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Decreased $FEV_1\%$ in asthmatic adults in Scottish homes with high Environmental Relative Moldiness Index values

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Summary

Background Exposures to indoor biological contaminants have been implicated in asthma's aetiology but their effect on lung function is not well quantified.

Objective The aim of this cross-sectional study of non-smoking, asthmatic adults in Scotland was to determine the correlation between the results from a standard spirometry test, forced expiratory volume in one-second percent ($FEV_1\%$), and quantitative estimates of some biological exposures.

Methods A population ($n = 55$) of non-smoking, adult asthmatics in Scotland was included in this study and each completed a questionnaire that allowed the determination of the Asthma Control Questionnaire scores (ACQ) and St. George's Respiratory Questionnaire scores (SGRQ), as well as corticosteroid use. Spirometry testing was completed and the pre-bronchodilator $FEV_1\%$ value calculated. At about the same time, floor dust samples were collected in the living room and in the bedroom. These dust samples were analysed for mould contamination, as described by the Environmental Relative Moldiness Index (ERMI) values and by $(1, 3)$-β-D-glucan concentrations, for endotoxin, and for dust mite, cat, and dog allergen concentrations. The asthmatics' $FEV_1\%$ values were tested for correlation (Pearson) to questionnaire-based estimates of health. Also, each biological exposure was tested for correlation (Pearson) to the $FEV_1\%$ values.

Results $FEV_1\%$ results were correlated with ACQ scores (ρ -0.586, $P < 0.001$), SGRQ scores (ρ -0.313, $P = 0.020$), and weakly with corticosteroid use (ρ -0.221, $P = 0.105$). The ERMI values in the homes (average 5.3) were significantly correlated with $FEV_1\%$ values (ρ -0.378, $P = 0.004$). There was no correlation between $FEV_1\%$ and concentrations of endotoxin, $(1, 3)$-β-D-glucan, or any of the allergens.

Conclusion and clinical relevance Although these results do not prove that mould exposures caused the deficit in lung function observed in this study, it might be advisable for asthmatics to avoid high ERMI environments.

Keywords adults, asthma, ERMI, $FEV_1\%$, lung function
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Introduction

World-wide, 300 million people have asthma [1], including approximately 1.1 million children and 4.3 million adults in the UK [2]. In Scotland, 1 in 14 people are currently receiving treatment for asthma. Surveys indicate that many patients with severe asthma have poor symptom control and reduced lung function [3–5]. Asthma has been associate with various biological exposures, including mould, endotoxin, and allergens (dust mite, insect, and animal). Depending on the study, each of the agents has been reported to cause, have no effect on, or be protective of the asthma. It is beyond the scope of this paper to review all of this literature. However, only a few studies have examined these biological exposures and their association with lung function as quantified by spirometry testing and the resulting $FEV_1\%$ values.

Mould exposures have often been estimated using the cell product $(1, 3)$-β-D-glucan. The studies of

(1, 3)-β-D-glucan's link to lung function generally report a lack of a relationship to FEV$_1$% measurements. For example, Thorn and Rylander [6] found no effect of different exposures of 0–19 ng (1, 3)-β-D-glucan per m^3 of air on FEV$_1$%. Similarly, Blanc et al. [7] found no correlation between (1, 3)-β-D-glucan concentration in the dust and FEV$_1$% values. However, the high ERMI values in homes of asthmatic children in New Orleans were linked to reduced FEV$_1$% values [8].

Gram-negative bacterial exposures, estimated by measurement of endotoxin concentrations, were inversely associated with FEV$_1$% values [7]. But in other studies, endotoxin exposures were found to be protective in a manner consistent with the hygiene hypothesis [9]. But high occupational levels of endotoxin caused FEV$_1$% values to be reduced [10]. However, inhalation of 2 μg of endotoxin did not induce any changes in FEV$_1$% values [11].

Dust mite allergen concentrations were not correlated with FEV$_1$% values in a study of adult asthmatics in the U.S. [7]. Chiang et al. [12] also found no correlation between the concentration of dust mite allergen antibodies and lung function. However, Omenaas et al. [13] found that exposure of dust mite allergen correlated with reduced FEV$_1$% values in Norwegian adults. These diverse findings might be explained by host genetics. Abdelmotelb et al. [14] showed that the number of copies of the α-tryptase gene might be critical to the effect of dust mite exposures on lung function.

The effect of exposures to insect and animal allergens on FEV$_1$% values has been examined in some studies. Weiss et al. [15] found that exposures to cockroach, but not dust mite or cat allergens, were correlated with a decline in FEV$_1$% test results. Jaén et al. [16] showed that exposure to cat allergen was associated with lower FEV$_1$ values, but only for women.

