Pulmonary Function Reductions Among Potentially Susceptible Subgroups of Agricultural Workers in Colorado and Nebraska

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Objective: Organic dust inhalation has been associated with adverse respiratory responses among agricultural workers. We evaluated factors that may confer increased susceptibility to these health effects. **Methods:** We quantified personal work shift exposures to inhalable dust, endotoxin, and its 3-hydroxy fatty acid constituents, and evaluated changes in pulmonary function among 137 grain elevator, cattle feedlot, dairy, and corn farm workers. **Results:** Increased dust exposure was associated with work shift reductions in lung function. Although interpretation is limited because of small samples, a suggestion of stronger exposure–response relationships was observed among smokers, as well as workers reporting pesticide/herbicide application, asthma, or allergies, and those with genetic polymorphisms (TLR4) ($P_{\text{interaction}} \leq 0.05$). **Conclusions:** A better understanding of factors leading to increased susceptibility of adverse respiratory outcomes is needed to optimize exposure reduction strategies and develop more comprehensive wellness programs.

Increased risk of respiratory morbidity and mortality has been described among agricultural workers, and continues to be a concern.[1–3] A relatively high lifetime prevalence of lung disease has also been observed in agricultural workers.[3,4] Inhalation of organic dusts in the agricultural environment has been associated with adverse respiratory responses including acute organic dust toxic syndrome, chronic asthma and asthma-like syndrome, and chronic obstructive airway disease.[5–7] Although certain conditions are well defined, the specific causes and pathophysiologic mechanisms are not completely understood.[3,4]

Endotoxins are composed of lipopolysaccharides that are components of Gram-negative bacterial cell walls.[6,8,9] These agents are a common constituent of agricultural dusts and contribute significantly to its pathogenicity.[5–7] However, the measurement of endotoxin is not completely standardized,[10–12] and dust constituents are often not differentiated in field studies.[13] We have previously described a relationship between 3-hydroxy fatty acid constituents of endotoxin (3-OHFA) in agricultural dust and nasal airway inflammation,[14] which could help explain mechanisms leading to increased respiratory morbidity, including changes in pulmonary function.[3,4,9,15]

Epidemiologic studies have described interindividual variation in human responses to aerosols, resulting in certain populations being at increased risk for aerosol-related health effects. Stratified analyses are often conducted in which a greater association between exposure and health is observed in one subgroup compared with another, providing evidence that one population may be more susceptible to the health-damaging effects of air pollutants.[9,16–20] Some agricultural workers exhibit heightened susceptibility to organic dust's respiratory effects.[21–25] Conversely, adaptation to endotoxin exposure, with a dampening of inflammatory response has been demonstrated in some previously unexposed workers,[26] as well as in clinical trials.[27] A better understanding of factors capable of modifying the health effects of dust or endotoxin exposure will aid in the identification of biological mechanisms of effects as well as assist in the identification of potentially susceptible populations to optimize disease prevention strategies. In this study, personal exposure to inhalable airborne dust was quantified over one work shift among grain elevator, cattle feedlot, corn farm, and dairy workers. Endotoxin in personal airborne dust samples was measured using the recombinant factor C (rFC) assay[28] and gas chromatography mass spectroscopy to quantify endotoxin's 3-OHFA constituents.[29] The relationships between each dust or endotoxin exposure measurement and cross-shift changes in lung function parameters were examined among the entire population and potentially susceptible subgroups. We assigned potential effect modifiers as extrinsic factors (ie, smoking, reported pesticide/herbicide use, living on a farm, and time on the job) or intrinsic factors (ie, obesity, age, ethnicity, reported asthma or allergies, and polymorphisms in TLR4).

METHODS

Agricultural workers for the four groups (cattle feedlots, dairies, grain elevator facilities, and corn farms) were recruited using a variety of methods to accommodate the requirements of the producer organizations. The study received institutional review board approval, and all participants provided informed consent in either English or Spanish. Data were collected between the spring of 2005 and fall of 2006. Study participation was completed over the course of one work shift. Approximately 350 workers from 26 work sites were originally recruited to participate in the study; 174 workers participated. Relationships between dust/endotoxin exposures and cross-shift pulmonary function changes were evaluated among 137 male workers with complete exposure and pulmonary function data.

Personal breathing zone samples for inhalable particulate matter were collected using Institute of Medicine sampling cassettes loaded with 25-mm PVC filters with a 5-μm pore size (SKC, Eighty

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Four, PA) and attached to personal sampling pumps (MSA, Pittsburgh, PA). The pumps were calibrated at a flow rate of 2 L/min using an electronic soap bubble flow meter (Gilibrator, Sensidyne, Clearwater, FL). The flow rates were checked postsampling to ensure they were within ±5% of the original flow rate. Inhalable dust samples were analyzed by weighing the internal cassette and filter as a single unit using a Mettler MT5 balance (Mettler-Toledo, Columbus, OH). Field and lab blanks were analyzed in a similar manner.

