IS BRONCHIAL HYPERREACTIVITY PROTECTIVE IN GRAIN DUST INDUCED AIRWAY INFLAMMATION?

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ABSTRACT

To better understand the relative importance of bronchial hyperreactivity (BHR) on the physiologic and inflammatory response in the lung following acute inhalation challenge to corn dust extract (CDE), we performed exposure studies on 5 subjects with BHR and compared the physiologic and inflammatory response to 5 subjects with normal airway reactivity (NAR). Subjects with BHR more frequently complained of chest tightness and dyspnea within the first hour following inhalation of CDE in comparison to subjects with NAR. Similarly, subjects with BHR developed a significantly greater percent declines in FEV1 at time points 10, 20, and 30 minutes following exposure to CDE. This exaggerated decline in FEV1 improved over time, as there were no significant differences in the percent decline in FEV1 at time points 1, 2, 3, 4, and 24 hours post exposure between subjects with BHR and NAR. Increases in total cell, neutrophil, TNFα, IL-6, and IL-8 were detected in bronchoalveolar lavage fluid 4 hours following inhalation of CDE in subjects with BHR and NAR, but no significant differences were detected between these groups. These results suggest that although subjects with BHR develop an exaggerated initial decline in FEV1 following exposure to CDE, the physiologic and inflammatory response to CDE is similar in subjects with and without BHR.

Key Words: grain dust, bronchial hyperreactivity, inhalation exposure, airway inflammation, airflow obstruction

INTRODUCTION:

Epidemiologic studies of grain workers have demonstrated an excess of respiratory symptoms and airflow obstruction associated with chronic exposure to grain dust (1-5). Studies looking at specific host factors, such as atopy or the presence of specific antibodies to grain dust have not been consistently associated with either acute (6) or chronic (7) airway responses to grain dust. Other host factors, such as smoking, age, and duration of employment have been associated with greater longitudinal declines in lung function (7). In addition, it appears that acute changes in airflow over a workshift or workweek are predictive of accelerated longitudinal declines in airflow (4,7,8). In fact, in grainworkers with non-specific bronchial hyperreactivity (BHR), there is an association between workshift changes in FEV1 and longitudinal declines in FEV1, while no association was seen in workers with normal airway reactivity (NAR) (9). These findings would suggest that (BHR) may be an important host factor contributing to the pathogenesis of chronic airflow limitation due to grain dust.

The purpose of the present investigation is to further investigate the role of BHR as it relates to the acute physiologic and inflammatory events due to acute grain dust inhalation. Using an acute exposure model of grain dust induced airway inflammation, our goal was to compare the acute

physiologic and inflammatory changes following exposure to grain dust in subjects with and without BHR. Our hypothesis is that both the physiologic and inflammatory changes following exposure to grain dust were more pronounced in subjects with BHR.

METHODS:

We used a single-blinded, crossover design to determine whether bronchial reactivity affected the acute physiologic and inflammatory changes following acute inhalation of corn dust extract (CDE) in normal, otherwise healthy volunteers.

Study Subjects: The study protocol used was approved by the human subjects committee at this institution. Healthy, never-smoking subjects without any history of prior cardiac disease or history of occupational exposure to grain dust were recruited. To be considered eligible for participation, all study subjects were required to have a normal physical examination, 12 lead electrocardiogram, chest x-ray, and pulmonary function tests (spirometry, lung volumes, diffusing capacity, and arterial blood gases). A standard histamine challenge test was performed on each subject, which included five inhalations each of 0.03, 0.06, 0.12, 0.25, 0.5, 1.0, 2.5, 5.0, and 10.0 mg/ml concentrations of buffered histamine at room temperature delivered according to the guidelines established by the American Academy of allergy, Committee on Standardization of Bronchoprovocation (10) . The cumulative dose (in breath units) of histamine causing a 20% fall in baseline FEV1 as compared with diluent (sterile isotonic saline solution) or up to a maximum dose of 97.3 breath units was determined. Bronchial

hyperreactivity was defined as a 20% or greater decrease in FEV1 as compared to diluent FEV1 with a cumulative dose of histamine less than 47.3 breath units. The slope of the dose-response curve was calculated by dividing the maximal percent drop in FEV1 by the cumulative breath units causing this decline (11). Individuals in the study who were screened and found to have BHR were limited to subjects who were never previously diagnosed with asthma or who had a history of stable, mild asthma but with only occasional (less than twice per week) use of inhaled beta-agonists. Subjects on antihistamines, theophylline, inhaled corticosteroids, or other chronic medications were excluded from participation. Subjects on inhaled beta agonist were instructed to discontinue the drug for 24 hours prior to both the histamine challenge and each inhalation exposure. Subjects with BHR were matched with subjects demonstrating normal airway reactivity (NAR) and of similar age, gender, and body height and weight.