The aims of this cross-sectional study of non-smoking, adult asthmatics in Scotland were to determine the correlation between FEV$_1$% values and questionnaire-based measures of respiratory health and to determine the correlation between FEV$_1$% values and some biological exposures in the home. The dust samples and environmental and anonymous clinical data that were obtained in an earlier study [17] were utilized in this analysis.

Methods

Study population

The original study was approved by the Lanarkshire Research Ethics Committee and all participants gave written informed consent. This study was conducted from 2006 to 2008 and the details have been published [17]. The original study design was to test whether an added home ventilation system might improve the respiratory health of asthmatic adults who were all allergic to house dust mites. However, because dust samples were collected from each home and because spirometry testing was performed on each adult at baseline (before the intervention), there was an opportunity to use the baseline data and samples in a cross-sectional study of the effect of some biological exposures on the respiratory health of the asthmatic adults.

A volunteered smoking history was taken and serum samples obtained to determine the cotinine concentration (Cozart Bioscience Ltd, Abingdon, UK) in order to confirm smoking status [17]. As smoking is the major exposure affecting pulmonary function [18], smokers were excluded from this evaluation of the relationship between biological exposures and respiratory health.

Persons 16–60 years of age with asthma were recruited for the original study [17]. At the time of the initial baseline clinical visit, a health questionnaire was completed and spirometry testing performed (before the use of a bronchodilator) by each person using a Vitalograph Spirometer (Buckingham, UK). The FEV$_1$% of predicted normal was calculated using the European Community for Coal and Steel, 1993 updated reference formula [19] which also adjusts for age, gender, and height and is incorporated into the output from the spirometer.

Sampling

At about the time of the clinical visit, dust samples were collected from the living room floor and separately from the bedroom floor of each home. The open areas (not covered by lamps, furniture etc.) in each room were vacuumed at a rate of 1 m^2 per min using a Dyson, 1500 watt, model–DC14 (Dyson, London, UK) vacuum cleaner [17]. For example, if there was 2 m^2 of open floor space in a room, it was vacuumed for 2 min to collect that sample.

The Dyson DC14 is a bagless vacuum cleaner that uses an extremely effective HEPA filter and captures all particles above 0.3 microns in size, as measured by a laser particle scanner. The dust was collected in the vacuum's reservoir which was meticulously cleaned between each sampling event. The dust in the reservoir was emptied into a sealable bag, placed in a refrigerated cooler, and returned to the laboratory. At the laboratory, the dust samples were screened through a 2-mm-pore sieve to remove large particles and then stored at −20°C before further processing and analysis.

Dust analysis

The concentration of dust mite allergens, cat allergen, dog allergen, (1–3)-β-glucan, and endotoxin was

quantified in each living room and bedroom sample, as previously described [17]. For the ERMI analysis, 5.0 ± 0.1 mg of the total, combined living room and bedroom dust samples were extracted using a bead beater (after the internal reference organism was added), as previously described [20].

The 36 ERMI moulds were quantified in each dust extract by QPCR analysis [20]. Briefly, the standard reaction assays contained 12.5 μL of Universal Master Mix (Applied Biosystems Inc., Foster City, CA, USA), 1 μL of a mixture of forward and reverse primers at 25 μM each, 2.5 μL of a 400 nM TaqMan probe (Applied Biosystems Inc.), 2.5 μL of 2 mg/mL fraction V bovine serum albumin (Sigma Chemical, St. Louis, MO, USA), and 2.5 μL of DNA-free water (Cepheid, Sunnyvale, CA, USA). To this mix was added 5 μL of the DNA extract from the sample. All primer and probe sequences used in the assays have been published [21]. Primers and probes were synthesized commercially (Applied Biosystems, Inc.).

The ERMI value for each home was calculated, as shown in Eqn. 1, by taking the 'Sum of the Logs' of the concentrations of the 26 Group 1 species (s_1) and subtracting the 'Sum of the Logs' of the concentrations of 10 Group 2 species (s_2) [22].

$$\text{ERMI} = \sum_{i=1}^{26} \log_{10}(s_{1i}) - \sum_{j=1}^{10} \log_{10}(s_{2j}) \quad (\text{Equation 1})$$

(The 26 'Group 1' species are found in water-damaged homes and the 10 'Group 2' species are found in homes independent of water damage and that generally, but not exclusively, come from outdoors.)

