The Role of Endotoxin in Grain Dust–induced Lung Disease

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To identify the role of endotoxin in grain dust–induced lung disease, we conducted a population-based, cross-sectional investigation among grain handlers and postal workers. The study subjects were selected by randomly sampling all grain facilities and post offices within 100 miles of Iowa City. Our study population consisted of 410 grain workers and 201 postal workers. Grain workers were found to be exposed to higher concentrations of airborne dust ($p = 0.0001$) and endotoxin ($p = 0.0001$) when compared with postal workers. Grain workers had a significantly higher prevalence of work-related (cough, phlegm, wheezing, chest tightness, and dyspnea) and chronic (usual cough or phlegm production) respiratory symptoms than postal workers. Moreover, after controlling for age, gender, and cigarette smoking status, work-related respiratory symptoms were strongly associated with the concentration of endotoxin in the bioaerosol in the work setting. The concentration of total dust in the bioaerosol was marginally related to these respiratory problems. After controlling for age, gender, and cigarette smoking status, grain workers were found to have reduced spirometric measures of airflow (FEV_1, FEV_1/FVC, and FEF_{25-75}) and enhanced airway reactivity to inhaled histamine when compared with postal workers. Although the total dust concentration in the work environment appeared to have little effect on these measures of airflow obstruction, higher concentrations of endotoxin in the bioaerosol were associated with diminished measures of airflow and enhanced bronchial reactivity. Our results indicate that the concentration of endotoxin in the bioaerosol may be particularly important in the development of grain dust–induced lung disease. Schwartz DA, Thorne PS, Yagla SJ, Burmeister LF, Olenchock SA, Watt JL, Quinn TJ. The role of endotoxin in grain dust–induced lung disease.

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Occupational and environmental exposure to grain dust can cause a spectrum of clinical syndromes, including asthma, acute and chronic changes in airway reactivity, grain fever, bronchitis, and progressive irreversible airflow obstruction (1). In North America alone, over 5 million agricultural workers are exposed to grain dust each year (1–4). Among grain handlers, the prevalence of work-shift changes in FEV_1 ($\geq$ 10% decline) varies between 3.9 and 11% (2–4). Grain fever, an acute illness consisting of fevers, chills, headache, myalgias, chest tightness, dyspnea, and a transient decline in airflow, has been reported in up to 25% of grain workers (5–7). Grain dust exposure causes seasonal decrements in airflow (8–10) and an accelerated longitudinal decline in FEV_1 (11–14). Chronic bronchitis occurs in a high prevalence (23 to 37%) of grain handlers (6, 11, 14, 15). The effect of cigarette smoking appears to be additive (12, 16), however, nonsmokers also develop grain dust–induced airway disease (15, 17, 18). Moreover, acute changes in airflow across either the work shift or work week are consistently associated with accelerated declines in lung function (9, 12, 13), and chronic occupational exposure to grain dust is associated with impaired lung function even among retired grain workers (19).

Exposure-response studies have shown that declines in lung function across the work shift are directly related to higher ambient concentrations of grain dust (4, 20). Importantly, 15 yr cumulative average dust exposures have been found to be directly related to chronic phlegm production, breathlessness on exertion, and lower spirometric measures of lung function (14). However, the concentration of inhaled endotoxin is strongly associated with the development of airflow obstruction among other agricultural workers, such as swine confinement workers (21), poultry workers (22), and those exposed to cotton dust (23–26). Furthermore, we have recently shown that, among swine confinement operators and nonconfinement farmers, higher ambient concentrations of endotoxin are associated with longitudinal declines in measures of airflow (27). These studies suggest that the ambient concentration of endotoxin may be particularly important in the development of impaired lung function in grain workers.

To identify the role of endotoxin in grain dust–induced lung disease, we conducted a population-based, cross-sectional inves-

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tigation among grain handlers in eastern Iowa. Our results indicate that working in the grain industry and exposure to higher ambient concentrations of endotoxin are associated with an increased prevalence of respiratory symptoms and diminished measures of airway function.