Dust samples were extracted in sterile, pyrogen-free water containing 0.05% Tween-20 for 1 hour at room temperature (22°C) with continuous shaking. A portion of each extract was analyzed for endotoxin using the PyroGene rFC assay; results were quantified in endotoxin units (EU) (Lonza, Walkersville, MA).[30] Another portion of the extract was lyophilized and stored at −70°C for determination of 3-OHFA endotoxin constituents via a gas chromatography mass spectroscopy method modified for these environments.[29] Samples and standards derivatized with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) were analyzed using an HP 5890 Series II Plus gas chromatograph equipped with an HP-5MS column (Hewlet-Packard, Palo Alto, CA) and an HP 5972 Mass Selective Detector. Selected ion monitoring was used for individual 3-OHFA, and results were quantified in picomoles (pmol). Calibration was accomplished via lab-fortified matrix blanks at anticipated 3-OHFA concentrations in dust samples. The limit of detection and the limit of quantitation were determined by signal-to-noise (S/N) ratio on the basis of the chromatograms of controls and 0.5- and 1-ng spikes (S/N > 3 for limit of detection and >10 for limit of quantitation). The 3-OHFA constituents with carbon chain lengths between 8 and 18 were quantified and reported as both total carbon chain length 3-OHFA components (total 3-OHFA) and even-numbered carbon chain length 3-OHFA components (even-chain 3-OHFA).

Self-administered, structured, written questionnaires were used to gather information before and after the work shift about respiratory health, mucous membrane irritation symptoms, tobacco smoke exposure, workplace exposures, home environment, height and weight, and duration of employment. These questionnaires were constructed using the American Thoracic Society standardized questionnaire.[31] Additional questions for identifying organic dust toxic syndrome were based on the methods of Rylander et al.[32]

Spirometry was performed in triplicate with the subjects in a seated position using the Puritan Bennett Renaissance spirometer (Puritan Bennett; Wilmington, MA). Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and their ratio (FEV_1/FVC) were measured. The best value was reported according to American Thoracic Society standards. Spirometry was performed before the work shift began and again at the end of the work period. Interpretation of the spirometry findings was done using prediction equations from Hankinson et al[33] and the interpretation algorithm of the American Thoracic Society and European Respiratory Society task force for the standardization of lung function testing.[34]

Blood samples were collected from the antecubital vein using 8 mL Qiagen PAXgene tubes and frozen until analyzed. DNA extraction and genotyping were performed using the Puregene system (Gentra Systems, Minneapolis, MN) and the TaqMan allelic discrimination assay for TLR4 299 and 399 on an ABI 7900 Sequence Detection system (SDS) (Applied Biosystems, Foster City, CA), respectively, at National Jewish Health (Denver, CO) using standard methodology. Allele calls were made using the ABI SDS software. We included plasmids containing TLR4 299 (Asp299Gly) or 399 (Thr399Ile) or both as controls. Participants with heterozygous mutations for TLR4 299 and 399 were identified as positive for each respective polymorphism and participants without mutations (wild-type) for TLR4 299 and 399 were identified as negative for the respective polymorphism. No homozygous mutations were observed.

Statistical analyses were performed using the SAS computer program (version 9.1, SAS Institute Inc, Cary, NC). Exposure variables were normalized to the volume of air sampled. Correlations among exposure variables were evaluated via Spearman rank correlation coefficients (r). Lung function values were standardized for each participant using age- and height-adjusted reference equations.[33] Percent change in predicted lung function values over the course of the work shift were calculated using the following equation:

$$[(\text{postshift\% predicted value} - \text{preshift\% predicted value})/\text{preshift\% predicted value}] \times 100.$$

General linear models were used to assess the multivariable relationships between dust and endotoxin exposures and lung function. Inhalable dust, endotoxin, total 3-OHFA, and even-chain 3-OHFA were assessed separately in exposure–response models. On the basis of *a priori* decisions, multivariable models were adjusted for age and current smoking. We evaluated differences in adjusted mean lung function changes across quartiles of dust/endotoxin groups using the least significant difference method. P values were used to assess the differences between the mean lung function change in the lowest and highest quartiles of exposure. To more closely approximate the distribution of exposure concentrations within quartiles, we assessed trend across the quartiles by using the midpoint of the quartile as a continuous variable in the model. Similar results were obtained when natural log-transformed exposure data were treated as continuous variables. Exposures were categorized into quartiles to provide consistency with our previous report about inflammatory markers in this cohort[14] and to allow a more direct comparison with other similar studies.[35] In addition, regression diagnostics were used to assess the potential for influential observations. Analyses were performed with and without one potential outlier (a grain elevator employee). Although the parameter estimates were slightly attenuated after removal of the potential outlier, the interpretation and statistical significance of results did not change; therefore, the data for this employee were not removed from primary analyses.

Effect modification was assessed by stratifying the population by the potential effect modifier and presenting lung function changes across quartiles of exposure separately for each modifier. The entire population was used in evaluating the interactions between the full shift dust/endotoxin concentrations and the potential effect modifiers (via P values for the parameter estimate obtained from multiplying the exposure concentration by the potential effect modifier). Potential effect modifiers included current smoking, self-reported pesticide/herbicide application, living on a farm, time at the job (assessed with a 1-year cut point), obesity status, age (youngest two tertiles vs the oldest tertile), Hispanic or Latino ethnicity, self-reported allergy or asthma or both, and the presence of a genetic polymorphism in the TLR4 gene (299 or 399).

