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Experimental Human Exposure to Inhaled Grain Dust and Ammonia: Towards a Model of Concentrated Animal Feeding Operations

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Background *Ammonia and endotoxin-rich dust are present in high concentrations in swine confinement facilities; exposure to this environment is linked to workers' respiratory problems. We hypothesized that experimental exposure to ammonia and dust would impair pulmonary function, and that these exposures would be synergistic.*

Methods *We exposed six normal subjects and eight subjects with mild asthma to ammonia (16–25 ppm) and/or endotoxin-rich grain dust (4 mg/m^3). Pulmonary function and exhaled NO_x were measured before and after exposure.*

Results *There was no significant change in pulmonary function in the normal subjects following any of the exposure conditions. Among asthmatics, a significant transient decrease in FEV_1 was induced by grain dust, but was not altered by ammonia; increased bronchial hyperreactivity was also noted in this group.*

Conclusion *In a vulnerable population, exposure to grain dust results in transient airflow obstruction. Short-term exposure to ammonia does not increase this response.* Am. J. Ind. Med. 46:345–348, 2004. © *2004 Wiley-Liss, Inc.*

KEY WORDS: asthma; airway inflammation; swine confinement; concentrated animal feeding operation

INTRODUCTION

Until the late 20th century, livestock husbandry in the United States and worldwide was mostly on diversified farms; animals were usually raised in relatively small numbers or, as in the case of the American West, in low concentrations on spacious grazing lands. Economic considerations have changed this paradigm over the past two decades with the introduction of high efficiency, highly concentrated animal confinement centers (concentrated animal feeding operations, CAFOs). This trend has been particularly evident in the swine industry in the Midwestern United States, where the total number of farms raising hogs has been decreasing despite an increase in the annual production of animals [Iowa State University and The University of Iowa Study Group, 2002]. Concentrating animals in this manner has engendered concerns over effects on the environment and the health of workers in these centers as well in the surrounding communities [American Thoracic Society, 1998].

CAFOs lead to the accumulation of large volumes of animal waste products, typically collected through grates in the floor into pits under the building and later pumped into holding lagoons outside, but adjacent to, the facilities. Gases

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released from the fermenting manure include ammonia, frequently in high concentrations; long-term exposure to levels greater than 10 ppm have been shown to be deleterious to both animal and human health [Donham and Popendorf, 1985]. Hydrogen sulfide, methane, and an array of volatile organic compounds are other significant products. In addition to the gases and vapors, dust levels within the CAFOs are high, frequently far higher than the 10 mg/m^3 threshold limit value (TLV) for ''nuisance'' dust. Dust in swine facilities consists of a mixture of grain dust, feces, insects, animal hair, and other organic products, and contains a high concentration of endotoxin and measurable concentration of ammonia, approximately 2.5–4.0 mg N/g dust. Endotoxin has significant adverse effects on respiratory health in both humans and animals and is significantly responsible for the unfavorable respiratory effects of inhaling grain dust [Michel et al., 1992; Kline et al., 2000; George et al., 2001].

Workers in concentrated animal feeding operations (CAFO) have a significantly increased prevalence of chronic respiratory disease and symptoms, including cough, wheeze, sputum production, dyspnea, reduced airflow, and nonspecific bronchial hyperreactivity. A longitudinal study of swine confinement workers found an accelerated decline in pulmonary function over time, in comparison with unexposed workers [Vogelzang et al., 1998b]. This decline correlates well with the concentration of endotoxin in the dust [Vogelzang et al., 1998a]. Animals raised in these conditions similarly experience adverse effects on their health. It has been suggested that ammonia acts in synergy with endotoxin to adversely affect respiratory health in humans and animals. A mixture of endotoxin and ammonia has been shown to have a synergistic effect on pulmonary vascular responses in animal studies, but no similar studies have been carried out in humans [Jolie et al., 1999; Mombarg et al., 2002]. In addition, the organic dust particles, known to carry endotoxin, may also bind the ammonia and act as a carrier, allowing deposition deep into the lung; hygroscopic ammonia otherwise is generally absorbed in the proximal airways. We hypothesized that exposing human subjects to inhaled grain dust and ammonia would promote airway inflammation and bronchospasm, greater than either exposure alone and that these responses would be more pronounced in asthmatic than in normal subjects.

MATERIALS AND METHODS

Human Subjects

The protocol for this study was reviewed and approved by the Institutional Review Board (Human Studies Research) of the University of Iowa. Subjects were recruited by advertisement in local print media and posters. Inclusion criteria for normal volunteers included age over 18, lifetime nonsmoking history, no history of any medical problems (including atopy), and no medications other than oral contraceptives; these subjects were also required to demonstrate an absence of bronchial hyperresponsiveness, as defined by a methacholine PC20 $>$ 15 mg/ml. Inclusion criteria for asthmatic subjects were identical, except that they had a history of physician-diagnosed asthma, met criteria for intermittent asthma, and only required the use of rescue inhalers on an as-needed basis; these subjects were required to demonstrate a methacholine PC20 $<$ 8 mg/ml.