METHODS

Study Population

The study population consisted of a population-based cohort of grain handlers and postal workers who were located in eastern Iowa. The grain handlers were selected by randomly sampling all grain facilities within 100 miles of Iowa City (Iowa City region) and screening all of the grain workers at each of the selected sites. In total, 553 grain facilities are present in the Iowa City region with a total work force of approximately 7,000 workers. These grain facilities were stratified by the number of grain dust-exposed employees (< 10 and ≥ 10) and by the type of industry (grain elevator, corn mill, and soybean processing plant). Study sites were randomly selected within the six strata (size of work force and type of industry) such that each stratum was equally represented. Within each site, workers were considered eligible for the study if they worked directly with a grain product. Because we thought that more variability in exposure would exist between sites than within study sites, we limited the number of subjects surveyed to 15 workers at each study site. At each site, we used unequal subsampling fractions to limit the number of study participants to no more than 15 subjects per site. In those sites with less than 15 grain workers, all exposed individuals were studied. In total, our cohort of grain handlers consisted of 410 workers who were screened from 121 grain facilities.

Our unexposed study group consisted of a population-based sample of postal workers who worked in the Iowa City region and lived in the same communities (towns) as our exposed study subjects. All postal workers, except for those who also worked or had worked in the agricultural industry, were eligible for participation. No more than 15 postal workers were selected from each site. In total, our cohort of postal workers consisted of 201 individuals who were screened from 24 post offices.

Field Visits

The field visits included the health and environmental evaluations and were evenly distributed through 12 months for both grain facilities and post offices. Similar proportions of grain workers and postal workers were evaluated during each season. A mobile survey vehicle was used to perform these evaluations at each work site. The team staffing the mobile unit was composed of a certified respiratory therapist and an industrial hygiene technician. The field team was equipped with medical and exposure questionnaires, a spirometer, skin testing supplies, and environmental sampling equipment.

Health Evaluation

The health evaluation was performed by a certified respiratory therapist who was trained to interview, perform pulmonary function tests, and apply skin tests. This evaluation consisted of completing a modified American Thoracic Society (ATS) questionnaire (28), spirometry testing before and after 4 h of regular work, and standard skin tests to assess atopy status. The ATS questionnaire (28) was used to assess the presence of chronic respiratory symptoms. Additional questionnaires were used to assess acute work-related symptoms, collect demographic data, and characterize the cigarette smoking history. Spirometric evaluations were conducted using a Spirotech S520 spirometer (Spirotech, Atlanta, GA) which was statically and dynamically calibrated on a daily basis while in use. This spirometer meets ATS specifications and all spirometric measures were performed in accordance with ATS guidelines (29). Nose clips were used on all study subjects, and tests were conducted in a sitting position in a temperature-controlled environment. Spirometry measures were made before a work shift and after completing 4 h of normal work. All subjects were asked to refrain from the use of dust masks or respirators during the work period. Study subjects were asked not to smoke cigarettes for 1 h before spirometry testing and during the work period. The predicted normal values used were those of Morris and colleagues (30) for spirometry.

Airway responsiveness was assessed immediately after the postworkshift spirometry using an abbreviated bronchial challenge with inhaled histamine (31, 32). The abbreviated histamine protocol consisted of one breath of 0.16 mg/ml, three breaths of 0.16 mg/ml, one breath of 2.12 mg/ml, four breaths of 2.12 mg/ml, three breaths of 10.6 mg/ml, and four breaths of 10.6 mg/ml of histamine. The dosing schedule of the abbreviated protocol minimizes the concentrations of histamine to complete an airway challenge and still approximates the total dosing of the standard histamine challenge (31). Spirometry was assessed 30 s and 3 min after each inhalation challenge. A sustained ($\geq$ 10 min) drop of FEV_1 of 20% was considered a positive result, and the challenge test was terminated. Otherwise, the test was continued until the subject inhaled four breaths of 10.6 mg/ml of histamine. The dose-response slope of bronchial reactivity (% change in FEV_1/cumulative dose of histamine) was computed and used as a continuous measure of airway responsiveness in all study subjects (33).

All subjects were skin tested by the respiratory therapist using a standard battery of 10 aeroallergens and 10 additional allergens common in eastern Iowa (Greer Laboratory). Skin tests were applied intradermally (positive = wheal 5 mm greater than diluent) at extract concentrations of 100 PNU/ml for pollens and danders, 1,000 PNU/ml for molds, and 10,000 PNU/ml for house dust. Histamine (2.5 mg/ml) was used as a positive control. Skin tests were read at 15 min and the diameter of the wheal was recorded. Study subjects were considered atopic if at least 1 of the 20 aeroallergen skin tests was positive.

