Article Type: Original Article-Asthma and Rhinitis

Decreased FEV₁% in Asthmatic Adults in Scottish Homes with High Environmental Relative Moldiness Index Values

C. McSharry¹, S. Vesper², L. Wymer³, S. Howieson³, R. Chaudhuri¹, G. R. Wright¹,

N. C. Thomson¹

¹Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK.

²United States Environmental Protection Agency, National Exposure Research Laboratory, Cincinnati, OH, USA.

³Department of Architecture and Building Science, University of Strathclyde, Glasgow, Glasgow, UK.

Running heading: Decreased FEV₁% in Asthmatic Adults

Corresponding author: Dr. Charles McSharry, Room B340, Institute of Infection, Immunity and Inflammation, Sir Graeme Davies Building, University of Glasgow, 120 University Place, Glasgow G12 8TA, UK. Tel: (44) 0 141 330 2282

Email: Charles.McSharry@glasgow.ac.uk

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cea.12482

This article is protected by copyright. All rights reserved.
Abstract

Background  Exposures to indoor biological contaminants have been implicated in asthma’s etiology 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 (FEV₁%), 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 FEV₁% value calculated. At about the same time, floor dust samples were collected in the living room and in the bedroom. These dust samples were analyzed for mold contamination, as described by the Environmental Relative Moldiness Index (ERMI) values and by (1, 3)-β-D-glucan concentrations; and for endotoxin, and for dust mite, cat, and dog allergens concentrations. The asthmatics’ FEV₁% values were tested for correlation (Pearson) to questionnaire-based estimates of health. Also, each biological exposure was tested for correlation (Pearson) to the FEV₁% values.

Results  FEV₁% results were correlated with ACQ scores (rho -0.586, p<0.001), SGRQ scores (rho -0.313, p= 0.020), and weakly with corticosteroid use (rho -0.221, p= 0.105). The ERMI values in the homes (average 5.3) was significantly correlated with FEV₁% values (rho -0.378, p= 0.004). There was no correlation between FEV₁% 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 mold 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, FEV₁%, ERMI, lung function

Introduction

Worldwide, 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, 4, 5]. Asthma has been associated with various biological exposures, including mold, 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 FEV₁% values.

Mold exposures have often been estimated by 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 FEV₁% measurements. For example, Thorn and Rylander [6] found no effect of different exposures of 0 to 19 ng (1, 3)-β-D-glucan per m³ of air on FEV₁%. Similarly, Blanc et al. [7] found no correlation between (1, 3)-β-D-glucan concentration in the dust and FEV₁% values. However, the high ERMI values in homes of asthmatic children in New Orleans was linked to reduced FEV₁% values [8].

This article is protected by copyright. All rights reserved.
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.

This article is protected by copyright. All rights reserved.
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, since 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]. Since 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 to 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–DC 14 (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 were 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 was extracted using a bead-beater (after the internal reference organism was added), as previously described [20].

The 36 ERMI molds 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), 1μl 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) and 2.5 µl of DNA free water (Cepheid, Sunnyvale, CA). 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 Eq. 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].

$$ERMI = \sum_{i=1}^{26} \log_{10}(s_{1i}) - \sum_{j=1}^{10} \log_{10}(s_{2j})$$  \hspace{1cm}  (Eq.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, i.e. ACQ, SGRQ scores and corticosteroid use, were determined via their respective Pearson correlation coefficients. Likewise, relationship between FEV$_1$% results and the biological exposures, i.e. 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) 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 (rho -0.586, p< 0.001), SGRQ scores (rho -0.313, p= 0.020), and weakly with corticosteroid use (rho -0.221, p= 0.105) (Table 2). The ERMI values in the homes were significantly correlated with FEV$_1$% test results (rho -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].

This article is protected by copyright. All rights reserved.
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 answering a questionnaire, i.e. 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 mold 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 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 etiology and there are likely many causes. However, if mold 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 molds 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 mold exposures are linked to some cases of asthma.
Many of the previous studies of the relationship between mold exposures and asthma have utilized methods that are not reliably quantified, e.g. visual inspection or moldy odor. 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 mold contamination in U.S. homes [22]. Its wider geographic applicability remains to be seen.

Although mold exposures have been linked to asthma in many studies [28, 29, 30], the linkage to lung function, as quantified by spirometry testing, has not often been included in such studies. Norbäck et al. [2011] did show that the presence of dampness in homes was a risk factor for lung function decline, especially in women [31]. Although our results in Scotland suggest that there is a quantitative link between mold 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 [30, 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 FEV₁% 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 done 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 mold exposure to poor respiratory health and asthma. Therefore, it might be prudent for asthmatics to avoid high ERMI environments.

Acknowledgements

We thank Teresa Ruby for preparation of the figure, Miss Lisa Jolly and Mrs Iona Donnelly for laboratory biomarker measurements, the Lanarkshire GPs and practice staff for their help with patient recruitment, and the study participants and their families for their helpful participation.

The original study [17] was funded by MRC, UK. The development of the Multiplex Array for Indoor Allergens (MARIA) has been supported in part by the National Institute of Environmental Health Sciences, Small Business Innovation Research contract ES55545.

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. Since mold specific quantitative PCR technology is patented by the US EPA, the Agency has a financial interest in its commercial use.

References


This article is protected by copyright. All rights reserved.
Dissemination Committee report. *Allergy* 2004; **59**:469-78.


5 Witt CA, Sheshadri A, Carlstrom L, *et al.* Severe Asthma Research Program (SARP).

   Longitudinal changes in airway remodeling and air trapping in severe asthma. *Acad Radiol* 2014; **21**:986-93.

6 Thorn J, Rylander R. Airways inflammation and glucan in a row house area.

   *Am J Respir Crit Care Med* 1998; **157**:1798-1803.


