

Short communication

House-dust-mite allergen concentrations (Der f 1) and mold spores in apartment bedrooms before and after installation of insulated windows and central heating systems

Background: It has been hypothesized that changes in heating systems and insulation of homes in developed countries have generated an indoor climate favorable to organisms that excrete allergens inducing sensitization and allergic disease. The purpose of this study was to determine the influence of the installation of highly insulated windows and central heating systems on indoor climate, and mite-allergen (Der f 1) and mold spore concentrations.

Methods: The bedrooms of 98 apartments were examined before and 7 months (mean) after installation of insulated windows and central heating systems. The air-exchange rate, temperature, and humidity were measured. In settled dust on carpets and mattresses, the number of colony-forming mold spores and the Der f 1 concentration were determined. The inhabitants completed a questionnaire about their lifestyles and housing conditions.

Results: The air-exchange rate decreased from geometric mean 0.73 to 0.52 per hour ($P=0.029$). Temperature (mean 13.4 vs 17.5°C, $P<0.001$), and absolute humidity (mean 4.6 g vs 6.2 g H₂O/kg air, $P<0.001$) increased. Relative humidity remained nearly unchanged (mean 47.6 vs 49.1%). Der f 1 concentrations on carpets (geometric mean 0.65 vs 1.28 µg/g dust, $P<0.001$) and mattresses (geometric mean 1.56 vs 2.40 µg/g, $P=0.002$) increased. Among the fungi that were analyzed, only the thermotolerant species *Aspergillus fumigatus* increased (geometric mean 20 vs 60 colony-forming units/g carpet dust, $P=0.02$).

Conclusions: The findings of this study suggest that the installation of insulated windows and central heating systems is associated with an increase of Der f 1 concentrations in carpet and mattress dust and of *A. fumigatus* in carpet dust in apartment bedrooms.

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In recent decades, many apartments in economically advanced countries have been equipped with highly insulated windows and central heating systems. During the same period, the prevalences of asthma and allergic sensitization have increased in these parts of the world. Many investigators have hypothesized that these developments are causally related to each other (1). The insulation of windows is associated with reduced ventilation and higher absolute indoor humidity, and consequently with higher mite-allergen concentrations in house dust (2, 3). Increased exposure to indoor allergens results in increased

prevalences of allergic sensitization to these allergens (1) and possibly asthma (4). However, the typical feature of modern housing is the combination of insulated windows and central heating. Central heating systems are associated with a higher mean temperature than single heating units (5). This reduces relative humidity and may be counterproductive with respect to mite growth (5, 6).

The purpose of this study was to determine the effect of the combined exchange of windows and heating systems on indoor climate, and mold spore and house-dust-mite (HDM) allergen concentration in

house dust. Exposure to HDM allergen was assessed for the major allergen Der f 1 of *Dermatophagoides farinae*, the most abundant HDM species in the city of Dresden (5).

Material and methods

Study design

In 1996, 765 apartments in 66 apartment houses constructed between 1959 and 1969 in a 3 km² area of Dresden, in the former German Democratic Republic, were reconstructed by two house-building societies. The old heating systems, mostly single-oven coal stoves, were replaced by central heating systems with heating elements in every room including the bedroom. The old poorly insulated windows (heat transmission coefficient [HTC] $k = 2.5\text{--}3.0 \text{ W}/[\text{m}^2 \cdot \text{K}]$) were replaced by highly insulated windows (HTC $k = 1.5\text{--}1.7 \text{ W}/[\text{m}^2 \cdot \text{K}]$).

In a short letter, all tenants were asked to participate in the study, and the inhabitants of 167 homes agreed and took part in both years. The first set of samples was collected between January 1996 and April 1996, and the second set after renovation between December 1996 and March 1997. The mean (minimum–maximum) time between installation and re-examination was 6.6 (1.6–9.7) months for heating systems and 7.8 (1.4–12.6) months for windows. Ninety percent of the apartments were examined at least 4 months after the installation of central heating systems and 3 months after the installation of insulated windows. Only one test site was re-examined less than 2 (1.6 and 1.4) months after renovation. Both investigations were performed by the same two trained fieldworkers (M.H. and K.B.).