Environmental Evaluation

The purpose of these environmental evaluations was to measure ambient conditions suspected of inducing acute respiratory symptoms related to work exposures and chronic pulmonary responses. The environmental evaluation consisted of an exposure questionnaire and personal sampling for aerosols and endotoxin.

The exposure questionnaire was administered by the industrial hygiene technician on the morning of the testing session. This questionnaire focused on current and remote exposures that have occurred either as a result of their work or home activities. This questionnaire also queried other known, nonagricultural exposures associated with the development of airflow obstruction.

The decision to use aerosol monitors was weighed heavily toward the technical considerations and to address the hypothesis that the concentration of particulate material or the level of endotoxin was responsible for the respiratory symptoms and airflow obstruction observed among grain handlers. Total and respirable dust samples were collected over their work shift in the breathing zone of each study participant on their side of preferred dexterity. For dust sampling, the MSA type FWS-D polyvinyl chloride membrane filter with pore size 0.5 μm (Mine Safety Appliances Co., Pittsburgh, PA) was used in a standard 37 mm polystyrene filter cassette with a cellulose backing pad. The filters were preweighed and reweighed after sampling using a Cahn Electrobalance (to within 2 μg) to determine the dust loading over the sampling period. Ambient air was drawn through dust samplers using MSA Flow Lite Model H constant flow personal sampling pumps calibrated to draw 1.7 L/min. These pumps weigh just 800 g each and were affixed to an equipment belt worn by the participant. Total concentration of airborne dust was determined using standard methods (34), and airborne endotoxin concentrations were measured using the commercially available assay which employs the chromogenic limulus amebocyte lysate (LAL) methodology (35). It has been shown for many of the most common gram-negative bacteria that the activity in the LAL assay is a reasonable measure of pulmonary potency and pyrogenicity in humans and animal models (35, 36). Measurements of endotoxin were performed by the National Institute of Occupational Safety and Health (NIOSH) Division for Respiratory Disease Studies (Morgantown, WV).

Characterization of Smoking History

Participants were classified as never smokers ($<$ 20 packs of lifetime cigarettes and no cigarettes in the month before the initiation of the study), former smokers ($\geq$ 20 lifetime packs of cigarettes but stopped at least one month before the initiation of the study), and current smokers (smoked within one month of the initiation of the study). In the classification of pack-years of cigarette smoking, never smokers were classified as having zero pack-years of cigarette smoking.

Statistical Analysis

Univariate comparisons were made to determine whether demographic

TABLE 1

DEMOGRAPHIC CHARACTERISTICS OF STUDY SUBJECTS*

	Grain Workers (*n* = 410)	Postal Workers (*n* = 201)
Age, yr	36.6 ± 11.2	43.4 ± 8.9
Male, %	94	59
Smoking status, %		
Never	52	49
Former	18	30
Current	30	22
Pack-years of cigarette smoking	9.4 ± 16.1	11.6 ± 16.7
Atopy, %	26	23

* Values for continuous variables are expressed as mean ± standard deviation; values for categorical variables are expressed as a percentage.

TABLE 3

RESPIRATORY SYMPTOMS IN STUDY SUBJECTS

	Grain Workers (*n* = 410) (%)	Postal Workers (*n* = 201) (%)	p Value
Work-related			
Cough	40.9	9.5	0.0001
Phlegm	30.5	5.7	0.0001
Wheezing	9.4	1.9	0.01
Chest tightness	14.3	1.9	0.0004
Dyspnea	14.1	0.9	0.0002
Chronic symptoms			
Usual cough	19.5	12.1	0.02
Usual phlegm	28.8	16.2	0.0008
Wheezing apart from colds	26.5	17.2	0.01
Dyspnea	2.4	3.0	0.70

or clinical variables were significantly different between grain workers and postal workers. A chi-square test with Yates correction factor was employed to test differences in the prevalence of categorical variables (such as respiratory symptoms) and Student's *t* test was used to examine differences of means in continuous variables (37). Multivariable models (logistic and linear regression; 38, 39) were used to control for variables that are known to affect respiratory symptoms (age, gender, and cigarette smoking) or lung function (age, gender, height, and cigarette smoking). This approach permitted us to determine the relationship between specific occupational exposures and either respiratory symptoms or measures of airflow while controlling for potentially important confounding variables. For purposes of the analysis, the concentration of dust and endotoxin was categorized to facilitate interpretation and comparison to other published studies (14). Standard approaches (38, 39) were used to create dummy variables to assess the statistical importance of categorical variables.

