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A CONTROLLED INTERVENTION STUDY CONCERNING THE EFFECT OF INTENDED TEMPERATURE RISE ON HOUSE DUST MITE LOAD

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Abstract: In epidemiological studies, increased indoor temperature - producing a lower relative humidity - is associated with low house dust mite (HDM) load. Twenty-eight dwellings were allocated for either intervention (12/15 completed) or control (11/13 completed). In the intervention group, participants were asked to increase the bedroom temperature by at least 3ºC compared to the self-assessed temperature of the previous winter. Dust samples were repeatedly collected from mattress and floor, and bedroom temperature and relative humidity were recorded hourly throughout one year. Dust was analysed for allergen (Der f 1 + Der p 1 + Der m 1 = Der 1) by ELISA and HDMs were counted. Changes in mite and in mite allergen concentration were the same in the control and intervention groups, and measured temperatures did not differ during intervention period in the groups (18ºC and 19ºC). Groups turned out not to be comparable with respect to initial (self-assessed) bedroom temperature (lowest in the intervention group). There was a significant seasonal variation, with doubled Der 1 concentrations in dust collected in July–November compared to January-May samples. No effect was obtained on mites or mite allergens, but this may be due either to a general lack of effect of increase in bedroom temperature, or to an insufficient increase in temperature in our intervention group. Seasonal variations in HDM and HDM allergens must be accounted for when data on exposure are interpreted.

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INTRODUCTION

House dust mite (HDM) allergy is common [19, 27] and the HDM load should preferably be kept at a minimum to alleviate the symptoms in HDM allergic patients and to avoid sensitization of high risk children [4, 28, 29]. There might be a dose dependent correlation between HDM concentration in beds and HDM sensitization rate in children; and a threshold of 2 µg/g dust of group 1 HDM allergen (Der 1) has been proposed, but lower or no threshold has also been suggested [7, 23, 26, 29, 31].

Cross-sectional studies show that low relative humidity (RH) is associated with low HDM load, especially when the RH is below 45–50% [12, 14, 22, 30, 33]. A low indoor humidity during the cold season seems sufficient to maintain low allergen levels [15]. The indoor RH can be reduced by raising the temperature [3], by increasing the exchange of air [11], or by producing less water vapour. Higher indoor temperatures are generally found to be associated with less HDMs [5, 8, 10, 12, 17], and in most of the studies an isolated effect of the temperature - not related to a decrease in RH - has been shown [5, 10, 12, 17]. The use of electrical heating blankets has been

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shown to reduce HDMs and their allergens in mattresses [20]. Documented guidelines for HDM avoidance measures are in demand. The first step in establishing such guidelines is to evaluate, whether a potential strategy actually does provide a significant reduction in HDM load. The next step would be clinical trials, assessing the effect on asthma, rhinitis or atopic dermatitis. An HDM intervention study with a deliberate increase in indoor temperature seems logical from the current knowledge [3, 5, 8, 10, 12, 17, 20], but a systematic study has so far not been performed.

The aim of this study was to evaluate whether an increased temperature during the winter in self-assessed cold bedrooms could decrease the HDM and HDM allergen exposure from the mattresses and from the floor. In addition, the study evaluates the seasonal changes in these parameters. Knowledge of the effect of temperature and season on HDM load could be useful in outlining HDM avoidance guidelines. Prospective clinical trials should follow, if an effect of a temperature rise was found.

MATERIAL AND METHODS

Inclusion of homes. An invitation to participate in the study was sent to 635 randomly selected 18–70 years old Danish citizens in a suburb of Copenhagen (Gladsaxe). Those willing to participate (N = 68) were visited from May–September 1999, provided that they had a bedroom they considered had been kept cold during the previous winter; and that they had lived in the same house and used the same mattress for at least one year and intended to do so the next year. During the visit, a mattress dust sample was collected by one of the authors (KES) [24]. To be included in the intervention study, the visited persons should have a minimum of 1 µg Der 1/gram of mattress dust; and they should be able and prepared to increase the temperature in the bedroom at least 3ºC compared to the previous winter. Twenty-eight fulfilled these criteria and were included.