Physiologic Testing

Pulmonary function testing was performed in a standard manner as dictated by the guidelines published by the American Thoracic Society [American Thoracic Society, 1995]. The spirometer (Sensormedics) was calibrated prior to each visit. Spirometry was performed using nose clips with the subject in the sitting position. Spirometry was carried out at baseline, then at 10 and 30 min and 1, 2, 3, 4, and 24 hr after inhalational exposures. Methacholine challenge tests were performed 4 hr after the completion of the exposure, according to ATS guidelines [Crapo et al., 2000]. Methacholine challenges were not carried out on subjects who demonstrated greater than 20% spontaneous reduction in FEV1 after exposure. DLCO was performed at baseline and 4 hr post exposure. Nitric oxide in exhaled breath (Eco Physics CLD77AM NO Analyzer) was measured at baseline, and at 10, 20, and 30 min following exposure.

Statistical Analysis

Between-group differences of continuous variables were compared using the Sign Rank test; chi-square and Fisher's exact tests were used to compare noncontinuous variables (e.g., presence/absence of increased bronchial hyperresponsiveness). A P value $<$ 0.05 was considered significant. Statistical comparisons were made with the SAS program statistical package (SAS institute 1999).

Exposure Procedures

Subjects were exposed to ammonia (16–20 ppm) and/or grain dust aerosol (4 mg/m^3; respirable fraction 1 mg/m^3; endotoxin content 4 μg/m^3) for 30 min at each session (approximately 1 week between sessions). Grain dust (collected from an eastern Iowa grain elevator) aerosol was generated by a Wright dust feed [Wright, 1950]. Adjusting the rotation speed of the generator can control the average steady state aerosol concentration of dust using this device. A linear relationship exists between the rotation speed and the average steady state dust concentration in the aerosol, thus allowing for precise control over dust levels in the mixing chamber and exposure hood. A positive-pressure air blower forces air through a 30-gallon steel drum (mixing

chamber), which is sufficiently large to achieve stable concentrations of gases and dust. The flow rate through the apparatus can be controlled and was maintained at a flow rate of 6 cfm (170 lpm); this ensures that the air inside the hood is free from outside contaminants despite even large inhalations. Air exiting the drum is directed to a Tyvek® hood with a plastic face shield (RACAL Air-Mate, 3M Inc., St. Paul, MN) that allows the air to flow down across the face and out through spaces near the neck. Dust produced in this manner contains approximately 25% respirable fraction (data not shown). An aerosol photometer (Model HAM, PPM Inc., Knoxville, TN) is used to display real-time measurements of the dust concentration, and an optical particle counter (1.100 Series, GRIMM Technologies Inc., Douglasville, GA) records the size distribution of the aerosol. During an exposure, a nose-clip was applied to ensure mouth breathing. The entire apparatus is operated within the human exposure chamber of the Clinical Exposure Facility, University of Iowa; to protect investigators from contaminants exhausted from the hood, the subject sits within a small plastic oxygen tent within the chamber. An exhaust line is connected from the tent to the chamber exhaust ductwork to exhaust dust and gas directly out of the tent and chamber. Characterization of this apparatus has recently been described [O'Shaughnessy et al., 2004]. Ammonia gas was delivered to the mixing chamber under the control of a flow-meter, and concentrations of ammonia were continuously monitored using an infrared spectrometer (MIRAN SapphIRe, Thermo Environmental Instruments, Franklin, MA).

RESULTS

Healthy Volunteers

Six healthy volunteers, age 25–45 (two males and four females) underwent the exposure protocols. These normal volunteers demonstrated no decline in spirometry (Fig. 1), bronchial hyperreactivity, nor a change in their DLCO (data not shown) following exposure to grain dust, ammonia, or the combination.

Asthmatic Volunteers

Eight individuals, age 18–52 (four males and four females), with physician-diagnosed intermittent asthma were evaluated for response to inhaled ammonia and grain dust. A methacholine PD20 < 8 was required; subject's PD20 ranged from 0.0625 to 6.67 with all but two subjects less than 4. In this group, a significant transient drop in FEV1 was seen at 10 min after exposure to ammonia and grain dust combined or to grain dust alone. This difference remained significant for up to 60 min in the subjects exposed to both dust and ammonia and for thirty minutes after exposure (Fig. 2) to grain dust alone, but no difference was seen following exposure to ammonia alone. Bronchodilator treatment was required in two cases where subjects' FEV1 dropped more than 50% but in all other cases recovery was spontaneous. There was no significant change in DLCO seen after any exposure. Exhaled NO levels measured up to 6 hr post exposure were not clearly affected. An increase in exhaled NO_x was seen in individuals with the greatest increase in bronchial hyper reactivity but the difference was not significant.

We defined a significant increase in bronchial hyperreactivity as either at least one level reduction in methacholine concentration required to induce a 20% decline in FEV1 or a spontaneous decrease in FEV1 by at least 20% after exposure. Using this criterion we found a significant increase in bronchial hyperreactivity in asthmatics 4 hr after exposure to dust alone or dust and ammonia in combination as compared to ammonia alone (Table I).