RESULTS

Grain workers were found to be demographically different than the postal workers (Table 1). Grain workers tended to be younger and more often male in comparison to postal workers. Although the prevalence of never cigarette smokers was similar in both populations, grain workers tended more often to be current cigarette smokers than postal workers. Given the younger age of grain workers, on average, grain workers tended to have fewer pack-years of cigarette smoking than postal workers. Importantly, the prevalence of atopy was similar in both study groups.

As expected, grain workers are clearly different than postal workers in relation to specific occupational exposures (Table 2). Almost half of the grain workers worked in grain elevators where they would have been exposed to mixed grain products, whereas the remaining grain workers were employed in either corn mills or soybean processing plants. Although a few postal workers had a remote history of either work in agriculture or work in the grain industry, this work had not occurred within the previous 5 yr and was far less than that experienced by grain workers. Importantly, grain workers were exposed to higher concentrations of total and respirable dust and endotoxin than postal workers. Because 78% of the total study population had a respirable dust concentration below the limit of detection, we did not analyze the effect of respirable dust on either respiratory symptoms or lung function.

TABLE 2

EXPOSURE CHARACTERISTICS OF STUDY SUBJECTS*

	Grain Workers (*n* = 410)	Postal Workers (*n* = 201)	p Value
Grain facility			
Grain elevator	46%	0%	
Corn mill	33%	0%	0.0001
Soybean plant	21%	0%	
Years in agriculture	14.3 ± 11.0	2.2 ± 6.8	0.0001
Years in grain industry	9.9 ± 9.0	1.5 ± 6.0	0.0001
Total dust, mg/m^3	4.8 ± 11.3	0.07 ± 0.29	0.0001
Respirable dust mg/m^3	0.12 ± 0.3	0.06 ± 0.32	0.03
Total endotoxin, Eu/m^3†	2,858.7 ± 7,208.6	3.1 ± 22.4	0.0001
Respirable endotoxin, Eu/m^3†	83.2 ± 277.9	5.1 ± 61.2	0.0001

* Values for continuous variables are expressed as mean ± standard deviation; values for categorical variables are expressed as a percentage.

† 10 Eu = 1 ng endotoxin.

Grain workers had a higher prevalence of worker-related and chronic respiratory symptoms than postal workers (Table 3). This was most pronounced for work-related symptoms of cough, phlegm, wheezing, chest tightness, and dyspnea but was also evident for chronic symptoms of cough, phlegm, and wheezing. After controlling for age, gender, and cigarette smoking status, grain workers were much more likely to have work-related respiratory symptoms with the adjusted relative risks ranging from 3.8 for wheezing to 15.6 for dyspnea (Table 4). However, the specific type of grain facility did not appear to significantly influence the risk of developing work-related respiratory symptoms. Interestingly, higher concentrations of total dust were associated with an increased risk of work-related respiratory symptoms, however, a consistent dose-response relationship was not evident between the concentrations of total dust and the prevalence of respiratory symptoms (Table 4). In contrast, higher concentrations of total endotoxin were strongly associated with an increased prevalence of work-related respiratory symptoms. Although chronic respiratory symptoms of cough and phlegm production continued to be associated with working in grain facilities, this relationship was markedly decreased after controlling for differences in age, gender, and smoking status (Table 5). Additionally, the type of grain facility had little relevance to the presence of respiratory symptoms. Higher concentrations of total dust and endotoxin were associated with an increased risk of chronic phlegm production.

On average, grain workers had a slightly reduced FEV_1 compared with postal workers (Table 6). However, other spirometric measures of lung function and cross-shift changes in lung function were similar in the two study groups. Although little differences were observed in the percentage of grain workers and postal workers who had a 20% decline in FEV_1 in response to inhaled histamine, on average, grain workers had greater declines in the FEV_1 following inhalation of histamine in comparison to postal workers (Table 6). Interestingly, although atopy was associated