Protocol: Study subjects underwent two separate inhalation challenges, with exposures being separated by at least two weeks. Previously, we have demonstrated that lung function and lavage parameters return to baseline values after 96 hours(12). All subjects were exposed HBSS on the first visit and CDE on the second visit. Vital signs, pulmonary function, and symptomatology were recorded prior to and following each inhalation exposure. Nasal lavage was performed four hours

following inhalation challenge and immediately prior to bronchoscopy (Nasal lavage methods and results are discussed in a separate report).

Preparation of the corn dust extract (CDE): Corn dust used in this study was obtained from the air filtration system at an eastern Iowa grain facility. Corn dust extract (CDE) was prepared by mixing 3.0 grams of dust in 30 ml of sterile, pyrogen free Hank's balanced salt solution without calcium or magnesium (HBSS) (0.1% solution), vortexing for 2 minutes, and shaking for 1 hour at 4°C. The mixture was centrifuged at 800 x g for 20 minutes, and the supernatant solution was collected, resulting in the CDE. The CDE solution underwent filter sterilization through a 0.22 μm filter (Acrocap, Low Protein Binding Filter, Gelman Schences, Ann Arbor, MI). All solutions used for inhalation were derived from a stock solution, which underwent sterility testing (bacteria and fungi) and endotoxin assay prior to separation into individual aliquots. These aliquots were stored at -70°C until being used. Endotoxin concentration was measured by the endpoint chromogenic *Limulus* Amebocyte Lysate (LAL) Assay (QCL-1000, Whittaker Bioproducts, Walkersville, MD). The measured endotoxin concentration in the CDE prepared by this method was 4.0 μg/ml.

Inhalational Challenge: The solutions were administered via a nebulizer (DeVilbiss 646) and dosimeter (DeVilbiss, DeVilbiss Health Care Inc., Somerset, PA), operated at 20 psi air pressure.

Subjects controlled the timing of each nebulized dose and were instructed to inhale through the mouthpiece of the nebulizer and exhale through their nose. Using this delivery system and technique, a precise dose of inhalant was delivered, obtaining maximal airway mucosal exposure. For each exposure, subjects were administered 0.04 ml of inhalant per kilogram of body weight, given over a 60 minute period of time.

Pulmonary Function Testing: The pulmonary function tests consisted of serial spirometry using a spirometer (Spirotech S-600, Graseby Anderson, Atlanta, GA). These maneuvers were performed using standard protocols and the American Thoracic Society guidelines (13). The spirometer was calibrated prior to each visit. Spirometry was performed with noseclips, in a sitting position pre-exposure and at the following time points post-exposure: 10, 20, and 30 minutes, and 1, 2, 3, 4, and 24 hours.

Bronchoscopy: Bronchoscopy was performed 4 hours following each inhalation exposure, in accordance with the standards established by the American Thoracic Society for bronchoscopy in asthmatics (14). An Olympus P-10 (Olympus, Lombard, IL) fiberoptic bronchoscope was introduced into the chosen lung segment for lavage and wedged. Twenty ml of sterile, 0.9% saline (37°C) was injected through the bronchoscope and then collected. This was performed 5 more times for a total lavage volume of 120 ml. The return of the first 20 ml aliquot was separated from the

remaining lavage fluid. Lung segments chosen for BAL alternated between a subsegment of the right middle lobe following the first exposure and a subsegment of the lingula following the second exposure.

