Title: Comparative Airway Response to High- vs Low-Molecular Weight Agents in Occupational Asthma

Running title: Bronchial responses to occupational agents

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ABSTRACT

Airway responses to occupational agents in sensitized workers may vary, clinically and physiologically. We compared the patterns of change in airway responsiveness, type of response, and fall in expiratory flows following laboratory exposure to high or low molecular weight agents in sensitized workers.

Data on workers who underwent specific inhalation challenges with occupational sensitizers (117 exposed to HMW and 130 to LMW) were collected in their medical charts.

Maximum falls in FEV₁ were of similar magnitude for both types of agents. Compared with high molecular weight agents, low molecular weight substances induced more frequently late or dual responses and higher increases in airway responsiveness. After exposure to high molecular weight agents, there was a mean reduction in doubling concentrations of methacholine (± SD) of 0.5 ± 1.7 for early responses, compared to 2.8 ± 1.2 for late responses, and 1.4 ± 2.0 for dual responses. Isolated early responses were more frequently found in women, smokers, workers with a higher % predicted FEV₁ and higher PC₂₀, and in those with longer asthma duration.

Workers’ characteristics, as well as the type of agent they are sensitized to, may help predict the type of response after specific inhalation challenge.

Key Words: Airway inflammation, airway responsiveness, occupational asthma, Specific inhalation challenge.

Word count: 196
INTRODUCTION

Occupational asthma (OA) is one of the most common occupational ailments. It is defined as a disease characterized by variable airflow limitation and/or airway hyperresponsiveness due to causes and conditions attributable to a particular occupational environment and not to stimuli encountered outside the workplace[1]. The specific-inhalation challenge (SIC) is recognized as a good reference diagnostic test for OA[2]. This test is performed in specialized centers and involves exposing the worker to the suspected offending agent using incremental doses, in order to detect any fall in expiratory flows and/or any increase in airway responsiveness (AR)[3].

There are more than 300 known OA-causal agents and these are divided into high-molecular weight agents (HMW), such as flour, latex, laboratory animals, and grain dust, and low-molecular weight agents (LMW), such as isocyanates, plicatic acid, metals, and anhydrides[3]. HMW agents are considered to act as common airborne allergens through Immunoglobulin E (IgE)-mediated responses, while the mechanisms by which LMW substances induce asthma may be different, possibly involving non-specific immune mechanisms[4].

Up until now, the few studies available on patterns of response to occupational agents have demonstrated variable patterns of response to HMW and LMW agents in limited numbers of patients[5]. Furthermore, how worker’s characteristics may influence the pattern of response to sensitizing agents remains to be explored. The various types of responses identified include: A) early responses (EAR), usually defined as a fall in forced expiratory volume in one second (FEV₁) of 20% or more in the first hour following the end of exposure; B) late responses (LAR), characterized by a decrease of 15-20% or more between 2 and 8 hours after exposure; C) dual responses (DAR), a combination of both types of reactions with a recovery of FEV₁ between the
two reactions, without the use of a bronchodilator; and D) atypical responses (AAR), where such recovery is not observed [1;3].

HMW agents seem to mostly induce early or dual reactions while LMW agents produce early, late, dual or atypical asthmatic reactions[3]. Malo et al. previously looked at clinical and functional parameters among three groups of subjects with OA caused by Western red cedar, isocyanates, and HMW agents[5]. In this study, having a non-immediate reaction at the time of SIC, being continuously exposed to the agent, and being younger slightly increased the risk of developing symptoms in subjects with occupational asthma.

Herein, we present the results of an analysis of the comparative influence of HMW and LMW substances on patterns of response, expiratory flows and airway responsiveness in a large group of patients with OA proven through SIC. To the best of our knowledge, this is the first study of this magnitude with such a diversity of sensitizing agents.