Allocation and exclusion. The 28 persons were allocated to either an intervention group or to a control group by the minimization procedure [1]. The minimization variables were obtained from the initial screening of mattress dust: the concentration of Der 1 (50% weight in the minimization; categories: < 2 µg/g dust, from 2 to < 10 µg/g dust, 10–50 µg/g dust, > 50 µg/g dust), the dominating mite at microscopy (25% weight in the minimization; categories: *Dermatophagoides pteronyssinus*, *D. farinae*, no mites) and total HDM count (25% weigh in the minimization; categories: 0 mites, 1–100 mites/g dust, > 100 mites/g dust). Fifteen persons were allocated to the intervention group and 13 to the control group. During the study, 3 were excluded from the intervention group (1 moved from her apartment; 1 bought a new mattress; and 1 had serious water damage) and 2 from the control group (1 moved to another room with a newer carpet; and 1 bought a new mattress). Thus, 12 from the intervention group and 11 from the control group completed the study.

Adult specimens of *Dermatophagoides pteronyssinus*, *D. farinae* and *D. microceras* were identified from the descriptions in Hughes [13] and for subadults of pteronyssinus and farinae we used the figures given by Mumcouglu [21].

Collection of dust samples. The participants were visited in September and November of 1999 and in January, March, May and September of 2000. At each visit, one of the authors (KES) collected by vacuum cleaner 1 dust sample from the entire upper surface of the mattress and another from the nearest 2 m^2 floor (2 min/m^2). The dust sampling device contained a cellulose filter (ALK-ABELLÓ, Hørsholm, Denmark) [9] and was connected to a HEPA-filter vacuum cleaner (Nilfisk-Advance UZ964 (650 W), Åmål, Sweden). Dust samples were kept cool (up to 3 hours) and then stored at ¸ 20ºC until extraction. The participants were asked to take a floor sample with the same kind of device in July, but to use their own vacuum cleaner, and keep the sample frozen until handed over at the September 2000 visit.

Temperature and humidity. During the inclusion visits in May-September 1999 [24], the persons were asked about the typical day and night temperature for the winter season 1998/99, and a self-assessed mean temperature with 2/3 weight on daytime and 1/3 on nighttime was calculated. The indoor temperature and RH were recorded hourly, from September 1999–September 2000, with an electronic thermometer and hygrometer coupled to a data-logger (Tinytag Ultra TGU-1500, Chichester, England) and records were loaded on a portable PC on each visit. Absolute humidity (AH (H_2O/m^3 air)) was calculated as:

$$RH(\%)\times 0.01\times \exp((0.000002\times t^3)-(0.00043\times t^2)+(0.073916\times t)+1.54209)$$

(temperature = t (ºC)). Recordings from 8:00–23:00 were defined as daytime, while those from 00:00–7:00 were defined as nighttime.

Weekly mean outdoor temperatures were obtained from the Danish Meteorological Institute. We defined the heating season to be the period where the temperature was below 8ºC, to ensure that all participants heated their houses.

For each home the mean of records for the heating season 1999/2000 during daytime was subtracted, the participants assessed day bedroom temperature for the winter 1998/99, and likewise for the night temperature. The individual overall temperature difference was calculated as: 2/3 × day difference + 1/3 × night difference.

Intervention. The participants from the intervention group were asked to increase the mean bedroom temperature by at least 3ºC during the winter heating season 1999/2000 compared to the previous winter, but to make no other changes in bedroom or ventilation habits. The 3ºC increase was chosen, as it was thought to be the highest acceptable in the period - from October/November to April - where houses normally are heated in Denmark.

Table 1. Group characteristics.