Processing of Specimens: Immediately following bronchoscopy, the BAL samples were processed according to methods described previously (15). The BAL supernatant was frozen at -70°C for subsequent use. After washing the cells twice with HBSS, the cell pellet was suspended in RPMI-1640 medium and cell counts were performed. Cytospin preparations were made from the lavage cell resuspension, stained with Diff Quick staining (Baxter Scientific Products, Miami, FL), and cell differential counts were quantified counting 200 cells. Tumor necrosis factor (TNFα), interleukin-6, and interleukin-8 (IL-8) were measured in the BAL supernatant fluid using commercially available enzyme immunoassays (R&D Systems, Minneapolis, MN).

Statistics: CDE induced changes in lung function and biologic measures of inflammation (BAL cellularity and BAL and cytokines) were performed comparing normal and hyrperreactive airway subjects using non-parametric statistics (Wilcoxon Rank Sum Test)(16). Comparison of symptom frequency was performed, using Fisher's Exact test.

RESULTS:

A total of 11 subjects participated and completed the study (9 females, 2 males). Of the six subjects with BHR, only three subjects were able to inhale all of the CDE at the doses used in this study. One subject was removed from the study because of very severe bronchospasm (FEV1 < 50% baseline) following exposure to less than half of the calculated dose of CDE. One subject with BHR received 33% and another subject with BHR 90% of the calculated dose. In contrast, all five subjects with NAR inhaled all of the CDE without any difficulty. Comparison of subjects in the two groups demonstrated similar age, height, weight, and baseline lung function, except for the FEV1/FVC ratio, which was lower in the BHR group (table 1). As expected, there was a significant difference in the slope of the dose-response curve to histamine between BHR and NAR groups ($p<0.01$).

Respiratory and non-respiratory symptoms were reported by subjects following exposure to CDE, including chest tightness, dyspnea, cough, sputum production, malaise, and chills. None of these symptoms were reported following inhalation of HBSS. When comparing the frequency of these symptoms in subjects with and without BHR, only chest tightness and dyspnea were found to be significantly different between these groups (table 2). In subjects with BHR, chest tightness was uniformly experienced for at least the first hour post exposure, with reporting of chest tightness less frequent over time. Only one subject with NAR

experienced chest tightness. Similarly, four subjects with BHR experienced dyspnea, lasting at least one hour following inhalation of CDE, while no subjects with NAR complained of dyspnea. No significant differences in the number of subjects reporting cough (3 BHR, 2 NAR), chills (2 BHR, 2 NAR), sputum production (1 BHR, 0 NAH), or malaise (1 BHR, 1 NAR) at each of the time points queried.

Acute airflow obstruction developed following exposure to CDE in both subjects with and without BHR, occurring as early as 10 minutes post exposure and persisting for at least 4 hours post exposure (figure 1). In contrast, both groups did not develop clinically significant airflow obstruction to HBSS (data not shown). Unlike subjects with NAR, subjects with BHR developed abrupt declines in FEV1 immediately following inhalation of CDE (figure 1). At 10 minutes post exposure, the mean percent decline in FEV1 from baseline in subjects with BHR was 45%, which was significantly greater than subjects with NAR (11%) ($p<0.03$). Over the first thirty minute following exposure to CDE, subjects with BHR continued to have significantly greater declines in FEV1 in comparison to subjects with NAR, although the magnitude of difference declined over time due to gradual improvement in FEV1 in the BHR subjects. At 1, 2, 3, 4, and 24 hours post exposure, there was no significant difference in the mean percent decline in FEV1 from baseline between subjects with NAR and subjects with BHR ($p> 0.05$ at each time points measured). Interestingly, the

differences in percent decline in FEV1 between subjects with and without BHR correlated with the subjective reporting of chest tightness and dyspnea (table 2).