**METHODS**

**Subjects and Study Design**

A retrospective review of all the charts of subjects aged 18 and over with OA confirmed by a positive SIC between 2001 and 2005 in two Canadian referral centers for OA; Hôpital Laval (Quebec City) and Hôpital du Sacré-Coeur (Montreal), was conducted. Workers had experienced no upper or lower respiratory tract infection within the 4 weeks before the challenges and had a stable respiratory condition. Charts were reviewed by trained research assistants. Results of the respiratory function tests, methacholine and specific inhalation challenges, in addition to data on medical history were analysed.
**Definitions**

We defined EAR as a percent fall in FEV\(_1\) of 20% or more within one hour from the last inhalation of the sensitising agent during a SIC. A LAR was defined as a fall in FEV\(_1\) of 15% or more between 2 and 8 hours following challenge. A DAR was defined as the combination of an early and a late reaction. Finally, AAR was defined as an early fall in FEV\(_1\), progressing to a maximum reaction 5 to 6 hours later or a “square waved” response, similar to a dual reaction but with only partial recovery (<10%) between the immediate and late falls in FEV\(_1\) or in the form of a prolonged immediate reaction.

**Spirometry and Methacholine Challenge**

Spirometry was performed according to the recommendations of the American Thoracic Society[6]. Methacholine challenge tests were done according to the method reported by Juniper et al.[7]. Responses were expressed as the PC\(_{20}\), obtained from the log-dose response curve. The change in AR was expressed in number of doubling concentrations of methacholine.

**Skin Prick Tests**

Skin prick tests were performed with commercial extracts (Omega, Montreal, Quebec, Canada) from common inhalants (trees, grasses, ragweed, house-dust mites, hormodendrum, aspergillus, alternaria, cat, and dog). A positive response was defined as a weal diameter of 3 mm or more, at 10 minutes, in the absence of reaction to a control diluent, and with a positive response to histamine phosphate (10 mg/ml), as previously described[8]. A subject was considered to be atopic if at least one positive response was observed.
**Specific Inhalation Challenges (SIC)**

SIC were performed according to previously published methods[9;10]. For each series of challenges, a control day was performed, the subjects being initially exposed to a non-sensitizing agent (e.g. lactose, solvents). During SIC, \( \text{FEV}_1 \) was measured at baseline and every 10 minutes for the first hour, every 30 minutes for the second hour, and then hourly for at least eight hours after the end of exposure[10]. On the following days, the time or dose of exposure to the occupational agent was progressively increased. For agents causing OA through an IgE-mediated mechanism, such as HMW substances, increasing-doses exposure was performed progressively over one day, while the exposure for agents causing OA without a recognized IgE-mediated mechanism was completed over several days. Occasionally, concurrent treatment with inhaled corticosteroids (ICS) could be maintained during the investigation if considered necessary, but long-acting \( \beta_2 \)-agonists were stopped 72 hours before the tests, when possible. Short-acting inhaled \( \beta_2 \)-agonists were withheld at least 6 hours before the challenges.

**Statistical Analyses**

No statistical analyses were completed with subjects who had an atypical reaction since the number of subjects with such response was too low. Between-factors interaction was considered in the statistical model. Some variables were log-transformed to stabilize variance. The univariate normality assumptions were verified with the Shapiro-Wilk tests and the Brown and Forsythe's variation of Levene's test statistics was used to verify the homogeneity of variances. In order to determine the factors associated to the reaction type (early vs. other), a stepwise logistic regression analysis was performed. Data were expressed using mean ± standard deviation (SD). According to the data distribution in the comparison of LMW versus HMW sensitized subjects, Student’s t test or chi-square test was used. Data were analyzed using a two-way ANOVA.
model. One factor (way) was associated to the comparison among the three groups of subjects (early, late and dual reactions) while the other factor was associated to the comparison between results from LMW allergens and HMW allergens. The results were considered significant at a p-value $\leq 0.05$. All analyses were conducted using the SAS statistical package, version 9.1.3 (SAS Institute Inc, Cary, NC, U.S.A.).