	Intervention group	Control group
Participants included (N)	15	13
Participants completed (N)	12	11
Males (N)	3	6
Age of participants (years)	56 (34–69)	50 (18-70)
One-family house (N)	11	11
Size of household (N)	2 (2–4)	2 (1–6)
Age of mattress (years)	12 (1–42)	5 (2–35)
Self-assessed bedroom temperature (1998/99)(ºC)	15 (10–18)*	17 (11–20)*
If carpet in proximity to bed (N)	8	7

Continuous data are shown as median (range). * $p < 0.05$ between gropus.

Table 2. Measured temperature and humidity in bedrooms.

	Intervention group	Control group
Heating season		
Temperature (ºC)	19.3 (1.39)	18.4 (2.35)
Relative humidity (%)	46.1 (3.87)	48.6 (7.16)
Absolute humidity (g/m^3)	7.78 (0.61)	7.68 (0.77)
Non-heating season		
Temperature (ºC)	21.0 (0.75)	20.7 (1.12)
Relative humidity (%)	55.0 (2.89)	56.1 (4.08)
Absolute humidity (g/m^3)	10.3 (0.48)	10.3 (0.52)

Results given as mean (STD) of each homes mean recordings. No statistical difference between groups.

The participants from the control group were asked to make no change at all.

At each visit in the heating season KES discussed with the participants from the intervention group the temperature curves obtained from the previous two months, and observed if the agreed temperature has been reached. If not, the participant was asked to increase the temperature further. Participants in the control group were asked if they felt they still had the same bedroom temperature as the year before.

Processing of dust samples. After being thawed at room temperature, 0.1 g dust was dispersed in 5 ml 80% lactic acid stained with lignin pink, in an 8.5-cm circular Petri dish, providing a 1–2 mm layer of lactic acid and dust for microscopy [16]. The rest of the sample (including the filter paper) was extracted with 0.125 M ammonium hydrogen carbonate + 0.1% sodium azide [31] added 0.1 M of the protease inhibitor ε-amino caproic acid in a 20-ml polypropylene syringe by slow rotation for 2 hours. Dust was extracted 1:10 w/v, except for dust samples < 0.5 g where 5 ml buffer was used. The ooze was squeezed out of the syringe, leaving the filter and part of the coarse particles behind, and centrifuged for 20 min. at 1,223 g. The supernatant was filtered using a 0.8 µm cellulose acetate filter and stored at 20ºC until analysis.

If the initial sample contained less than 0.3 g dust, dust for mite identification was not removed before extraction. Instead, the total sample was extracted; and thereafter the syringe and filters were cleaned with distilled water. The water was then removed by suction through a 0.45 µm mixed cellulose acetate and nitrate filter supported by a glass filter (Millipore7, Bedford, MA, USA). The concentrate on the filter was dispersed in lactic acid, as described above, for mite quantification.

The concentrations of Der p 1, Der f 1 and Der m 1 in the supernatant were individually assessed in duplicate by ELISA at ALK-ABELLÓ, Hørsholm, Denmark [18]. The detection limits, 1 ng/ml for Der p 1 and 2 ng/ml for Der f 1 and Der m 1, were used for calculations when the allergen concentration was unmeasurable. Der 1 was calculated as the sum of Der p 1, Der f 1 and Der m 1.

After incubation at room temperature for at least 1 day, the mites in the lactic acid preparation were counted in a stereo microscope with a magnification of × 25 (Olympus type SZ 4045 TR, Tokyo, Japan) by one of the authors (TEH). Species identification took place by × 100 or more by normal phase-contrast (Olympus type BX 40, Tokyo, Japan).

Statistical analysis. Groups were compared using Student's t test for HDM (-allergen) concentrations, AH, recorded temperature, and change in heating season temperature from the self assessments of 1998/99 to the mean of records from 1999/2000; Mann-Whitney for RH and basic continuous characteristics; and Fishers exact test for basis categorical characteristics. Transformation to log(mite+1) and log(allergen) was used.