Of the 167 apartments visited in both years, only 98 were analyzed, because in 69 apartments the tenants had made significant alterations during the renovation period. Inclusion criteria were that the same inhabitants had lived in the apartment from 6 months before until the end of the investigations, using the same room as a bedroom, the same mattresses, and the same bedroom carpets. In apartments where only mattresses or carpets had been replaced, these particular items were excluded from the analysis. Among the 98 apartments, 51 had evaluable Der f 1 sample pairs from both carpets and mattresses, nine from carpet samples only, and 38 from mattress samples only.

The 98 selected apartments did not differ from the rest of the 167 homes in building material, floor level, heating system before renovation, and number of inhabitants or bedroom users (χ^2 test for 2×2 tables). Due to technical defects or methodical faults, data on temperature and humidity could be evaluated at only 93 of the 98 test sites. Air-exchange rates (AER) were

evaluable at 78 of the 98 test sites. Due to limited laboratory capacities, fungal spore counts were performed in only 49 of the 98 apartments. These 49 apartments did not differ from the other test sites with respect to the above characteristics (χ^2 test for 2×2 tables).

The study was approved by the ethics committee at the Universitätsklinikum Carl Gustav Carus der Technischen Universität Dresden.

Sampling of carpet and mattress dust and laboratory analysis of Der f 1 concentrations

Dust sampling and determination of Der f 1 concentrations (Sandwich ELISA, ALK, Copenhagen, Denmark) were performed as described earlier (5). In the first year, one portion of the dust extract was analyzed immediately and a second portion was stored at -20°C for 12 months. In the second year, the extracts were also frozen and stored at -20°C for at least 4 weeks. Both extracts from a particular site were then analyzed on the same plate. Assay sensitivity was 2 ng/ml or 0.02 $\mu\text{g/g}$ dust. The intra-assay coefficient of variation (CV) in this study determined by double positive controls on every plate ranged between 0.1% and 8.2%. The interassay CV calculated with the results for these positive controls was 5.8%.

Laboratory analysis of viable mold spores

An amount of 100 mg of dust was suspended in 20 ml of a peptone solution (1000 mg/l) with Tween 80 (200 mg/l). A volume of 100 μl of the undiluted suspension and two 10-fold dilutions (1:10/1:100) were applied to agar dishes (DG 18-agar with 50 mg/l chloramphenicol) in triplicate in order to cultivate mesophilic fungi. Thermotolerant fungi were cultivated from 30–50 mg of native dust spread onto the agar dish. Colony-forming units (CFU) of mesophilic fungi were counted after 5 days at 25°C ; CFU of thermotolerant fungi after 3 days at 37°C . The following species were distinguished (7): *Cladosporium* spp. (25°C), *Penicillium* spp. (25°C), the *Alternaria* complex (all phaeohyphomycetes with dictiospores; 25°C), *Aspergillus* spp. without *A. fumigatus* ($25^\circ\text{C}/37^\circ\text{C}$), and *A. fumigatus* (37°C).

Measurements of temperature and humidity

Mean temperature and relative humidity (RH) in the bedroom were measured three times a day (morning, afternoon, and bedtime) over three continuous days of the week in which dust was sampled and the AER was measured. We used a negative temperature coefficient (NTC) sensor for temperature and a capacitive humidity sensor for RH (Testo 610, Testo GmbH, Lenzkirch, Germany). Absolute humidity (AH) was

Table 1. Bedroom climate before and after renovation

	No. of sample pairs	Before renovation	After renovation	P value*
Temperature (mean [95% CI] in °C)	93	13.4 (7.6–19.2)	17.5 (12.9–22.1)	<0.001
Relative humidity (mean [95% CI] in %)	93	47.6 (27.8–67.5)	49.1 (30.2–67.9)	0.187
Absolute humidity (mean [95% CI] in g/kg air)	93	4.6 (2.5–6.7)	6.2 (3.7–8.7)	<0.001
Air-exchange rate/h (geometric mean [95% CI])	78	0.73 (0.62–0.87)	0.52 (0.43–0.63)	0.029

95% CI: 95% confidence interval for mean (normally distributed values) or geometric mean (nonnormally distributed values).