8 Vesper SJ, Wymer L, Kennedy S, *et al.* Decreased pulmonary function measured in children exposed to high environmental relative moldiness index homes.

   *Open J Respir Med* 2014; **7**:83-86.

This article is protected by copyright. All rights reserved.


16 Jaén A, Sunyer J, Basagaña X, et al. Specific sensitization to common allergens and pulmonary function in the European Community Respiratory Health Survey.


This article is protected by copyright. All rights reserved.


Table 1. Baseline demographic, clinical and home characteristics.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Mean + Standard Deviation or % of total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>42 + 23</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>65</td>
</tr>
<tr>
<td>Race (Caucasian)</td>
<td>98</td>
</tr>
<tr>
<td>Cotinine (ng/ml serum)</td>
<td>3.1 + 2.6</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
</tr>
<tr>
<td>Duration of asthma (years)</td>
<td>20 + 13</td>
</tr>
<tr>
<td>Atopic dermatitis (positive)</td>
<td>15</td>
</tr>
<tr>
<td>Allergic hay fever (positive)</td>
<td>78</td>
</tr>
<tr>
<td>Allergic eczema (positive)</td>
<td>29</td>
</tr>
<tr>
<td>Dose of inhaled corticosteroid (μg)*</td>
<td>715 + 412</td>
</tr>
<tr>
<td><strong>Home</strong></td>
<td></td>
</tr>
<tr>
<td>Age of home (years)</td>
<td>43 + 9.5</td>
</tr>
<tr>
<td>With carpets (positive)</td>
<td>85</td>
</tr>
</tbody>
</table>
Table 2. Pearson correlations of the forced expiratory volume in one second percent predicted (FEV₁%) and other with other measures of respiratory health. All spirometry tests were completed before use of a bronchodilator. Abbreviations: IQR, interquartile range; ACQ, Asthma Control Questionnaire score; SGRQ, St. George’s Respiratory Questionnaire score.

<table>
<thead>
<tr>
<th></th>
<th>Median (IQR)</th>
<th>Pearson’s rho</th>
<th>p – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁%</td>
<td>88 (74, 99)</td>
<td>1.0</td>
<td>not applicable</td>
</tr>
<tr>
<td>ACQ score</td>
<td>1.4 (1.1, 2.4)</td>
<td>-0.586</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SGRQ score</td>
<td>27.3 (16.3, 39.6)</td>
<td>-0.313</td>
<td>0.020</td>
</tr>
<tr>
<td>Dose of inhaled corticosteroid (μg)*</td>
<td>800 (400, 1000)</td>
<td>-0.221</td>
<td>0.105</td>
</tr>
</tbody>
</table>

* Beclometasone equivalent

Table 3. Pearson correlation between forced expiratory volume in one second percent predicted (FEV₁%) and exposure variables (median). Abbreviations: IQR, interquartile range; ERMI, Environmental Relative
Moldiness 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.

<table>
<thead>
<tr>
<th></th>
<th>Median (IQR)</th>
<th>Pearson’s rho</th>
<th>p – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERMI</td>
<td>5.26 (2.6, 8.6)</td>
<td>-0.378</td>
<td>0.004</td>
</tr>
<tr>
<td>Living Room Der p 1 (μg/gm)</td>
<td>0.6 (0.2, 3.7)</td>
<td>0.006</td>
<td>0.967</td>
</tr>
<tr>
<td>Living Room Der p 2 (μg/gm)</td>
<td>0.2 (0.1, 1.6)</td>
<td>0.045</td>
<td>0.755</td>
</tr>
<tr>
<td>Living Room Fel d 1 (μg/gm)</td>
<td>0.4 (0.1, 1.6)</td>
<td>-0.026</td>
<td>0.856</td>
</tr>
<tr>
<td>Living Room Can f 1 (μg/gm)</td>
<td>4.6 (1.1, 27.5)</td>
<td>-0.093</td>
<td>0.521</td>
</tr>
<tr>
<td>Bedroom Der p 1 (μg/gm)</td>
<td>0.3 (1.0, 1.5)</td>
<td>0.017</td>
<td>0.910</td>
</tr>
<tr>
<td>Bedroom Der p 2 (μg/gm)</td>
<td>0.2 (0.0, 1.9)</td>
<td>0.074</td>
<td>0.618</td>
</tr>
<tr>
<td>Bedroom Fel d 1 (μg/gm)</td>
<td>0.4 (0.1, 5.8)</td>
<td>-0.068</td>
<td>0.646</td>
</tr>
<tr>
<td>Bedroom Can f 1 (μg/gm)</td>
<td>4.1 (0.5, 20.6)</td>
<td>0.016</td>
<td>0.911</td>
</tr>
<tr>
<td>Living room endotoxin (EU/g dust)</td>
<td>10.4 (5.2, 16.7)</td>
<td>-0.271</td>
<td>0.063</td>
</tr>
<tr>
<td>Bedroom endotoxin (EU/g dust)</td>
<td>10.0 (5.2, 16.0)</td>
<td>-0.204</td>
<td>0.169</td>
</tr>
<tr>
<td>Living room glucan (ng/g dust)</td>
<td>435 (279, 631)</td>
<td>-0.173</td>
<td>0.240</td>
</tr>
<tr>
<td>Bedroom glucan (ng/g dust)</td>
<td>363 (277, 579)</td>
<td>-0.107</td>
<td>0.472</td>
</tr>
</tbody>
</table>
Fig 1. Scatter plot of Environmental Relative Moldiness Index (ERMI) values (n=55) and the regression line (solid black) through the corresponding forced expiratory volume in one second percent predicted (FEV₁%) values and the 95% confidence interval (dashed lines).