RESULTS

Subjects’ Characteristics

Out of the 247 charts reviewed, 117 subjects were sensitized to HMW and 130 to LMW agents. Subjects’ characteristics are summarized in Table 1. These characteristics were similar in both groups except for gender and smoking habit. Indeed, a higher proportion of men were sensitized to LMW (76%) compared with HMW agents (53%) ($p<0.0001$), while this proportion was similar in women. There were more non-smokers and ex-smokers among subjects sensitized to LMW and more smokers in those sensitized to HMW ($p<0.0001$). The prevalence of atopy and the latency period between the onset of exposure and the onset of asthma symptoms were similar in both groups. Subjects were away from work for variable time periods, ranging from days to years.

There were no significant differences in the proportion of workers sensitized to LMW using ICS (89, 68%) compared with those with HMW-related occupational asthma (72, 62%) ($p>0.05$). Taking ICS or not had no influence on the maximum fall in FEV$_1$ during SIC and on the change in the provocative concentration of methacholine inducing a 20% fall in FEV$_1$ (PC$_{20}$), before vs.
after the challenge (p=0.4). The use of ICS was not associated with a specific type of asthmatic reaction (p=0.1, data not shown).

**Sensitizing Agents**

The various sensitizing agents responsible for OA are shown on Table 2. In the group sensitized to HMW agents, flour and seafood were the more prevalent (13.8% and 12.6% of workers, respectively). For LMW agents, isocyanates, wood dust, and metals were the most commonly found (17.8%, 6.9% and 6.9%).

**Magnitude of the fall in FEV1 after SIC**

On control days, no significant respiratory symptoms or change in FEV1 were noted. There were no significant differences between the mean maximum fall in FEV1 during SIC between reactions to LMW (mean±SD: 28.6±13.8) and HMW agents (28.1±12.2, p>0.05). In both groups, dual reactions were associated with a greater maximum fall in FEV1 than early reactions (LMW: 38.0±9.1% vs 30.3±12.4%, respectively and HMW: 34.9±10.3% vs 28.7±11.2%, respectively) (p=0.002, Figure 1). The number of more severe reactions (fall in FEV1 greater than 40%) was similar in both groups (LMW: 19 occasions (14.6%); HMW: 16 occasions (13.7%)).

**Patterns of Response Following SIC**

Following SIC, patterns of response were different for HMW and LMW agents. Percentages of workers presenting early, late, dual and atypical responses were respectively 61%, 9%, 14% and
0% following HMW agent challenges, and 32%, 23%, 17% and 3% with LMW agents (p<0.001, ANOVA, Table 2). Subjects exposed to HMW presented early reactions more frequently (61%) than subjects exposed to LMW (32%), whereas subjects exposed to LMW agents showed more frequently late (23%) and atypical reactions (3%) than those exposed to HMW agents (9% and 0%, respectively) (p<0.05).

Immediate reactions were common in smokers (odds ratio (confidence interval): 2.42 (1.06-5.52)) and ex-smokers (2.09 (1.04-4.21)) (p=0.04), while non-smokers presented more often non-immediate reactions (p=0.005). Patients with an immediate reaction following SIC had a longer asthma duration (mean ± SD: 7.4±0.8) than those having a non-immediate reaction (5.3±0.6, p=0.027). The baseline percent of predicted FEV\textsubscript{1} was higher for those with an immediate reaction (mean±SD: 91.3±1.3) than for non-immediate reactions (86.7±1.4, p=0.02). The same pattern was observed for the baseline percentage of predicted forced vital capacity (FVC) (104.0±3.2 vs 96.8±1.3, p=0.04).

Furthermore, subjects with a higher baseline PC\textsubscript{20} had at greater risk of experiencing immediate reactions (odds ratio (confidence interval): 1.02 (1.00-1.04)). There were no other significant differences in the prevalence of the various types of responses in both groups in relationship to the duration of exposure to the offending agents, the latency period, age, duration of asthma, and atopy.