TABLE 4

RELATIONSHIP BETWEEN SPECIFIC OCCUPATIONAL EXPOSURES AND WORK-RELATED RESPIRATORY SYMPTOMS*

	Cough	Phlegm	Wheezing	Chest Tightness	Dyspnea
Grain worker	5.2 (2.8, 9.9)	5.8 (2.4, 14.2)	3.8 (0.8, 17.0)	7.1 (1.6, 31.4)	15.6 (2.0, 119.0)
Grain facility					
Grain elevator	5.3 (2.4, 11.4)	5.5 (2.2, 13.9)	3.6 (0.8, 17.2)	6.9 (1.5, 32.1)	16.8 (2.1, 133.1)
Corn mill	5.8 (2.7, 12.6)	7.2 (2.8, 18.2)	4.2 (0.9, 20.0)	8.3 (1.8, 37.9)	17.6 (2.3, 137.4)
Soybean plant	4.1 (1.8, 9.5)	4.1 (1.5, 11.1)	3.3 (0.6, 17.3)	4.9 (1.0, 24.6)	9.5 (1.1, 81.1)
Total dust, mg/m^3					
< 4	1.0	1.0	1.0	1.0	1.0
⩾ 4 to < 10	2.0 (1.1, 3.7)	1.9 (1.0, 3.5)	1.3 (0.5, 3.6)	1.9 (0.9, 4.3)	2.1 (0.9, 4.7)
⩾ 10	2.1 (1.1, 3.7)	2.3 (1.2, 4.1)	1.6 (0.7, 4.1)	2.6 (1.2, 5.3)	2.4 (1.1, 5.1)
Total endotoxin, Eu/m^3†					
0	1.0	1.0	1.0	1.0	1.0
> 0 to < 100	5.9 (2.5, 13.8)	5.7 (1.9, 16.8)	2.6 (0.5, 12.3)	3.1 (0.6, 14.7)	2.4 (0.5, 12.1)
⩾ 100 to < 1,000	6.6 (2.7, 15.8)	7.3 (2.4, 21.7)	3.9 (0.8, 18.7)	8.0 (1.8, 36.5)	8.3 (1.8, 37.7)
⩾ 1,000	9.6 (4.0, 22.6)	13.0 (4.4, 38.0)	4.7 (1.0, 21.5)	9.3 (2.1, 41.9)	10.3 (2.3, 46.3)

* Relative risks were computed using a logistic regression model while controlling for age, gender, and cigarette smoking status. Each of the specific occupational exposures (grain worker, grain facility, total dust, and total endotoxin) was individually tested in a separate multiple logistic regression model for each respiratory symptom. Values represent the adjusted relative risk with the 95% confidence interval in parentheses.

† 10 Eu = 1 ng endotoxin.

with enhanced bronchial reactivity, within our study population, atopy was not significantly associated with diminished measures of airflow (data not presented). After controlling for variables known to affect lung function, working in the grain industry was found to be associated with spirometric measures of airflow obstruction (FEV_1, FEV_1/FVC, and FEF_{25-75}), and a greater decline in FEV_1 in response to inhaled histamine (Table 7). Individuals working in either grain elevators or corn mills appeared to be more affected than those working in soybean processing plants. Although the total dust concentration in the work environment appeared to have little effect on these measures of airflow obstruction, higher concentrations of endotoxin in the bioaerosol were associated with diminished measures of airflow and enhanced bronchial reactivity (Table 7).

DISCUSSION

Our results indicate that occupational exposure to grain dust is associated with acute and chronic respiratory symptoms, objective measures of diminished airflow, and enhanced bronchial reactivity. Although the total concentration of dust was associated with some of these adverse effects, the concentration of endotoxin in the bioaerosol was more consistently and more strongly associated with these measures of respiratory impairment. In aggregate, our findings indicate that the concentration of endotoxin in the bioaerosol may be particularly important in identifying hazardous grain facilities. Moreover, reducing the concentration of endotoxin may be important in preventing lung disease in grain workers.