An acute inflammatory response in the lower respiratory tract was observed following exposure to CDE in comparison to saline for subjects with BHR and NAR (data not shown). The inflammatory response consisted predominately of increases in bronchoalveolar lavage (BAL) fluid concentrations of total cells and neutrophils (figure 2). BAL total cell, neutrophil, and eosinophil concentrations following exposure to HBSS were not significantly different between subjects with and without BHR: (mean $\pm$ SEM cells/ml) total cells: BHR: 1.53 $\pm$ 0.3 X 10^5, NAR: 2.12 $\pm$ 0.6 X 10^5 ($p>0.05$); neutrophils: BHR: 1.78 $\pm$ 0.9 X 10^3, NAR: 2.05 $\pm$ 1.1 X 10^3 ($p>0.05$); eosinophils: BHR: 1.2 $\pm$ 1.0 X 10^3, NAR: 0 $\pm$ 0 X 10^3 ($p>0.05$). In contrast, following exposure to CDE, significant differences were seen in BAL cellularity between subjects with and without BHR (fig 2). In subjects with NAR, BAL total cell (9.28 $\pm$ 1.6 X 10^5 cells/ml) and neutrophil (5.2 $\pm$ 1.4 X 10^5 cells/ml) concentrations were significantly greater than subjects with BHR (3.1 $\pm$ 0.9 X 10^5 cells/ml and 0.98 $\pm$ 0.42 X 10^5 cells/ml, resp.). No differences in BAL eosinophils were detected between groups (BHR: 8.8 $\pm$ 6.7 X 10^3, NAR: 6.3 $\pm$ 2.9 X 10^3, ($p>0.05$)). To account for the two subjects with BHR who were not able to complete all of the inhalant, when analysis was performed excluding these two subjects, only the BAL total

cell concentration was found to be greater in subjects with NAR ($p<0.03$; data not shown).

In subjects with and without BHR, exposure to CDE (in comparison to saline) resulted in increases in the concentration of BAL fluid TNFα, IL-6, and IL-8 (figure 3). In subjects exposed to CDE, the mean concentration of IL-8 was significantly greater in subjects with NAR in comparison to subjects with BHR ($p<0.04$), but mean concentrations of TNFα and IL-6 were not significantly different between these groups. However, when analysis was performed excluding the two subjects with BHR not finishing all of the CDE inhalant, no difference was detected in any of the concentrations of BAL cytokines measured ($p>0.05$).

DISCUSSION:

Our results indicate that subjects with BHR develop greater respiratory symptoms and airflow obstruction immediately following exposure to CDE in comparison to subjects with NAR. However, this initial marked decline in airflow obstruction appears to slowly improve over the first hour after inhalation exposure, resulting in a similar pattern of airflow reduction as is observed in normal subjects. In contrast to the distinct physiologic changes observed between subjects with and without BHR, subjects with BHR do not demonstrate a greater inflammatory response to CDE as measured by BAL cellularity and cytokine concentrations. In fact, our results suggest that subjects with NAR may tend to develop greater inflammatory cell recruitment in the distal airspaces than subjects with BHR.

At least two explanations could account for the reduced inflammatory response to CDE observed in subjects with BHR. One potential explanation for the reduction in inflammatory cell concentrations in subjects with BHR in comparison to normal controls is that two subjects with BHR were not able to inhale a full dose of the CDE. This decrease in BAL inflammatory cells and cytokines may be due to the reduction in total dose of endotoxin in the CDE delivered to the distal airspaces. Previously, we have demonstrated in subjects with NAR that a dose-response relationship exists between the concentration of

endotoxin in CDE and the inflammatory response in the lung (17). However, when subjects who were not able to inhale a complete dose of CDE were excluded from the analysis, the remaining subjects with BHR continued to have a reduced inflammatory response in comparison to study subjects with NAR. Alternatively, and potentially a more interesting explanation, is that airflow obstruction may actually provide protection when exposed to environmental stimuli. Although we did not measure FEV1 throughout the exposure period, it is likely that reductions in FEV1 were occurring during the period of inhalation challenge. This decrease in airflow may have altered the distribution of aerosol in the lung, preventing aerosol from being deposited in the distal regions of the lung in subjects with BHR. These observations suggest that BHR may act to protect individuals from environmental exposures, such as grain dust, by reducing the overall exposure, resulting in less inflammation in the lower respiratory tract. In contrast, subjects with NAR may be more likely to tolerate these exposures for longer periods of time, but as a consequence develop greater airway inflammation in the lower respiratory tract.