* Differences before and after renovation were tested for significance by paired *t*-test for normally distributed values (temperature and humidity) and by Wilcoxon matched pairs signed ranks test for nonnormally distributed values (air-exchange rate).

calculated with the simultaneously measured values of RH (ϕ [%]) and temperature (t [°C]):

$$\text{absolute humidity} = [\text{gH}_2\text{O/kgair}] = \frac{6.222}{\frac{1}{(\phi/100) * 0.28868 * (1.098 + t/100)^{8.02}} - \frac{1}{100}}$$

Determination of AER

The AER in the bedroom was determined by a tracer gas method (8). The concentration of a nontoxic tracer gas, hexafluorobenzene (HFB), emitted from a source placed in the bedroom was measured for 7 days. From the source emission rate (e), the measured HFB room concentration (c), and the bedroom volume (V), the AER was then calculated as follows:

$$\text{AER}(\text{h}^{-1}) = \frac{e(\mu\text{g/h})}{c(\mu\text{g/m}^3) * V(\text{m}^3)}$$

The reproducibility of the HFB measurements was ensured by performing duplicate measurements in every tenth dwelling. The mean CV for duplicate measurements was 4.4% (minimum 0.2%, maximum 9.5%) in the first study period, and 6.6% (minimum 0.8%, maximum 10.8%) in the second study period.

All laboratory work was done in the same laboratory in both years.

Statistical analysis

Differences for paired values (data from the same dwelling before and after reconstruction) were evaluated by the Wilcoxon matched-pairs signed-ranks test (nonnormally distributed values of AER, fungal spore

counts, and Der f 1 concentrations) or by the *t*-test for paired samples (normally distributed values of temperature and humidity). Differences in the after/before ratio of Der f 1 concentrations when comparing apartment characteristics were analyzed by the Mann–Whitney U-test. The associations of indoor climate with spore counts and Der f 1 concentrations were analyzed by Spearman's rank correlation test. The level of significance for all tests was $P < 0.05$ for two-tailed tests. Der f 1 concentrations below the limit of detection were uniformly estimated with 0.02 $\mu\text{g/g}$ dust for all calculations.

Results

The installation of central heating systems and highly insulated windows was accompanied by a decrease in the AER, and a significant increase in temperature and AH, but not in RH (Table 1). Der f 1 concentrations increased on 42/60 of the carpets and on 52/89 of the mattresses (Table 2). Der f 1 concentrations increased less on carpets older than 3 years. No differences were found for other apartment characteristics. There was no significant difference in cleaning habits (vacuuming the bedroom more or less than once a week) between the first and second sampling period.

While mesophilic fungi counts did not consistently increase or decrease in mattresses and carpets, the concentrations of thermotolerant fungi did. *A. fumigatus* concentrations on carpets increased in 20/32 apartments, while the concentrations of other thermotolerant *Aspergillus* species decreased in 26/32 apartments. Similar tendencies were found on mattresses (Table 3).

Table 2. Der f 1 concentrations in bedroom dust before and after renovation

	No. of sample pairs	Der f 1 before renovation ($\mu\text{g/g}$ dust) Geometric mean (95% CI)	Der f 1 after renovation ($\mu\text{g/g}$ dust) Geometric mean (95% CI)	P value (Wilcoxon matched-pairs signed-ranks test)
Carpets	60	0.65 (0.36–1.15)	1.28 (0.67–2.47)	<0.0005
Mattresses	89	1.56 (0.89–2.74)	2.40 (1.33–4.36)	0.0024

95% CI: 95% confidence interval for geometric mean.

There were no significant correlations either before or after reconstruction between temperature, humidity, or AER, and Der f 1 concentrations. Concentrations of *Aspergillus* spp. on mattresses were negatively correlated with absolute ($r = -0.57$) and relative ($r = -0.39$) humidity in the first, but not in the second, year. For *A. fumigatus*, similar but less strong associations were found ($r = -0.39$ for absolute and $r = -0.26$ for relative humidity in the first year). No consistent correlation was found between Der f 1 concentrations and fungal counts in both years.

Discussion

This study of 98 apartments found a decrease in the AER and an increase in room temperature and AH (at constant RH) 7 months (mean) after installation of insulated windows and central heating systems. In the same period, an increase of Der f 1 concentrations in carpet and mattress dust was observed. It is most suggestive to assume that the reconstruction measures induced the observed changes in the indoor climate, which consequently favored the thriving of the mite *D. farinae* and the expression of Der f 1.

At constant RH, an increase of temperature accelerates the rate of reproduction and the generation period from egg to adult in mites (9). However, like others (10), we did not find significant associations between temperature or humidity in the bedroom air and mite-allergen concentrations. This may have two causes:

- 1) Discontinuous measurements in the middle of the room may not optimally represent temperature and humidity on mattresses or floors.
- 2) *D. farinae* is less dependent on climatic conditions than other mites such as *D. pteronyssinus* (9).