RESULTS

Of the 174 who participated, 38 were not included in the final analysis. Thirty-two were excluded because of missing information about exposure, smoking, or pulmonary function tests. In addition, six women were excluded. Those excluded from analyses because they did not have complete exposure information ($n = 29$) were older and had lower baseline (preshift) pulmonary function values than those included in the analyses ($P < 0.05$). In addition, those excluded did not experience, on average, statistically significant cross-shift decrements in pulmonary function (as did those included in the analysis). Of the 137 included in analyses, a majority of participants worked in grain elevators (38.7%) or cattle feedlots (43.8%). Participant ages ranged from 18 to 72 years. Characteristics of the study

population, including all potential effect modifiers, and preshift (or baseline) lung function parameters are summarized in Table 1.

Geometric mean dust levels were similar among feedlot (2.66 mg/m^3; $n = 59$), dairy (2.37 mg/m^3; $n = 15$), and farm (2.86 mg/m^3; $n = 9$) employees and were elevated for grain elevator operators (5.09 mg/m^3; $n = 53$). Endotoxin concentrations were similar across all facility types with 3-OHFA constituents although slightly elevated for cattle feedlot employees (Table 2). Inhalable dust, endotoxin, and the 3-OHFA constituents were all moderately correlated ($r = 0.45$ to 0.65). Even-chain 3-OHFA concentrations were highly correlated with total 3-OHFA ($r = 0.99$). Although the geometric mean exposures were generally low relative to proposed occupational guidelines for dust and endotoxin in livestock environments, a significant number of participants experienced very high exposures in relation to proposed occupational guidelines (38% exceeded 4 mg/m^3 [conservatively adjusting, suggested level for the button sampler used in this study], and 86% exceeded 90 EU/m^3).[16,17,35,36]

TABLE 1. Population Characteristics and Pre-Work Shift Pulmonary Function Measures ($n = 137$)

Population Characteristics	n (%)
Age distribution, yr	
18–24	36 (26.3)
25–40	65 (47.5)
41–72	36 (26.3)
Facility type	
Grain elevator	53 (38.7)
Cattle feedlot	60 (43.8)
Dairy	15 (11.0)
Corn farm	9 (6.6)
Ethnicity	
Hispanic or Latino	44 (34.7)
Not Hispanic or Latino	83 (65.4)
Education (English or Spanish)	
Primary	18 (13.4)
Secondary/high school	65 (48.5)
Any postsecondary/college	51 (38.1)
Body mass index, kg/m^2	
<30 (nonobese)	100 (74.6)
≥30 kg/m^2 (obese)	34 (25.4)
Current smoker	
No	102 (74.5)
Yes	35 (25.6)
Currently live on a farm	
No	83 (61.9)
Yes	51 (38.1)
Applied pesticides/herbicides at work or home (within the past month)	
No	92 (68.2)
Yes	43 (31.9)
Reported doctor-diagnosed asthma or allergies	
No	102 (74.5)
Yes	35 (25.6)
Job duration, yr	
≤1	30 (22.1)
>1	106 (77.9)
Polymorphism in the TLR4 (*Asp299Gly*)	
No	111 (93.3)
Yes	8 (6.7)
Polymorphism in the TLR4 (*Thr399Ile*)	
No	114 (91.2)
Yes	11 (8.8)
Health Endpoints: Pre-Shift	**Mean (SD)**
FVC (% predicted)	98.7 (12.0)
FEV_1 (% predicted)	97.3 (13.0)
Ratio FEV_1/FVC (% predicted)	99.3 (7.7)

FEV_1, forced expiratory volume in one second; FVC, forced vital capacity.

Table 3 presents analyses examining mean pulmonary function changes (% change over the work shift of percent-predicted FEV_1 and FVC) across quartiles of exposures (dust/endotoxin/3-OHFA). Although strict dose–response relationships were not always observed, evidence suggests larger reductions in FVC and FEV_1 as exposures increased (Table 3). For example, among the entire population, we observed adjusted mean changes in percent-predicted FEV_1 of −0.95, −3.39, 0.04, and −6.22 across increasing quartiles of dust exposures, respectively ($P_{trend} = 0.01$; Table 3). The relationships observed among the total population appear to be driven primarily by those working in grain elevators; however, the small sample sizes limit interpretations for dairy and corn farm workers. In addition, among grain elevator employees, stronger associations were observed across quartiles of the even-chain 3-OHFA components. No evidence of associations was observed for dust/endotoxin exposures and the FEV_1/FVC ratio (results not presented).