**Airway Responsiveness**
Before SIC, \( \text{PC}_{20} \) was higher in workers experiencing immediate reactions (geometric mean (range): 3.36 (0.02-128)) than for non-immediate reactions (2.3 (0.04-64), \( p=0.03 \)). The post-challenge increase in airway responsiveness, in number of double concentrations of methacholine, was higher following exposure to LMW (mean±SD: 1.8±2.3) compared with HMW agents (0.8±1.8, \( p=0.0006 \)). Furthermore, if we compare this parameter with the type of reaction observed during SIC, the fall in doubling concentrations of methacholine after immediate responses following exposure to HMW (0.5±1.7) was lower than for late (2.7±1.2) and dual responses (1.4±2.0, \( p=0.0005 \)) (Figure 2). For the non-immediate reactions, there was a weak negative correlation (\( r=-0.32, p=0.018 \)) between the difference in \( \text{PC}_{20} \) before and after SIC and the maximum \( \text{FEV}_1 \) fall during the challenge. No correlation was found between these parameters for immediate reactions (\( p>0.05 \)).
This study shows that exposure to LMW agents induces late responses more frequently than HMW agents, while early responses are more common after exposure to the latter. Increases in airway responsiveness are of smaller magnitude with HMW-induced early responses than late responses. Men were more frequently exposed to LMW than to HMW whereas women were equally exposed to LMW and HMW. This is likely to be explained by the fact that LMW are often found in jobs where men are represented in a larger proportion than women. Compared with non-immediate responses, immediate responses were found more frequently in women, smokers, workers with a higher predicted FEV\(_1\) and PC\(_{20}\) and in those with a longer duration of asthma.

Definitions of EAR and LAR vary between studies, ranging from 15% to 20% fall in FEV\(_1\)[5;11]. In this study, we used a 20% fall in FEV\(_1\) for EAR and a 15% fall for LAR. The majority of our subjects experienced falls in FEV\(_1\) greater than 20% both for EAR and LAR, with very few subjects between 15% and 20%, making our data comparable to other studies using a 20% fall to define LAR.

Malo et al. previously studied the responses to three types of LMW agents and various HMW substances, with the aim of determining how these exposures influenced the development of symptoms in OA[5]. Western red cedar, isocyanates, and HMW agents induced a non-immediate response in respectively 45, 38 and 14% of workers, while they induced an immediate and dual reaction in 55%, 62% and 86% of the cases. Zammit-Tabona et al. showed that out of 6 subjects showing a specific asthmatic reaction to MDI challenge, 4 had a LAR and 2 had a DAR[12]. Our study extends these observations on a wider range of sensitizing agents. We found that LAR are
more often associated to LMW agents and EAR to HMW agents, in keeping with this previous
report. This is probably related to the fact that HMW agents usually induce OA through an IgE-
mediated mechanism[4], while for LMW agents, the mechanisms involved seem to be usually
different, although still to be determined[9;13-15]. In this respect, HMW agents act mostly as
common non-occupational allergens, IgE dependent mechanisms inducing early or dual
responses, as in the classical type of allergen-induced asthma[3]. Perrin et al.[16] reported that
LMW agents are more often associated with atypical reactions. In our study, although the
number of atypical reactions was smaller than the numbers reported by Perrin et al., all four
subjects having an atypical reaction were sensitized to LMW agents. The paper from Perrin et al.
focused on the characterization of the patterns of asthmatic reactions. All the patterns of
reactions were thoroughly analyzed by the physicians in charge of the study. Our study was a
retrospective study that relied on the information included in the medical charts of the subjects.
Therefore, some atypical reactions may have been confused with or interpreted as typical
reactions in our study. These findings emphasize the need of performing a progressive exposure
to LMW spread over a few days during the SIC in order to minimize potentially severe isolated
late asthmatic reactions.