DISCUSSION

We found that exposure to grain dust induced a transient decrease in FEV_1 and increased bronchial hyperreactivity in mild asthmatics, but not in healthy subjects; the addition of ammonia did not augment this response and the exposure to ammonia alone did not have this effect. No significant changes in DLCO or exhaled NO were observed in either group, suggesting that these responses represented transient

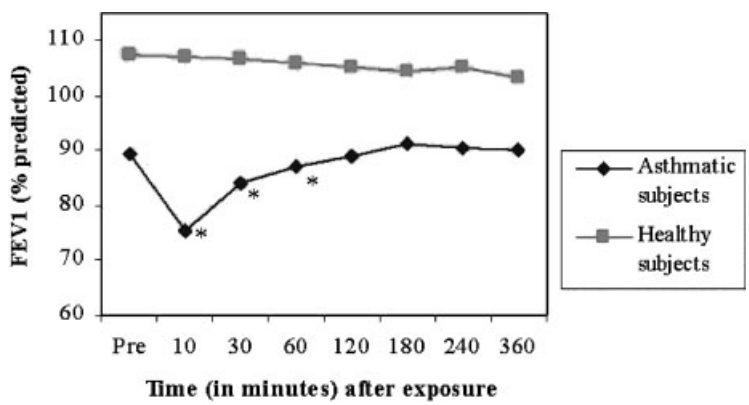

FIGURE 1. Inhalation of grain dust aerosol results in significant decline in FEV_1 in asthmatic, but not in healthy control subjects.
*$P < 0.05$, asthmatic vs. control subjects.

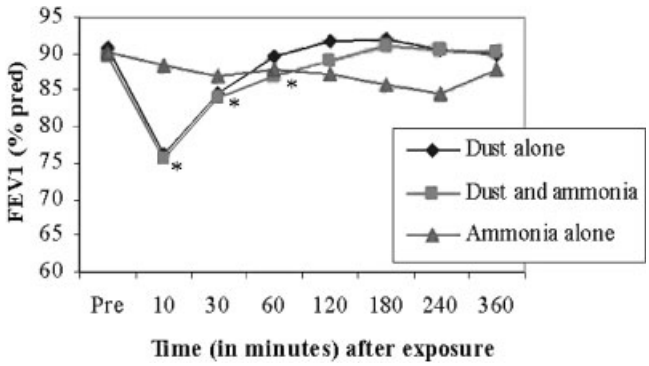

FIGURE 2. Inhalation by asthmatic subjects of grain dust or of grain dust and ammonia, but not of ammonia alone results in a significant reduction of FEV_1. * $P < 0.05$, ammonia alone versus grain dust or grain dust with ammonia.

bronchospasm, most likely caused by irritant properties of the dust, rather than substantial airway inflammation. Airway inflammation was not directly assessed, however, and exhaled NO has been best characterized as increasing in response to atopic inflammation; given the substantial inter-individual variability in exhaled nitric oxide, we may have missed a modest change in this secondary outcome marker.

The lack of synergy between ammonia and dust is contrary to current opinion. Our study was adequately powered to detect a 10% difference in FEV1 response with an alpha (type I error) of 0.05 and a power of 95%. However, several parameters of the study could have confounded these results. First, the ammonia and dust levels might not have been sufficiently high to cause an effect. Clearly the dust levels in the workplace are often far greater than those generated in this study, and often exceed 40 mg/m^3 in swine barns. Secondly, the duration of exposure was relatively short (30 min), compared with the 8-hr or greater workday. Finally, the environmental conditions within the chamber during exposure may have been suboptimal; in particular, the relative humidity within the mixing chamber was quite low (below 20%), whereas in the workplace, humidity may approach 100% within the confinement operations. Humidity may clearly affect partitioning of ammonia gas between the particulates (where it would be expected to penetrate deeper into the airways) and as a gas. In addition, temperatures in swine barns in the summer time frequently reach 90–100 F, considerably higher than in our chamber where temperatures are maintained between 65–70 F.

This study extends our previous work examining the effect of inhaled grain dust [Kline et al., 2000] by demonstrating that dry grain dust aerosols (with a lower delivered endotoxin content than the liquid extracts) can induce bronchospasm and enhance bronchial hyperresponsiveness in a vulnerable (mildly asthmatic) population. We identified no independent or a synergistic effect of inhaled ammonia gas, but would not rule out an undetected effect of ammonia due to study design issues noted above. Ongoing studies are evaluating the effect of environmental humidity and temperature on the ammonia-carrying capability of grain dust particles.

TABLE I. Bronchial Hyperreactivity Changes in Bronchial Hyperreactivity 4 hr After Exposure to Ammonia, Grain Dust, and Combination Grain Dust and Ammonia

Exposures	Increase	No increase	Significance
Ammonia	1	10	
Dust and ammonia	7	7	$P = 0.042$
Dust	4	3	$P = 0.047$

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