To study the season effect on the HDM loads, we performed two-way analyses of variance with season (July/September/November vs. January/March/May), and participant number as variables.

The statistical programs of SigmaStat 2.03 (SPPS Inc., Chicago, IL, USA) and Quattro Pro 8 (Corel, Ottawa, Canada) were used; $p < 0.05$ was regarded as significant.

RESULTS

No significant differences were found between the intervention and the control group concerning age, sex, type of housing, household size, age of mattress, and whether or not there was a carpet in proximity of the bed; but the intervention group had a significantly lower self-assessed pre-study bedroom temperature than the controls (Tab. 1).

The heating season, defined as described in material and methods, turned out to be from 8 November 1999–17 April 2000. The mean bedroom temperature for this period was 19.3ºC in the intervention group and 18.4ºC in the control group (Fig. 1). No significant differences were seen between the intervention and the control group in mean temperature, RH and AH in and outside the heating

Table 3. Changes in HDM and HDM allergen concentrations.

	September 1999–May 2000		September 1999–September 2000	
	Intervention group	Control group	Intervention group	Control group
Mattress				
Der 1 µg/g	0.52*	0.59*	126	154
Mites/g	6	0.33*	138	101
Der 1µg/m^2	0.39*	0.37*	153	157
Mites/m^2	0.45*	0.21*	167	102
Floor				
Der 1 µg/g	54	0.32*	8	125
Mites/g	28	58	173	139
Der 1µg/m^2	55	0.36*	117	135
Mites/m^2	29	0.67*	252	150

No significant differences between groups on any parameter. * $p < 0.05$ within group along time.

season 1999/2000 (Tab. 2). The mean difference between recorded temperature in the heating season 1999/2000 and the self-assessed temperature for the heating season 1998/99 was for the intervention group 4.9ºC and for the control group 1.6ºC, and thus a 3.3ºC higher increase in temperature in the intervention compared to the control group ($p < 0.001$).

Changes in HDM and HDM allergen concentrations in dust collected in either May (subsequent to the heating season) or September 2000 (12 months after initiation of the study), compared to September 1999, were not different for the intervention and the control group (Fig. 2, Tab. 3). No change in concentrations was seen from September 1999–September 2000 for neither the control nor the intervention group; whereas samples from May 2000 contained less HDM and HDM allergen compared to samples from September 2000 (Tab. 3).

The concentrations of HDM and HDM allergens showed annual variation, where significantly higher concentrations were found in dust collected in July/September/November compared to January/March/May. HDM allergen concentrations were doubled on floors, and likewise on mattresses for both mites and allergens, independent of whether the concentration was expressed as per weight or per area; and 4 times higher for mites on floors (Fig. 2).

DISCUSSION

Inspired by studies of mite cultures [3], epidemiological studies [5, 8, 10, 12, 17], and an intervention study with electric heating blankets [20], we wanted to test the effect of a new strategy to reduce the HDM exposure. The intervention should focus on increasing the temperature in (self-assessed) cold bedrooms. Studies of mite cultures indicate that HDMs can survive at low relative as well as low absolute humidity, if the temperature is kept low [3]. However, such investigations cannot with certainty predict what happens in peoples' homes. In the laboratory, several conditions are different from those in field studies [6]: temperature and humidity are recorded in the microclimate where the mites in fact reside; in general there is plenty of food; culture specimens are selected to thrive at the particular cultural environment; and only intended changes are induced. In contrast, environmental changes in a home would most often involve more than one parameter; e.g. if one is trying to increase the temperature one might decrease the ventilation in order to do so.