In a study comparing the allergens of both mites, we found no significant association between temperature or humidity and Der f 1 concentrations, whereas Der p 1 concentrations were significantly associated with both climatic factors (5). Other ecologic factors such as mold contamination were also not associated with Der f 1 concentrations in the study presented here.

Because we did not examine a “placebo group” of dwellings not being reconstructed in the same period, we cannot definitely exclude the possibility that the observed changes are the result of a systematic methodical difference, or that they reflect a general trend affecting dwellings independently of reconstruction measures. Potential sources of methodical variability were kept to a minimum: all analyses were done in the same laboratory by the same protocol. To exclude bias by systematic differences in assay charges or plate coating, sample pairs from one site were always examined on the same plate in immediate vicinity to each other. To check for potential storage effects, the first year’s samples were also examined soon after they were collected. The results were similar with these specimens (data not shown). We conclude that the observed differences cannot be explained by bias due to systematic differences in the laboratory procedures.

While counts of fungi with a temperature optimum at 25°C did not change, species with affinity for higher temperatures behaved differently. *A. fumigatus*, a thermotolerant species that also has a high water affinity (a_w 0.98–0.99), increased, whereas the concentrations of other thermotolerant species of *Aspergillus* characterized by a slightly lower water affinity (a_w 0.95–0.98) decreased. These differences suggest that the species that depends most on high temperature and humidity benefits most from the climatic changes due to the renovation. As for levels of mite allergen, no

Table 3. Mold spore concentrations in bedroom dust before and after renovation

	No. of sample pairs	Before renovation (CFU/g dust) Geometric mean (95% CI)	After renovation (CFU/g dust) Geometric mean (95% CI)	P value (Wilcoxon matched-pairs signed-ranks test)
Carpets				
<i>Cladosporium</i> spp. (25°)	37	2740 (1330–5640)	990 (380–2600)	0.03
<i>Penicillium</i> spp. (25°)	37	9820 (6160–15 640)	10 470 (7450–14 710)	0.67
<i>Alternaria</i> spp. (25°)	37	40 (10–120)	30 (10–100)	0.89
<i>Aspergillus</i> spp. (25°)	37	810 (260–2500)	2060 (840–5050)	0.45
<i>Aspergillus</i> spp. (37°)	32	80 (50–130)	20 (10–40)	0.003
<i>A. fumigatus</i> spp. (37°)	32	20 (10–40)	60 (40–90)	0.02
Mattresses				
<i>Cladosporium</i> spp. (25°)	49	130 (50–370)	60 (20–160)	0.23
<i>Penicillium</i> spp. (25°)	49	1500 (740–3020)	1250 (590–2660)	0.82
<i>Alternaria</i> spp. (25°)	49	10 (0–20)	10 (10–30)	0.32
<i>Aspergillus</i> spp. (25°)	49	590 (270–1320)	820 (400–1710)	0.95
<i>Aspergillus</i> spp. (37°)	42	30 (20–50)	10 (10–20)	0.003
<i>A. fumigatus</i> spp. (37°)	42	10 (0–10)	10 (10–20)	0.12

CFU: colony-forming units.

95% CI: 95% confidence interval for geometric mean. Temperatures in parenthesis indicate temperature at which spores were cultured *in vitro*.

consistent direct associations of fungal counts with air climate measurements were found.

Outdoor factors may affect the ecology of micro-organisms indoors. The yearly mean emission values for SO₂, NO₂, O₃, CO, benzene, and suspended particles were similar in both years at Dresden (11). The annual mean temperature in Dresden was 9.4°C in 1995 and 7.2°C in 1996. Mean RH was 75% in 1995 and 79% in 1996. The mean outdoor temperature was very similar during both sampling periods (January–April 1996: 0.6°C; December 1996–March 1997: 0.5°C), whereas RH was somewhat higher in the second period (77% RH, 81% RH) (personal communication, Deutscher Wetterdienst Dresden). It is not reasonable to assume that small differences in outdoor humidity within the optimal range for mite growth could explain the increase in Der f 1 concentrations.

In summary, this study found an increase of the mite allergen Der f 1 and the thermotolerant mold

A. fumigatus in apartments after installation of tightly closing windows and central heating systems. This change was accompanied by an increase of temperature and absolute humidity. A direct association between climatic changes and allergen increase could not be demonstrated. Explanations other than a renovation effect appear to be unlikely but cannot be definitely excluded due to the lack of a control group.

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