Because of the associations observed for FEV_1 and FVC, we conducted effect modification analyses for these lung function endpoints. Effect modification results for FEV_1 are presented in Fig. 1 (extrinsic effect modifiers) and Fig. 2 (intrinsic effect modifiers). Results for FVC were similar to those for FEV_1 and are presented in the appendix (Supplemental Digital Content, Fig. S1, http://links.lww.com/JOM/A84, and Fig. S2, http://links.lww.com/JOM/A85). In addition, because similar trends were observed when evaluating inhalable dust, endotoxin, total 3-OHFA, and even-chain 3-OHFA, we present only results for inhalable dust and even-chain 3-OHFA. Thus, we briefly highlight important results from Figs. 1 and 2. A greater reduction across quartiles of even-chain 3-OHFA exposure was observed among smokers compared with nonsmokers; the cross-shift change in FEV_1 in the highest quartile of 3-OHFA exposure was −4.9% for smokers and −3.3% for nonsmokers ($P_{interaction} < 0.05$; Fig. 1). Among the workers who reported applying pesticides or herbicides, the cross-shift change in FEV_1 for those in the highest quartile of inhalable dust exposure was −11.3% compared with −4.4% for those not reporting pesticide/herbicide use ($P_{interaction} = 0.05$; Fig. 1). Although living on a farm did not appear to modify the relationship between dust or endotoxin exposure and pulmonary function ($P_{interaction} = 0.53$ for inhalable dust and FEV_1), some evidence of effect modification by "time at the job" was observed ($P_{interaction} = 0.01$ for inhalable dust and FEV_1; Fig. 1); however, wide confidence intervals among those working for 1 year less on the job limit interpretation. We did not observe consistent evidence of effect modification by obesity for FEV_1 (Fig. 2) or FVC; however, a significant trend of decreasing FVC across quartiles of total 3-OHFA was observed only among obese participants ($P_{trend} = 0.04$; Supplemental Digital Content, Fig. S2, http://links.lww.com/JOM/A85). We did not observe consistent evidence of effect modification by age (Fig. 2), Hispanic ethnicity (results not presented), or reported asthma or allergies. However, a significant trend of cross-shift reductions in FVC across quartiles of inhalable dust exposure was observed only among those reporting asthma/allergies ($P_{trend} = 0.03$; Supplemental Digital Content, Fig. S2, http://links.lww.com/JOM/A85), which was consistent with trends observed for FEV_1 (Fig. 2). Evidence of effect modification by TLR4 polymorphism status was observed. Those with genetic polymorphisms in TLR4 299 (results not presented) or TLR4 399 tended to have greater

TABLE 2. Work Shift Air Quality Measures Among All Participants and by Facility Type.

Pollutant	*n*	Geometric Mean	GSD	Q1: Geometric Mean (GSD); *n*	Q2: Geometric Mean (GSD); *n*	Q3: Geometric Mean (GSD); *n*	Q4: Geometric Mean (GSD); *n*	Spearman Correlation Coefficients			
								Dust	Endotoxin	3-OHFA (all chains)	3-OHFA (even chains)
Dust, mg/m^3											
Total population	136	3.40	3.0	0.86 (2.6); 31	2.33 (1.2); 34	4.15 (1.3); 36	13.52 (1.7); 35	1.00			
Grain elevator	53	5.09	3.7	0.68 (2.5); 10	2.14 (1.2); 5	4.44 (1.3); 13	14.56 (1.8); 25				
Cattle feedlot	59	2.66	2.2	0.95 (1.6); 13	2.35 (1.2); 22	4.02 (1.3); 18	11.35 (2.1); 6				
Dairy	15	2.37	1.9	1.16 (1.2); 5	2.36 (1.2); 5	3.64 (1.2); 3	7.38 (1.1); 2				
Corn farm	9	2.86	3.6	0.71 (1.5); 3	2.61 (1.1); 2	4.32 (1.7); 2	16.62 (1.0); 2				
Endotoxin, EU/m^3											
Total population	136	895	6.8	69 (2.8); 32	447 (1.5); 35	1827 (1.5); 34	9347 (1.9); 35	0.60	1.00		
Grain elevator	53	800	8.4	50 (3.2); 15	546 (1.4); 11	1833 (1.5); 11	7973 (1.8); 16				
Cattle feedlot	60	971	7.1	94 (2.3); 15	424 (1.5); 17	1767 (1.6); 11	11824 (2.0); 17				
Dairy	15	1166	2.8	143 (NA); 1	436 (1.7); 4	1930 (1.5); 9	5243 (NA); 1				
Corn farm	8	624	4.5	44 (NA); 1	295 (1.2); 3	1732 (1.6); 3	3902 (NA); 1				
3-OHFA (total), pmol/m^3											
Total population	128	2031.6	4.3	321.9 (1.6); 32	1262.2 (1.5); 32	3343.3 (1.3); 33	13092.6 (2.4); 31	0.45	0.65	1.00	
Grain elevator	53	1772.9	5.7	288.3 (1.7); 17	1249.0 (1.4); 16	2909.4 (1.2); 6	19420.8 (2.4); 14				
Cattle feedlot	53	2515.9	3.8	359.0 (1.6); 11	1209.2 (1.6); 9	3663.6 (1.3); 17	9720.6 (2.2); 16				
Dairy	14	1428.0	2.6	346.7 (1.2); 3	1308.7 (1.6); 6	3265.9 (1.3); 4	6156.7 (NA); 1				
Corn Farm	8	2253.4	2.0	504.1 (NA); 1	1769.1 (NA); 1	3010.9 (1.4); 6	NA; 0				
3-OHFA (even chains), pmol/m^3											
Total population	128	1564.9	4.6	228.3 (1.7); 32	998.4 (1.5); 33	2538.9 (1.3); 32	11176.9 (2.6); 31	0.46	0.64	0.99	1.00
Grain elevator	53	1427.9	6.7	206.5 (1.8); 17	1053.5 (1.5); 16	2329.0 (1.3); 7	20002.2 (2.4); 13				
Cattle feedlot	53	1878.8	4.0	247.6 (1.7); 11	850.3 (1.5); 9	2730.9 (1.3); 16	7459.9 (2.2); 17				
Dairy	14	1102.2	2.7	264.8 (1.2); 3	998.6 (1.4); 6	2482.2 (1.4); 4	5588.7 (NA); 1				
Corn farm	8	1578.9	2.1	328.5 (NA); 1	1337.2 (1.1); 2	2310.0 (1.4); 5	NA; 0				