Endotoxin appears to play a primary role in the development of airway symptoms and airflow obstruction among grain workers. These findings are supported by analogous lines of investigation in individuals exposed to other types of organic dust. The concentration of inhaled endotoxin is strongly associated with the development of acute decrements in airflow among volunteers exposed to cotton dust (25), swine confinement workers (21), and poultry workers (22). In fact, the concentration of endotoxin in the domestic environment appears to adversely affect asthmatics, with higher concentrations of ambient endotoxin associated with greater degrees of airflow obstruction (40). Physiologically, inhaled lipopolysaccharide can cause airflow obstruction (41–44) but may also adversely affect the diffusion capacity of carbon

TABLE 5

RELATIONSHIP BETWEEN SPECIFIC OCCUPATIONAL EXPOSURES AND CHRONIC RESPIRATORY SYMPTOMS*

	Cough	Phlegm	Wheezing
Grain worker	1.8 (1.0, 3.2)	1.7 (1.0, 2.7)	1.4 (0.8, 2.3)
Grain facility			
Grain elevator	1.9 (1.0, 3.7)	1.6 (0.9, 2.8)	1.4 (0.8, 2.5)
Corn mill	1.9 (1.0, 3.8)	2.1 (1.2, 3.8)	1.7 (1.0, 3.1)
Soybean plant	1.2 (0.6, 2.8)	1.1 (0.6, 2.2)	0.9 (0.5, 1.9)
Total dust, mg/m^3			
> 4	1.0	1.0	1.0
⩾ 4 to < 10	0.7 (0.3, 1.7)	1.4 (0.7, 2.7)	1.5 (0.7, 3.1)
⩾ 10	1.2 (0.6, 2.6)	2.0 (1.1, 3.7)	2.3 (1.2, 4.4)
Total endotoxin, Eu/m^3†			
0	1.0	1.0	1.0
> 0 to < 100	1.7 (0.9, 3.4)	1.3 (0.7, 2.3)	0.8 (0.4, 1.4)
⩾ 100 to < 1,000	2.6 (1.2, 5.3)	1.6 (0.9, 3.1)	1.0 (0.5, 1.9)
⩾ 1,000	1.2 (0.6, 2.6)	2.3 (1.3, 4.2)	1.4 (0.8, 2.6)

* Relative risks were computed using a logistic regression model while controlling for age, gender, and cigarette smoking status. Each of the specific occupational exposures (grain worker, grain facility, total dust, and total endotoxin) was individually tested in a separate multiple logistic regression model for each respiratory symptom. Values represent the adjusted relative risk with the 95% confidence interval in parentheses.

† 10 Eu = 1 ng endotoxin.

TABLE 6

PULMONARY FUNCTION BY STUDY GROUP*

	Grain Workers (*n* = 410)	Postal Workers (*n* = 201)	p Value
Preshift spirometry			
FEV_1	97.0 ± 15.1	99.7 ± 15.7	0.04
FVC	93.5 ± 12.8	94.6 ± 14.1	0.30
FEV_1/FVC ratio	79.6 ± 6.9	80.1 ± 7.0	0.30
FEF_{25-75}	90.4 ± 26.9	93.6 ± 28.0	0.17
Cross-shift changes			
FEV_1	1.1 ± 4.7	1.2 ± 6.1	0.70
FVC	0.8 ± 4.5	1.0 ± 5.2	0.60
FEV_1/FVC ratio	0.4 ± 2.9	0.3 ± 3.2	0.70
FEF_{25-75}	2.7 ± 10.7	2.9 ± 13.9	0.80
Histamine challenge			
Dose-response slope	−3.4 ± 28.7	−0.7 ± 1.9	0.05
> 20% decline	11.0%	8.1%	0.30

* Preshift spirometric measures of FEV_1, FVC, and FEF_{25-75} are expressed as a percentage of the predicted values. Values for continuous variables are expressed as mean ± standard deviation; values for categorical variables are expressed as a percentage.

TABLE 7

RELATIONSHIP BETWEEN SPECIFIC OCCUPATIONAL EXPOSURES AND MEASURES OF AIRFLOW*

	FEV_1	FEF_{25-75}	FEV_1/FVC	Dose-Response Slope
Grain worker	−0.13 (0.6)‡	−0.21 (0.10)‡	−1.3 (0.6)‡	−7.0 (2.7)§
Grain facility				
Grain elevator	−0.12 (0.6)	−0.25 (0.12)‡	−2.0 (0.7)§	−4.6 (3.1)
Corn mill	−0.14 (0.7)‡	−0.18 (0.12)	−0.7 (0.7)	−11.4 (3.2)§
Soybean plant	−0.12 (0.8)	−0.20 (0.14)	−0.9 (0.9)	−4.1 (3.8)
Total dust, mg/m^3				
⩾ 4 to < 10	−0.11 (0.8)	−0.27 (0.15)	−1.1 (0.9)	1.4 (4.0)
⩾ 10	−0.06 (0.8)	−0.17 (0.15)	−0.8 (0.9)	0.4 (3.8)
Total endotoxin, Eu/m^3†				
> 0 to < 100	−0.08 (0.06)	−0.08 (0.12)	−0.1 (0.7)	−2.6 (3.3)
⩾ 100 to < 1,000	−0.15 (0.7)‡	−0.17 (0.13)	−0.5 (0.8)	−3.8 (3.4)
⩾ 1,000	−0.19 (0.7)§	−0.26 (0.13)‡	−1.1 (0.8)	−7.4 (3.1)‡