While the results of this investigation demonstrate that BHR does in fact cause early exaggerated physiologic changes, it is not clear from our investigation whether this exaggerated response to grain dust itself, may lead to chronic irreversible airflow obstruction following repeated exposures to

grain dust. One may speculate that the exaggerated physiologic response to inhalation of grain dust may over time lead to changes in airway epithelial permeability and integrity, basement membrane thickening, and airway smooth muscle hypertrophy in a manner similar to the changes resulting from chronic inflammmation in asthmatics. This may account for the irreversible airflow obstruction associated with chronic exposure to grain dust. On the other hand, this exaggerated physiologic response to grain dust may be responsible for the dropout of newly employed grain workers because of intolerable symptoms related to work, the so called "healthy worker" effect. Epidemiologic data have demonstrated that in some seasonal workers there are significant respiratory symptoms and dropout of the workplace due to asthma-like symptomatology (18). It is therefore possible that subjects with underlying severe BHR may avoid employment in the grain industry whereas individuals with only mild BHR may tolerate grain dust exposure, but may be at risk for greater longitudinal declines in lung function.

The mechanism by which CDE produces an initial exaggerated physiologic response in subjects with BHR was not explored in this study, but is clearly of interest. Extracts of grain dust have been shown to cause the release of histamine and leukotrienes from human lung tissue(19). Likewise, endotoxin, a major component found in grain dust, may cause the release of preformed mediators such as histamine (20), resulting in

bronchoconstriction. These substances may cause rapid, short term declines in airflow, which may be exaggerated in subjects with underlying BHR. Alternatively, inhalation of CDE may cause acute bronchoconstriction through neurally mediated mechanisms, such as through cholinergic pathways or non-adrenergic, non-cholinergic neuropeptide mediators. Previously, we were not able to demonstrate detectable levels of histamine, 15-HETE, PGE2, or LTB4 in BAL fluid of subjects with NAR four hours following exposure to CDE (15).

In conclusion, it appears that BHR is a major host factor which is responsible for causing exaggerated initial declines in airflow following acute grain dust exposure, but may be protective in reducing the magnitude of the acute inflammatory cell recruitment in the lower respiratory tract. Given these findings, one cannot attribute increased susceptibility to grain dust induced lung inflammation based upon an exaggerated inflammatory cell response following grain dust inhalation. It is possible that the mechanism underlying bronchial hyperresponsiveness in conjunction with repetitive bouts of bronchoconstriction and airway inflammation associated with chronic exposure to grain dust may be responsible for producing the chronic, irreversible airflow obstruction.

LEGEND:

Figure 1. The mean percent baseline FEV1 (± SEM) is depicted over time in subjects with bronchial hyperreactivity (BHR) and normal airway reactivity following inhalation of CDE. (*represents $p < 0.05$)

Figure 2. Bronchoalveolar lavage mean total cell and neutrophil concentrations (± SEM) are shown for subjects with bronchial hyperreactivity (open bars) and normal airway reactivity (hatched bars) following exposure to CDE. (*represents $p < 0.03$)

Figure 3. BAL mean concentrations (± SEM) of TNFα, IL-6, and IL-8 are shown for subjects with bronchial hyperreactivity (open bars) and normal airway reactivity (hatched bars) following exposure to CDE. (*represents $p < 0.05$)

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Table 1. Profile of Study Subjects

Variable	BHR*	NAR*	P value
age (years)	23.6 ± 1.6	26.8 ± 2.9	*
height (cm)	170.8 ± 5.7	167.4 ± 2.3	*
weight (kg)	72.8 ± 4.9	70.1 ± 7.3	*
male:female	1 : 4	1 : 4	*
FEV1 (L)	3.52 ± 0.47	3.42 ± 0.21	*
FVC (L)	4.89 ± 0.69	4.04 ± 0.23	*
FEV1/FVC	0.73 ± 0.02	0.85 ± 0.02	$p<0.01$
TLC (L)	6.57 ± 0.91	5.57 ± 0.49	*
RV (L)	1.61 ± 0.34	1.39 ± 0.25	*
DLCO	34.6 ± 5.04	32.7 ± 1.82	*
slope#	2.53 ± 0.75	0.04 ± 0.01	$p<0.01$