3-OHFA, 3-hydroxy fatty acid constituents of endotoxin; GSD, geometric standard deviation; NA, not applicable.

TABLE 3. Mean* Changes in FVC and FEV_1 Across the Work Shift by Quartiles of Dust, Endotoxin, or 3-OHFA (all Chains and Even Chains) Among All Participants and by Facility Type.

			FVC						FEV_1					
			Exposure Categorized into Quartiles						Exposure Categorized into Quartiles					
Pollutant	Site	*n*	Q1	Q2	Q3	Q4	*P* (Q1 vs Q4)	P_{trend} (across quartiles)	Q1	Q2	Q3	Q4	*P* (Q1 vs Q4)	P_{trend} (across quartiles)
Dust														
	Total population	136	−1.50	−1.19	−0.38	−4.15	0.14	0.04	−0.95	−3.39	0.04	−6.22	0.01	0.01
	Grain elevator	53	−0.75	2.20	−0.45	−4.91	0.25	0.05	0.99	−2.75	1.20	−7.62	0.05	0.01
	Cattle feedlot	59	−0.55	−0.97	0.47	−0.30	0.93	0.83	−0.96	−2.84	0.16	−0.19	0.77	0.48
	Dairy	15	−1.73	−3.55	−1.39	−3.69	0.71	0.74	−1.97	−4.09	−2.38	−8.67	0.19	0.16
	Corn farm	9	−8.49	−6.96	−7.21	−3.53	0.57	0.48	−5.64	−7.06	−6.48	−5.00	0.95	0.95
Endotoxin														
	Total population	136	0.07	−2.19	−2.06	−3.07	0.08	0.20	−0.48	−2.69	−2.97	−4.40	0.05	0.11
	Grain elevator	53	1.07	−1.85	−4.26	−4.63	0.10	0.19	0.74	−0.68	−4.93	−8.01	0.04	0.04
	Cattle feedlot	60	−0.75	−0.77	2.42	−1.51	0.70	0.47	−1.38	−2.65	1.48	−1.45	0.97	0.96
	Dairy	15	−1.33	−1.43	−3.09	−3.12	0.87	0.90	2.90	−0.87	−4.63	−12.51	0.14	0.15
	Corn farm	8	0.63	−11.94	−8.10	−5.78	0.27	0.44	5.66	−10.84	−8.12	−5.98	0.04	0.72
3-OHFA (total)														
	Total population	128	−1.05	−1.92	−2.00	−3.35	0.22	0.23	−2.09	−1.62	−3.68	−4.32	0.29	0.21
	Grain elevator	53	0.20	−0.79	−1.56	−7.62	0.02	0.01	−0.61	−0.18	−4.13	−10.03	0.02	0.01
	Cattle feedlot	53	−2.14	−0.56	−0.71	0.36	0.29	0.35	−3.06	−0.68	−2.90	0.59	0.11	0.09
	Dairy	14	0.06	−4.95	−1.06	−6.02	0.39	0.67	−2.06	−3.96	−4.87	−9.30	0.33	0.27
	Corn farm	8	−15.45	−10.93	−6.48	–	–	0.06	−14.15	−13.63	−4.90	–	–	0.08
3-OHFA (even chains)														
	Total population	128	−0.87	−2.00	−2.51	−2.94	0.27	0.35	−1.92	−1.52	−4.55	−3.78	0.38	0.34
	Grain elevator	53	0.24	−0.53	−0.85	−8.83	0.01	0.002	−0.65	−0.01	−3.98	−10.74	0.02	0.0047
	Cattle feedlot	53	−1.47	−1.26	−2.20	1.64	0.17	0.06	−2.27	−1.50	−4.31	1.63	0.06	0.01
	Dairy	14	0.14	−4.98	−1.21	−5.44	0.43	0.62	−2.07	−3.86	−7.98	−9.43	0.32	0.26
	Corn farm	8	−15.23	−6.03	−7.60	–	–	0.30	−13.65	−2.79	−7.59	–	–	0.66

3-OHFA, 3-hydroxy fatty acid constituents of endotoxin; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity.
*Least-square mean from the general linear model analysis with adjustment for age and smoking status.