* Multiple linear regression analyses were performed to evaluate the relationship between specific occupational exposures and measures of airflow. For spirometric measures of airflow, age, gender, height, and cigarette smoking status were included as covariates. For the dose-response slope to inhaled histamine, age, gender, cigarette smoking status, and atopy were included as covariates. Because atopy was not found to be associated with spirometric measures of airflow, it was not included as a covariate in the regression models that examined the occupational determinants of spirometric measures of airflow. Each of the specific occupational exposures (grain worker, grain facility, total dust, and total endotoxin) was individually tested in a separate regression model for each measure of airflow. Values represent the regression coefficient with the standard error in parentheses.

† 10 Eu = 1 ng endotoxin.

‡ $p < 0.05$.

§ $p < 0.01$.

monoxide (43, 44). In an animal model of grain dust-induced lung disease, we have recently shown that responsiveness to endotoxin is critical to the development of grain dust-induced inflammation in the lower respiratory tract (45). In aggregate, these findings indicate that endotoxin may be the principal mediator of the acute inflammatory and physiologic response to inhaled grain dust.

Depleting endotoxin from organic dusts should markedly alter the physiologic and biologic response following inhalation. Washing cotton removes up to 95% of the endotoxin in the dust (46). Importantly, experimental exposure to washed cotton dust significantly decreased the degree of airflow obstruction compared with decrements in airflow following exposure to untreated cotton dust (47). Although other substances may be removed by the washing process, the consistent relationship between the respirable concentration of inhaled endotoxin and airflow obstruction following exposure to cotton dust (25) suggests that endotoxin may be particularly important in the development of cotton dust-induced airflow obstruction. Interestingly, depletion of endotoxin from extracts of grain dust significantly reduces the capacity of macrophages to release chemotactic activity for neutrophils (48). These findings, in conjunction with those presented in this report, suggest that the biologic and physiologic response to inhaled organic dusts is primarily related to the dose of inhaled endotoxin in the organic dust. However, further, more specific, studies are needed to fully address this hypothesis.

Our findings suggest that the concentration of endotoxin in the bioaerosol may be more important to monitor and control than is the concentration of total or respirable dust. Although this is supported by previous findings in cotton workers (23), a longitudinal study among grain handlers (14) suggests that monitoring total dust levels may be sufficient. Given the inherent limitations of our cross-sectional study, it is clearly premature to recommend that the endotoxin concentration in the bioaerosol be monitored and regulated. However, our results do indicate the need for more directed research in this area of investigation.

Interestingly, no association was observed between cross-shift changes in lung function and specific occupational exposure. Although experimental exposure to endotoxin can cause a decline in FEV_1 (25, 41–44), only modest endotoxin-related cross-shift declines were observed in swine confinement workers (21), and, in one investigation, cotton textile workers did not demonstrate an acute exposure–response relationship between endotoxin concentration and lung function (23). Perhaps lipopolysaccharide is less available/accessible to lung inflammatory cells when mixed with other components of the organic dust. Thus, we speculate that constitutive components of an organic dust may actually minimize exposure to an injury from the contaminants inherent in organic dust.

In summary, we have found that grain workers are at risk of developing lung disease, primarily involving the airways. Moreover, grain dust–induced airway disease appears to be more pronounced in those exposed to higher concentrations of endotoxin. However, we have not shown that endotoxin causes airway disease in grain handlers. Clearly, other exposures associated with microbial contamination of grain dust may be responsible for the development of airway disease. It is conceivable that endotoxin serves as a good surrogate marker for these more pathogenic components of grain dust. Despite these reservations regarding causality, our findings do suggest that control of endotoxin concentrations may minimize the development of respiratory impairment in grain workers.

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