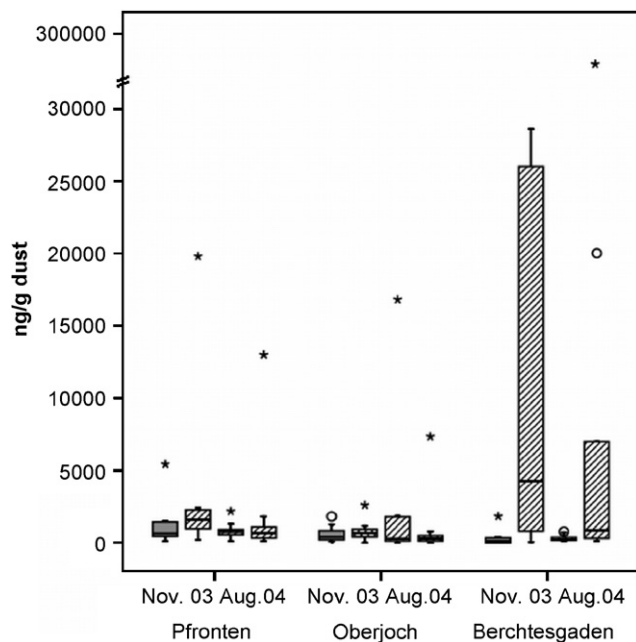


Fig. 3. Mite (*Der f 1*) allergen levels in each 10 mattresses in different locations (hospitals (□); homes (▨)) at different time points. Results were expressed as median and interquartile range (IQR).

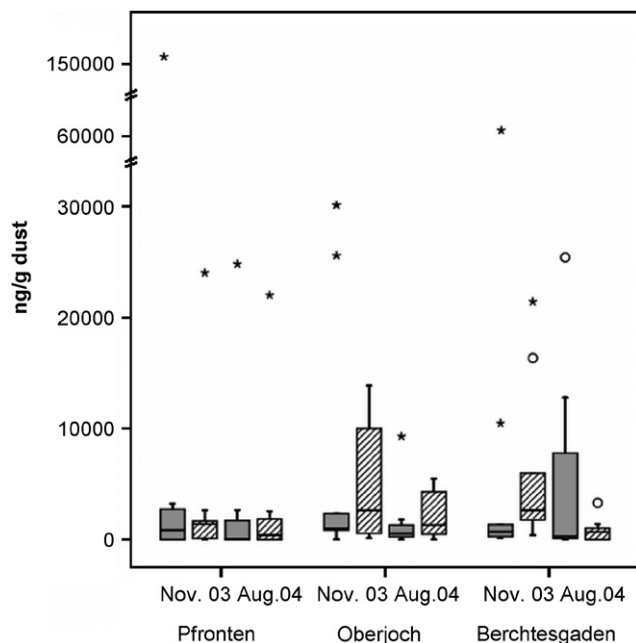


Fig. 4. Cat (*Fel d 1*) allergen levels in each 10 mattresses in different locations (hospitals (□); homes (▨)) at different time points. Results were expressed as median and interquartile range (IQR).

Bacterial endotoxins are lipopolysaccharides found in the outer membrane of predominantly Gram-negative bacteria. High exposure in childhood has been discussed as being protective against the development of asthma

and the atopic state; whereas, continued exposure in later life may be pro-inflammatory and favors the development of asthma. In our study, the endotoxin levels in hospitals offering rehabilitation for asthmatic adults and children varied between 51,880 and 1,172,000 EU/g dust (median: 321,720 (230,500; 518,700) EU/g dust); whereas the endotoxin levels in adjacent homes varied between 52,400 and 3,440,000 EU/g dust (median: 243,980 (156,520; 399,020) EU/g dust) (Fig. 1) with significantly lower values in the homes ($p < 0.05$).

Because the no-response threshold to an acute inhalation is assumed to be less than 5000 EU (Michel et al., 1997), efforts must be made to reduce endotoxin exposure in hospitals. Although there is to our awareness no data about endotoxin levels in hospitals, endotoxin levels in homes have been measured in a few studies: In a British study investigating the exposure to endotoxin in asthmatic and healthy children in their homes, the median concentration of endotoxin in fine dust was 10,988,000 EU/g dust in mattresses (Tavernier et al., 2005). Another study showed that median levels of endotoxin per gram of mattress dust in farm children, Rudolf Steiner school children and reference children were below 100,000 EU/g (Schram et al., 2005). Mattress dust samples from East and West Germany homes showed a mean endotoxin level of about 6500 EU/g dust in Leipzig (infants) and about 5000 EU/g dust in Munich (infants) (Gehring et al., 2004). Comparisons of endotoxin levels between studies are limited though, due to differences in sampling, extraction and storage procedures. Seasonal variation of endotoxin levels in settled house dust (with higher concentrations in summer) was found by some authors, but not by others (Rizzo et al., 1997). We found no significant differences between endotoxin levels in the dust collected before and after the winter.