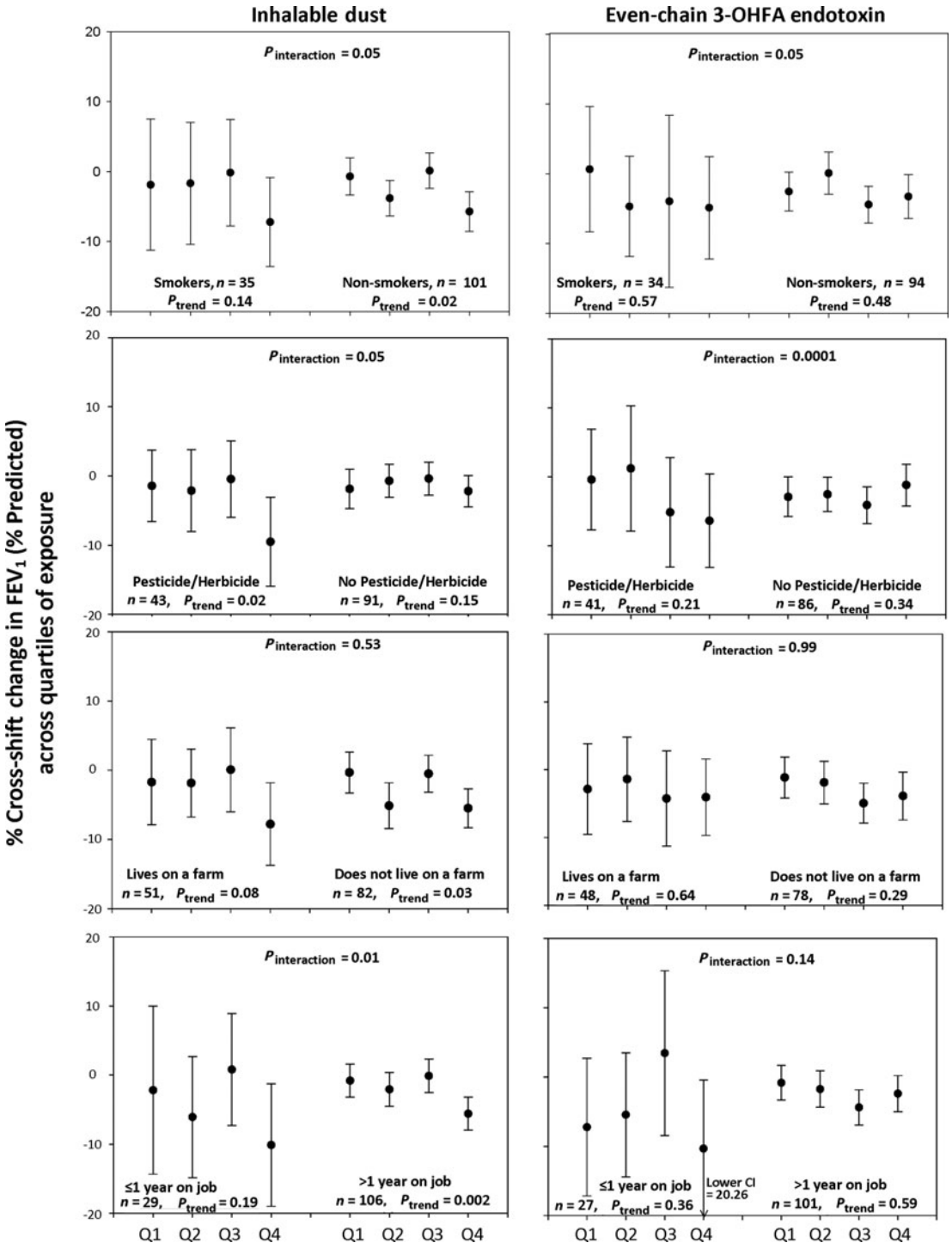

FIGURE 1. Effect modification of associations between personal inhalable dust (left column) or even-chain 3-hydroxy fatty acid constituents of endotoxin (3-OHFA) endotoxin (right column) exposure quartiles and pulmonary function by extrinsic/environmental risk factors. Models adjusted for age and smoking. Error bars indicate 95% confidence intervals.

reductions in FEV_1 (Fig. 2) or FVC (Supplemental Digital Content, Fig. S2, http://links.lww.com/JOM/A85) across quartiles of inhalable dust exposure. Modification of the relationships between lung function and either 3-OHFA or inhalable dust exposure by genetic polymorphism status should be interpreted with caution because of the small numbers of participants with mutations, as well as the fact that the statistical trends across quartiles of exposure appear to be driven largely by the fourth quartile.