According to previous observations[5], sensitization to red cedar or isocyanates may occur after
a shorter time interval than for HMW agents. However, we found no differences in the latency
period between the onset of symptoms and the duration of exposure between HMW and LMW.
In our study, the LMW group was composed of a large variety of agents, in contrast to previous
studies that included only red cedar and isocyanates[5;17]. The length of time necessary to
become sensitized may depend, among other factors like genetic[18] or concentration of agents
inhaled[9], upon the nature of each agent rather than on the molecular weight of the agent only.
Following SIC, we observed in this group a smaller increase in AR following EAR induced by HMW agents compared with LAR and DAR. This difference in changes in airway responsiveness is possibly due to the mode of exposure to the offending agent, although the magnitude of the asthmatic response, in keeping with the method used, aiming at a 20 fall in FEV$_1$, was similar in both groups. It could be due to the fact that airway responsiveness was measured at the end of the last SIC in case of EAR, whereas the methacholine challenge was performed the next day in subjects with a LAR since a bronchodilator had to be administered at the end of the day to reverse the asthmatic reaction. However, in allergic asthma, it has been shown that LAR are associated with a more intense airway inflammatory response than EAR, such responses being considered responsible for the increase in AR following exposure to the offending agent[19;20]. The intensity and type of inflammatory response induced by the various agents may explain the differences observed in this parameter.

The higher prevalence of isolated immediate responses in women may be due to the higher prevalence of sensitization (and exposure) to HMW agents compared with men. The reason why smokers show a higher frequency of isolated immediate responses than non-smokers is unclear. There may be an influence of smoke-related substances on the pattern of inflammation, and previous observations had shown some inhibitory effects of allergic and immune mechanisms, either in asthma or in allergic alveolitis, as examples[21;22]. Otherwise, increased immediate responses observed in subjects with higher predicted FEV$_1$ and PC$_{20}$ may indicate an influence of airway caliber and responsiveness on the pattern of airway response to occupational agents. We may suggest that those with increased airflow limitation or airway responsiveness have more bronchial inflammation and maybe remodeling than the others, and that this may influence
responses to occupational agents. With respect to increased immediate responses with a longer duration of asthma, it has been previously shown that the latency period before the beginning of symptoms and withdrawal from work was longer in workers exposed to HMW substances, which induce more frequent immediate responses[9].

In conclusion, we showed that there are significant differences in the type of airway changes induced by LMW and HMW agents. These results could help to better understand the physiopathology of OA and predict the outcome of these workers. However, how occupational agents may cause OA, particularly for LMW agents, remains to be studied. The analysis of the type of airway response induced by these agents and the outcome of workers following withdrawal will help us understand the mechanisms involved. Further studies should focus on comparing the clinical/physiological responses in occupational asthma versus work-aggravated asthma, as well as their relationships with bronchial inflammatory responses. In this respect, the non-invasive methods of assessment of airway inflammation developed in the last years may help find answers about these processes.

Acknowledgments

We wish to thank Serge Simard for the statistical analyses.
### Table 1

**Subject’s Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>LMW</th>
<th>HMW</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>$n ,(%)$</td>
<td>130 (53)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>$\text{mean} \pm \text{SD}$</td>
<td>41.0±11.4</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>$n ,(%)$</td>
<td>99 (76) **</td>
</tr>
<tr>
<td>Male</td>
<td>31 (24)</td>
<td>55 (47)</td>
</tr>
<tr>
<td>Female</td>
<td>62 (53)</td>
<td>55 (47)</td>
</tr>
<tr>
<td><strong>Atopy</strong></td>
<td>$n ,(%)$</td>
<td>78 (60)</td>
</tr>
<tr>
<td><strong>Smoking habit</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>$n ; \text{mean} , P-Y \pm \text{SD}$</td>
<td>49 ; 0±0 **</td>
</tr>
<tr>
<td>Smokers</td>
<td>$n ; \text{mean} , P-Y \pm \text{SD}$</td>
<td>17 ; 17.5±9.0 **</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>$n ; \text{mean} , P-Y \pm \text{SD}$</td>
<td>62 ; 12.2±13.0 **</td>
</tr>
<tr>
<td><strong>Duration of asthma (years)</strong></td>
<td>$\text{mean} \pm \text{SD}$</td>
<td>6.0±8.0</td>
</tr>
<tr>
<td><strong>Duration of exposure (years)</strong></td>
<td>$\text{mean} \pm \text{SD}$</td>
<td>11.5±10.9</td>
</tr>
<tr>
<td><strong>Latency period * (years)</strong></td>
<td>$\text{mean} \pm \text{SD}$</td>
<td>7.3±8.9</td>
</tr>
<tr>
<td><strong>Baseline FEV$_1$</strong></td>
<td>$\text{mean} \pm \text{SD}$</td>
<td>3.0±0.8</td>
</tr>
<tr>
<td>(L)</td>
<td>88.2±18.2</td>
<td>88.7±18.5</td>
</tr>
<tr>
<td>(% predicted)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Baseline FVC</strong></td>
<td>$\text{mean} \pm \text{SD}$</td>
<td>4.2±1.0</td>
</tr>
<tr>
<td>(L)</td>
<td>98.3±15.9</td>
<td>102.5±40.1</td>
</tr>
<tr>
<td>(% predicted)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Baseline PC$_{20}$ Methacholine (mg/ml)</strong></td>
<td>Geometric mean (range)</td>
<td>3.5 (0.02-128)</td>
</tr>
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</table>