In our study, the levels of *Der p 1* varied between 0 and 1270 ng/g (median: 0 (0; 208) ng/g) in the three hospitals and between 0 and 1515 ng/g (median: 163 (0; 391) ng/g) in the adjacent homes with significantly lower values in the hospitals (Fig. 2). These values are below the levels of mite exposure (generally $< 0.5 \mu\text{g}$ of *Der p 1*/g of dust) that will result in significant symptoms according to Platts-Mills (Platts-Mills, 2004), yet some authors believe there may not be any threshold value (Cullinan et al., 2004). *Der p 1* concentrations in the mattresses of British hospitals showed a mean of about 100 ng/g of dust (Custovic et al., 1998). In homes in the Netherlands (Terreehorst et al., 2003), house dust of mattresses contained $5.07 \mu\text{g/g}$ dust (mean), while concentrations were rather low in another German study (< 10 – 164 ng/g) (Carrer et al., 2001). In Erfurt, East Germany, allergen levels in mattress dust samples had a mean of 84 ng/g *Der p 1*; whereas, allergen levels in Hamburg, West Germany, were 344 ng/g *Der p 1* (Fahlbusch et al., 1999).

The concentrations of Der f 1 were higher than Der p 1 concentrations in our study, with a range between 0 and 16,794 ng/g Der f 1 (median: 409 (152; 839) ng/g) in hospitals and between 0 and 287,440 ng/g Der f 1 (median: 791 (320; 2074) ng/g) in homes (significantly lower in the hospitals). Another study has demonstrated that concentrations of Der f 1 in mattress dust were 2.5–3.5 times higher than Der p 1 levels (Gross et al., 2000). Data from other hospitals is not available. In German homes, Der f 1 concentrations in mattresses had medians between 677 and 1354 ng/g Der f 1 (Topp et al., 2003). In mattress dust samples, the mean of Der f 1 was 161 ng/g in Erfurt, Germany, and 98 ng/g dust in Hamburg (Fahlbusch et al., 1999).

The concentrations of Der p 1 and Der f 1 were lowest in the hospitals using dust mite-proof covers. This agrees with studies showing a significant reduction in Der p 1 and Der f 1 after 12 months of use of these covers for mattresses (Carrer et al., 2001). Nonetheless, mite-proof bedding covers for allergen avoidance can only be regarded as partial strategy, because it has been shown that this single avoidance measure is clinically ineffective in patients with asthma and allergic rhinitis (Terreehorst et al., 2003; Woodcock et al., 2003).

The concentrations of Fel d 1 ranged between 0 and 153,040 ng/g dust (median: 663 (117; 2426) ng/g) in hospitals, in adjacent dwellings between 0 and 24,000 ng/g dust (median: 1227 (361; 2824) ng/g) with no significant differences between hospitals and dwellings. These levels of Fel d 1 in the mattresses are sometimes above the threshold level defined as the minimal level at which most patients with cat allergies will experience symptoms ($>8 \mu\text{g/g}$ Fel d 1). Mattresses of British hospitals had a Fel d 1 range around 2000 ng/g of dust (Custovic et al., 1998). In German homes there were rather low concentrations between 146 and 397 ng/g Fel d 1 (Carrer et al., 2001). In mattress dust samples, the mean of Fel d 1 was 212 ng/g in Erfurt, Germany, and 91 ng/g dust in Hamburg (Fahlbusch et al., 1999). The results show that the dispersion of allergens from cats to a pet-free place is likely to occur by deposition from people who have been in direct or indirect contact with pets, because direct contact to cats was reported in only 10% of the homes. Clothing is a known source of cat allergens (Berge et al., 1998). The higher values in November compared to August might be due to different clothing (coats) in the autumn. Efforts must be made to reduce the concentrations of Fel d 1, especially in hospitals. As an intervention measure individuals owning a cat should wear special clothing during their stay in the hospitals. It was shown that the levels of airborne cat allergen at school can be reduced by requiring the use of school clothing or banning owners of pets (Karlsson et al., 2004).

Mite allergens were especially low in hospitals compared to homes in the same climate. Amelioration of atopic diseases treated in these hospitals has been

reported (Eberlein et al., 2007). It is debatable whether the reduced allergen levels lead to clinical improvement of symptoms. Other important factors include the involvement of the patient, the relevance of other allergens (e.g. cat allergens), and environmental pollution (e.g. low pollution in the alpine climate). It is difficult in real life to distinguish the efficacy of a single intervention in comparison to the others, because the management of allergic diseases involves a multicomponent strategy (including drugs, allergen avoidance, immunological interventions and patient education). The combined strategy that was undertaken in these hospitals is likely to produce good clinical results (Liccardi et al., 2005).

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