DISCUSSION

Study results support previous reports of acute cross-shift reductions in pulmonary function among agricultural workers. [35,37–41]

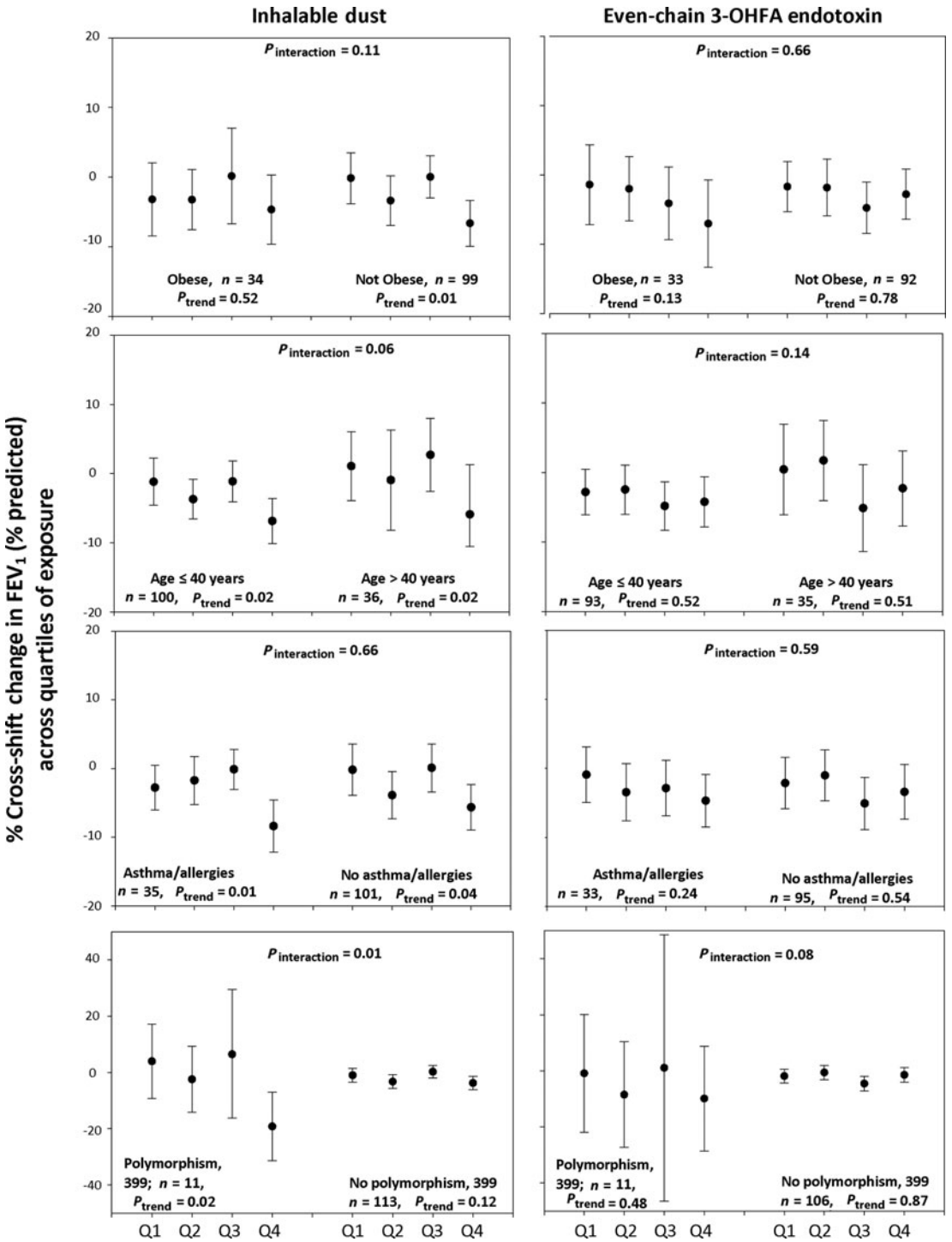

FIGURE 2. Effect modification of associations between personal inhalable dust (left column) or even-chain 3-hydroxy fatty acid constituents of endotoxin (3-OHFA) endotoxin (right column) exposure quartiles and pulmonary function by intrinsic risk factors. Models adjusted for age and smoking. Error bars indicate 95% confidence intervals. Note scale change for presentation of TLR4 399 polymorphism results.

Results for the current study appear to be driven by associations observed among grain elevator operators, although small sample sizes limit interpretations among corn farm and dairy employees. Endotoxins are important known proinflammatory components of bioaerosols and, thus, were a focus of measurement for this study. There has been a significant body of work regarding acute and chronic respiratory effects of endotoxin in swine and poultry production environments, leading to recommended occupational exposure guidelines.[16,35,36,42] Other agricultural settings, such as dairies, grain elevators, and cattle feedlots, have not been well characterized, and

no endotoxin-specific exposure guidelines have been developed for these work environments. Geometric mean endotoxin concentrations for all four work settings exceeded the proposed Dutch standard[36] of 90 EU/m^3 and the guideline of 600 EU/m^3 suggested by Donham et al.[16] In addition, although studies have reported reduced lung function with elevated endotoxin exposures,[35,37,38,43,44] very few studies have characterized the relationship between 3-OHFA endotoxin constituents and airway responses among agricultural workers. In this study, we observed similar relationships for lung function reductions with both dust and endotoxin. However, the stronger associations between the even-numbered carbon chain length 3-OHFA constituents and lung function reductions suggest that this particular component of dust may be a better exposure indicator for assessing the inflammatory potential of bioaerosols, at least in grain elevator facilities.