Unfortunately, the study performed was inconclusive since the study groups were not comparable and since the intended intervention on temperature was not clearly demonstrated. No difference between groups on HDMs and HDM allergens were observed, but this may be due either to a general lack of effect of increase in bedroom temperature, or to an insufficient increase in temperature in our intervention group. To make the design of the study as close as possible to daily clinical practice, we used

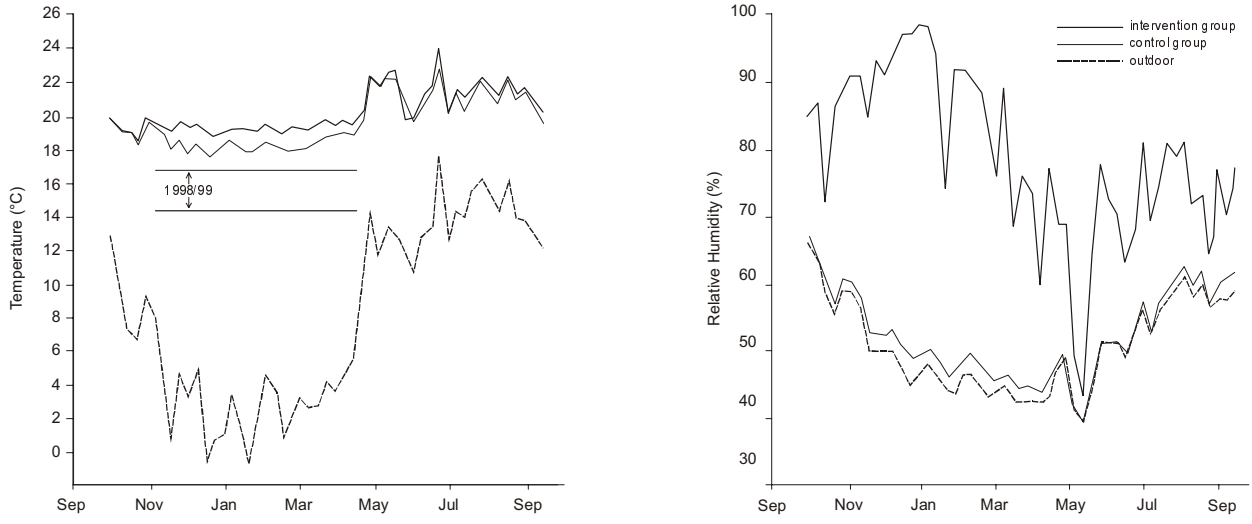

Figure 1. Measured mean temperature and relative humidity 1999/2000. The self assessed mean temperature winter 1998/99 are indicated.