Statistical analyses

Associations between $FEV_1\%$ and other measures of respiratory health, that is ACQ, SGRQ scores, and corticosteroid use, were determined via their respective Pearson correlation coefficients. Likewise, relationship between $FEV_1\%$ results and the biological exposures, that is ERMI values, allergens from dust mite, cat, and dog, $(1, 3)$-β-D-glucan, and endotoxin, were determined via their respective Pearson correlation coefficients. In addition, multiple linear-regression analysis was used to further investigate the relationship between $FEV_1\%$ and the combination ERMI score and living room endotoxin levels, given the latter's marginally significant relationship with $FEV_1\%$. Regression analysis of $FEV_1\%$ on ERMI was performed and graphed along with the corresponding 95% confidence interval. Analyses were performed in SAS version 9.3 (SAS Institute, Cary, NC, USA) and R version 2.14 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The demographic, clinical or home characteristics of the non-smoking, adult asthmatics and their homes are shown in Table 1. The $FEV_1\%$ test results were correlated with ACQ scores (ρ -0.586, $P < 0.001$), SGRQ scores (ρ -0.313, $P = 0.020$), and weakly with corticosteroid use (ρ -0.221, $P = 0.105$) (Table 2). The ERMI values in the homes were significantly correlated with $FEV_1\%$ test results (ρ -0.378, $P = 0.004$) (Table 3). There was no correlation between $FEV_1\%$ and the concentrations of endotoxin, $(1, 3)$-β-D-glucan, or any of the allergens (Table 3).

The average ERMI value in these homes in Scotland was 5.3 (standard deviation 4.5), and the average $FEV_1\%$ test result was 85.4% (standard deviation 18.6%). The regression analysis scatter plot of $FEV_1\%$ test results on ERMI values showed their inverse relationship (Fig. 1). Living room endotoxin levels alone were marginally related to $FEV_1\%$ values ($P = 0.063$) and their inclusion in the multiple linear-regression analysis, along with ERMI values, only increased the R^2 of the regression from 14% to 17%.

Discussion

The demographic, clinical or home characteristics of the non-smoker, adult asthmatics and their homes are shown in Table 1. Except for cotinine concentrations, these values were comparable to the entire cohort (smokers and non-smokers) in the original study (data not given) [17].

We found that $FEV_1\%$, as an objective measure of respiratory health, was correlated with other measures of respiratory health which are based on recall in

Table 1. Baseline demographic, clinical and home characteristics

Demographic	Mean $\pm$ SD or % of total
Age (years)	42 ± 23
Gender (female)	65
Race (Caucasian)	98
Cotinine (ng/mL serum)	3.1 ± 2.6
Clinical	
Duration of asthma (years)	20 ± 13
Atopic dermatitis (positive)	15
Allergic hay fever (positive)	78
Allergic eczema (positive)	29
Dose of inhaled corticosteroid (μg)*	715 ± 412
Home	
Age of home (years)	43 ± 9.5
With carpets (positive)	85
With cat (positive)	13
With dog (positive)	17

*Beclomethasone equivalent.

Table 2. Pearson correlations of the forced expiratory volume in one-second percent predicted (FEV$_1$%) and other with other measures of respiratory health. All spirometry tests were completed before use of a bronchodilator

	Median (IQR)	Pearson's ρ	P-value
FEV$_1$%	88 (74, 99)	1.0	Not applicable
ACQ score	1.4 (1.1, 2.4)	−0.586	**<0.001**
SGRQ score	27.3 (16.3, 39.6)	−0.313	**0.020**
Dose of inhaled corticosteroid (μg)*	800 (400, 1000)	−0.221	0.105

IQR, interquartile range; ACQ, Asthma Control Questionnaire score; SGRQ, St. George's Respiratory Questionnaire score.
*Beclomethasone equivalent.
P-values highlighted in bold indicate statistical significance.

Table 3. Pearson correlation between forced expiratory volume in one-second percent predicted (FEV$_1$%) and exposure variables (median)

	Median (IQR)	Pearson's ρ	P-value
ERMI	5.26 (2.6, 8.6)	−0.378	**0.004**
Living Room Der p 1 (μg/gm)	0.6 (0.2, 3.7)	0.006	0.967
Living Room Der p 2 (μg/gm)	0.2 (0.1, 1.6)	0.045	0.755
Living Room Fel d 1 (μg/gm)	0.4 (0.1, 1.6)	−0.026	0.856
Living Room Can f 1 (μg/gm)	4.6 (1.1, 27.5)	−0.093	0.521
Bedroom Der p 1 (μg/gm)	0.3 (1.0, 1.5)	0.017	0.910
Bedroom Der p 2 (μg/gm)	0.2 (0.0, 1.9)	0.074	0.618
Bedroom Fel d 1 (μg/gm)	0.4 (0.1, 5.8)	−0.068	0.646
Bedroom Can f 1 (μg/gm)	4.1 (0.5, 20.6)	0.016	0.911
Living room endotoxin (EU/g dust)	10.4 (5.2, 16.7)	−0.271	0.063
Bedroom endotoxin (EU/g dust)	10.0 (5.2, 16.0)	−0.204	0.169
Living room glucan (ng/g dust)	435 (279, 631)	−0.173	0.240
Bedroom glucan (ng/g dust)	363 (277, 579)	−0.107	0.472

IQR, interquartile range; ERMI, Environmental Relative Moldness Index; Der p 1 and Der p 2, *Dermatophagoides pteronyssinus* allergens 1 and 2; Fel d 1, cat allergen; Can f 1, dog allergen; EU, endotoxin units.
The p-value highlighted in bold indicates statistical significance.

answering a questionnaire, that is corticosteroid use, ACQ and SGRQ scores. The ACQ score does include the FEV$_1$% value, so it is not surprising that the FEV$_1$% values are the most highly correlated with ACQ scores. The mould exposure, as described by the ERMI values in each home, was the only biological assessment that was correlated with FEV$_1$% results.