*represent mean ± SEM * > 0.05

#slope of the histamine dose-response curve

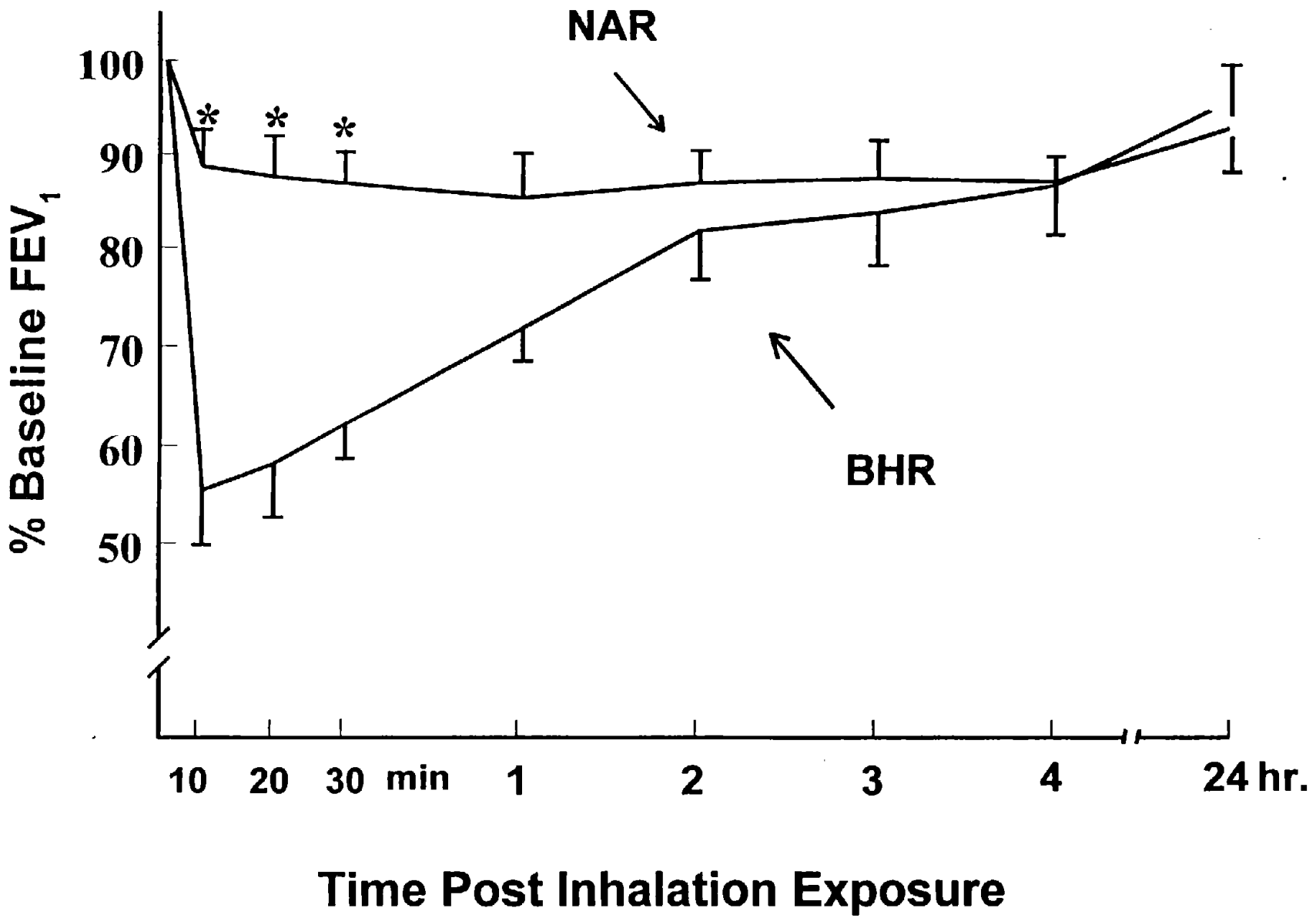
Table 2. Frequency of symptoms reported following inhalation exposure to CDE[+]

Symptoms	BHR	NAR	P value
chest tightness			
10min	5	1	<0.05
20min	5	1	<0.05
30min	5	1	<0.05
1hr	5	1	<0.05
2hr	3	1	*
3hr	2	1	*
4hr	2	1	*
dyspnea			
10min	4	0	<0.05
20min	4	0	<0.05
30min	4	0	<0.05
1hr	4	0	<0.05
2hr	1	0	*
3hr	1	0	*
4hr	1	0	*