* Onset of exposure – onset of asthma symptoms

** p < 0.0001

P-Y = pack-years
<table>
<thead>
<tr>
<th>Types of Occupational Agents and Types of Responses Following SIC</th>
<th>LMW agents</th>
<th>n workers</th>
<th>Early reaction</th>
<th>Late reaction</th>
<th>Dual reaction</th>
<th>Atypical reaction</th>
<th>Unspecified in chart</th>
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<tr>
<td><strong>LMW agents</strong></td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>Isocyanates</td>
<td>44 (17.8)</td>
<td>18</td>
<td>11</td>
<td>6</td>
<td>1</td>
<td>8</td>
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<tr>
<td>HDI</td>
<td>22 (8.9)</td>
<td>9</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>1</td>
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<tr>
<td>MDI</td>
<td>9 (3.6)</td>
<td>5</td>
<td>3</td>
<td>1</td>
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<td>1</td>
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<tr>
<td>TDI</td>
<td>5 (2.0)</td>
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<td>2</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Unspecified</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
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<tr>
<td>Metals</td>
<td>17 (6.9)</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td></td>
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<tr>
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<td>16 (6.5)</td>
<td>7</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>1</td>
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<td>Wood dust</td>
<td>17 (6.9)</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td></td>
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<tr>
<td>Haidresser products</td>
<td>7 (2.8)</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Epoxy</td>
<td>4 (1.6)</td>
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<td>0</td>
<td>2</td>
<td>0</td>
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<td></td>
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<td>3 (1.2)</td>
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<td>2</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td>130 (52.6)</td>
<td>41</td>
<td>30</td>
<td>22</td>
<td>4</td>
<td>33</td>
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<tr>
<td><strong>HMW agents</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flour</td>
<td>34 (13.8)</td>
<td>27</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Plants and Grain dust</td>
<td>16 (6.5)</td>
<td>10</td>
<td>0</td>
<td>5</td>
<td>0</td>
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<tr>
<td>Seafood / Fish</td>
<td>31 (12.6)</td>
<td>21</td>
<td>3</td>
<td>7</td>
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<td>0</td>
<td></td>
</tr>
<tr>
<td>Latex</td>
<td>7 (2.8)</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Animal-derived allergens</td>
<td>20 (8.1)</td>
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<td>7</td>
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<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Leather</td>
<td>2 (0.8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Enzymes</td>
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<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Talc</td>
<td>1 (0.4)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
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</tr>
<tr>
<td>Unknown</td>
<td>4 (1.6)</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>117 (47.4)</td>
<td>71</td>
<td>11</td>
<td>17</td>
<td>0</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td><strong>Grand total</strong></td>
<td>247 (100)</td>
<td>112</td>
<td>41</td>
<td>39</td>
<td>4</td>
<td>51</td>
<td></td>
</tr>
</tbody>
</table>
Legends

Figure 1
Magnitude of the asthmatic response during SIC.

Figure 2
Mean fall in double concentrations of methacholine according to the type of response.
Figure 1

![Bar chart showing mean fall in FEV1 (% predicted) for LMW and HMW with early, late, and dual categories with p = 0.002 for both groups.]
Figure 2

Mean fall of doubling concentration of methacholine (nb)

LMW

HMW

* p = 0.0005

early
late
dual