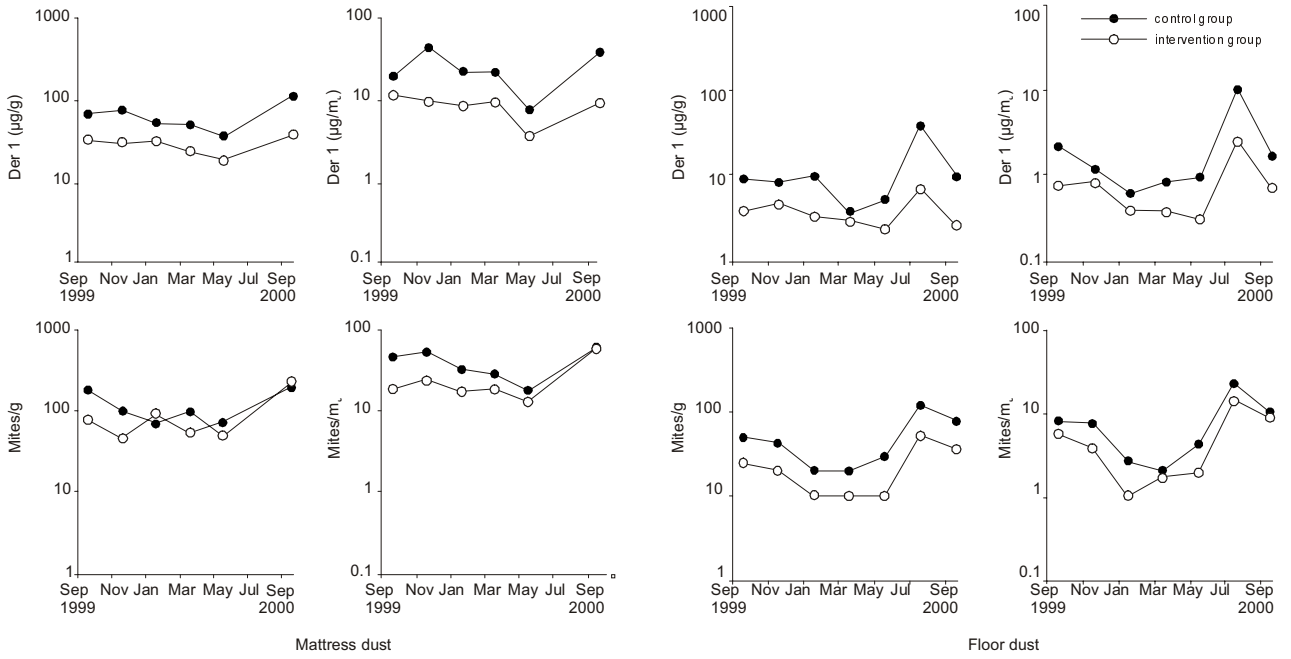

Figure 2. Mean concentrations of house dust mites and their allergens in mattress and floor dust during the study.

retrospectively self-assessed bedroom temperature as an inclusion criterion instead of temperatures controlled in a standardised way during a winter period. We knew this approach could make it more difficult to ascertain the real magnitude of a subsequent intervention. Both groups ended up by having the same temperature during the intervention period, but the intervention group reported a significantly lower pre-study temperature, and if we could rely fully on the reported temperatures, the intended increase in temperature of 3ºC, was, in fact, obtained in the intervention group. However, the statistical evaluation of effect in groups not surely comparable at inclusion is questionable; and in addition, from the control group (who claimed to have the same temperature as the last winter), we saw in the self-assessment a clear tendency to underestimate the real sleeping temperature.

The measured humidity during the study period was not significantly different between the groups, and it could be hypothesised that if an increase in temperature had occurred, it had been obtained by reducing the ventilation. For the conclusion it would have been valuable if the temperature, humidity and ventilation had been recorded in the pre-intervention season in addition to the intervention period. However, this was not possible for practical reasons. For future studies, the risk of unbalanced groups might be minimised by including the self-assessed winter bedroom temperature or spot measurements from the last part of the pre-study winter in the minimization procedure.

We found a lower HDM exposure in winter/spring in accordance with previous findings [2, 8, 15, 20]. Whether this was due to lower indoor RH observed in the same period remains to be established. It is also worth noticing that the increase in the HDM load took place mainly outside the heating season, where no intervention measures were prescribed. This seasonal variation in HDMs and HDM allergens must be accounted for when data on exposure are interpreted.

The HDM load may be assessed by HDM allergen analysis or HDM counting. The HDM allergen analysis reveals how much allergen the patients are exposed to, while the HDM counting quantifies the allergen producing source. HDM concentrations might more rapidly reflect indoor climatic and seasonal changes than HDM allergens, since a change in mites would be expected before a change in the produced allergens, which additionally seems to be very stable [25] and therefore will be accumulated. The fact that we obtained consistent results from both HDM allergen analysis and HDM counting, increases the reliability of our results. Calculations of the concentration per area as well as per weight were performed, since changes in dust amounts and thereby concentration per area, might reflect seasonal variation or the effect of repeated vacuuming, especially of the mattresses. Conclusions were identical, however.

We were not able to conclude from this intervention study whether an increased bedroom temperature in the heating season had an effect on the HDM load, since we are not sure the intended temperature increase was achieved, and even if this were the case, groups were not comparable at inclusion. The seasonal variation, with HDM and HDM allergen concentrations being highest summer to autumn, has to be taken into account when the severity of the HDM exposure is estimated.

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