[+] represents number of subjects reporting symptoms at time points post inhalation exposure

* > 0.05

figure 1


NAR
BHR
100
90
80
70
60
50
% Baseline FEV1
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10
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30
min
1
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24 hr.
Time Post Inhalation Exposure

figure 2

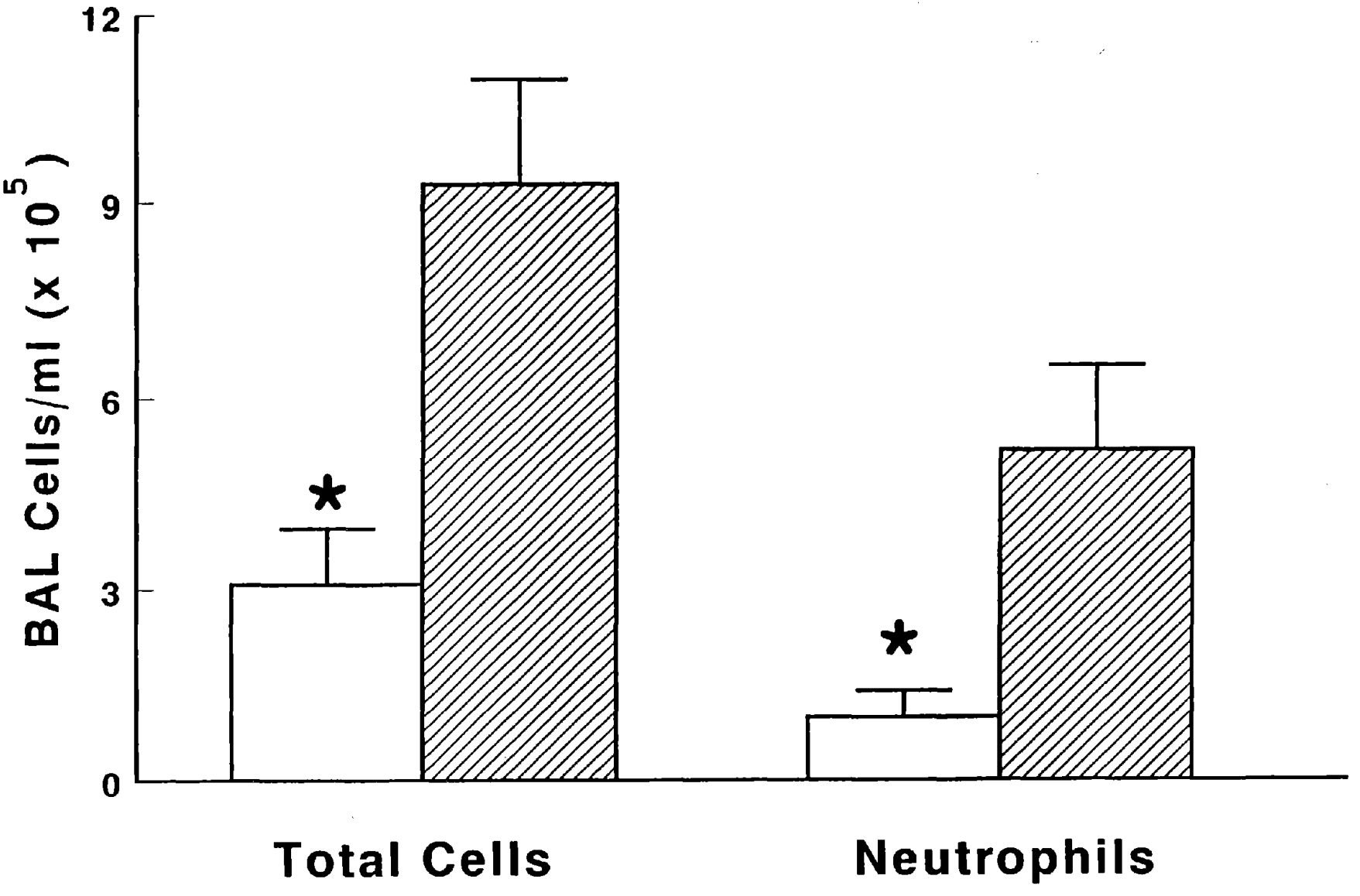

figure 3