The average ERMI value in these Scottish homes of asthmatic adults was 5.3. In a study of asthmatic adults in the U.S., specifically California, the average ERMI value was 6.0 [23]. No other study of adult asthmatics
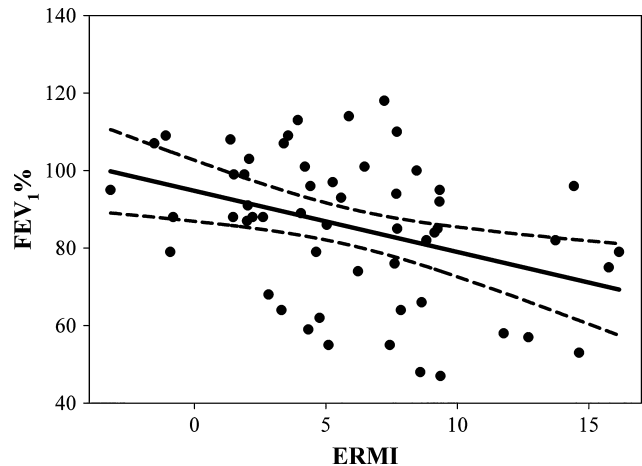

Fig. 1. Scatter plot of Environmental Relative Moldness Index (ERMI) values ($n = 55$) and the regression line (solid black) through the corresponding forced expiratory volume in one-second percent predicted (FEV$_1$%) values and the 95% confidence interval (dashed lines).

has utilized the ERMI metric. However, the homes of asthmatic children in three cities (Boston, Kansas City and San Diego) in the U.S., had an average ERMI value of 8.7 [24]. Also, infants exposed to homes with ERMI values above 5 were found to be twice as likely to develop asthma by age seven [25]. So the average ERMI value measured in the homes of asthmatic adults in Scotland was consistent with results from the homes of asthmatic adults and children in the US.

We recognize that asthma has a complex aetiology and there are likely many causes. However, if mould is causing some cases of asthma, then elucidating the agent's causal mechanism would help clarify the relationship.

Recently, Millien et al. [26] demonstrated that some moulds can cause chronic airway surface mycotic infections (ASMI) in a mouse model of asthma. These ASMI result in lung damage, which includes enhanced airway epithelial and vascular endothelial cell permeability. ASMI set into motion a cascade of events that led to asthma-like symptoms in these mice. If this model is confirmed in humans, then it would help to explain why high mould exposures are linked to some cases of asthma.

Many of the previous studies of the relationship between mould exposures and asthma have utilized methods that are not reliably quantified, for example visual inspection or mouldy odour. The ERMI is a standardized metric developed by the U.S. Environmental Protection Agency, in conjunction with the U.S. Department of Housing and Urban Development, to describe mould contamination in U.S. homes [22]. Its wider geographic applicability remains to be seen.

Although mould exposures have been linked to asthma in many studies [27–29], the linkage to lung function, as quantified by spirometry testing, has not

often been included in such studies. Norbäck et al. [30] did show that the presence of dampness in homes was a risk factor for lung function decline, especially in women. Although our results in Scotland suggest that there is a quantitative link between mould exposures and reduced lung function, this study cannot be considered as causal proof because of the study's many limitations.

The limitations of this study included the relatively small sample size, the fact that the sampled population was not randomly obtained (but part of earlier study), and many chemicals associated with decreased lung function [31] were not measured. Also, we did not quantify every possible biological exposure, including specific bacteria [32], viruses [33], or horse allergens [34] that might affect $FEV_1\%$ values.

In addition, dust samples were used in this study, as opposed to air samples, which might have provided different results [35]. Also, we recognize that the ERMI scale was created for US housing and improvements to the scale might be made for Europe by a random European sampling of homes, as was carried out in the US to create the ERMI [36].

In spite of these many limitations, this study in Scotland adds to the growing scientific literature linking mould exposure to poor respiratory health and asthma. Therefore, it might be prudent for asthmatics to avoid high ERMI environments.

Acknowledgements

Conflict of interest

The U.S. Environmental Protection Agency (EPA) through its Office of Research and Development collaborated in the research described here. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use. As mould-specific quantitative PCR technology is patented by the US EPA, the Agency has a financial interest in its commercial use.

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