Understanding variation in response to agricultural dust exposures may provide insight regarding underlying biological mechanisms, as well as identify at-risk individuals and potential prevention measures.[10] We examined a variety of extrinsic/environmental factors (eg, smoking, pesticide use) and intrinsic factors (eg, obesity, TLR4 genetic polymorphisms) to evaluate the potential for effect modification of the exposure–response relationship between agricultural dust and pulmonary function.

Agricultural workers are exposed to a complex myriad of environmental exposures that have been identified as respiratory irritants[3,45] and which may interact with dust exposures to further exacerbate adverse respiratory conditions. We identified several environmental (extrinsic) factors that may confer increased susceptibility to agricultural dust exposures. There were greater exposure-associated reductions in lung function among current smokers than nonsmokers. This observation supports previous studies demonstrating that smoking can modify the relationship between agricultural exposures and respiratory health.[46–51] Pesticide use is another environmental/extrinsic factor related to respiratory morbidity among agricultural workers,[52–57] and it has been suggested that pesticide use may modify endotoxin-induced inflammatory responses.[56] To our knowledge, this is the first human study to observe stronger endotoxin-induced reductions in lung function among those reporting pesticide or herbicide application than those not reporting application. Research incorporating more detailed questions regarding pesticide/herbicide application, including the specific chemical constituents, may help to further elucidate the potential interaction with dust or endotoxin.

An independent effect of "living on a farm" with increased respiratory symptom prevalence was observed among California farm operators.[58] Residential exposures due to living on a farm could signify additional types and durations of environmental exposures that could exacerbate the effects of agricultural dusts on respiratory health.[58] Thirty-eight percent of our study population reported living on a farm. We did not observe consistent evidence of a modifying effect of this factor on dust/endotoxin-induced respiratory effects. However, our survey may have been too general to fully ascertain take-home exposure to pesticides that has been identified as a potentially significant problem.[59,60]

To assess the potential role of endotoxin tolerance in our study population, we previously evaluated the influence of "time on the job" on the relationship between endotoxin and nasal inflammatory markers in this population.[14] Although a strong modifying response was not observed, participants with a shorter frequency or duration of agricultural employment tended to have elevated levels of some inflammatory markers in response to 3-OHFA exposures. Here, we report a similar modifying effect of "time on the job" ($\leq$1 year vs $>$1 year) on the relationship between personal dust exposures and cross-shift changes in lung function. Although a statistically significant interaction was observed only between time on the job and dust exposures, we cannot rule out the possible influence of endotoxin tolerance among those working on the job for longer durations because of the moderately high correlations among dust and endotoxin/3-OHFA exposure concentrations. Our findings that extended farm work may confer a degree of tolerance to the adverse health effects of endotoxin are consistent with other studies.[26,61–63] Years in the grain industry has also been shown to modify the relationship between longitudinal declines in FEV_1 and the TNF-α (tumor necrosis factor alpha) 308 genotype.[64] In addition, it is possible that the modifying effect of time on the job was partially because of the "healthy worker effect" wherein more experienced workers may exhibit resistance to the health-damaging effects of dust/endotoxin inhalation or self-select out of the industry.

We evaluated several intrinsic factors that may confer increased susceptibility to the adverse respiratory effects from agricultural dust or endotoxin exposure. A growing body of evidence suggests chronic inflammatory conditions, such as obesity, may modify the effect of inhaled air pollutants.[20] Although not statistically significant, there was some indication that obesity modified the effect of 3-OHFA exposure on changes in lung function in our study population. Greater effects that were observed among obese than nonobese populations could, in part, be due to elevated inhalation rates, and therefore increased dose.[20,65] Interestingly, Pahwa et al[66] reported a modifying effect of obesity on the relationship between TLR4 mutations and lung function in a community-based population. Older populations and ethnic minorities are also considered potentially susceptible to air pollution health effects among the general population.[20] We did not observe consistent modifying effects of these parameters, which could reflect differences in evaluating populations working in agricultural settings when compared with the general population. Epidemiologic studies have also evaluated the effects of preexisting respiratory diseases on air pollution–induced health effects.[20] Although our design does not allow for the analysis of the timing of allergies or asthma onset, we did observe a trend of lung function reductions across dust exposure quartiles among the subgroup of workers reporting allergies or asthma, which is supported by other studies.[67–69] Generalization of this finding to broader populations may be limited because more severe cases of respiratory diseases are less likely to be captured within the agricultural workforce.

Research describing the interactions between genetic polymorphisms and endotoxin exposures on respiratory health are inconsistent, and few field studies have been conducted.[70] In a controlled experiment, an attenuation of the effect of inhaled lipopolysaccharide on FEV_1 decrement was observed among those with a TLR4 polymorphism (Asp299Gly and Thr399Ile), although this appeared dependent on cumulative dose of lipopolysaccharide.[71] The authors suggested that humans with these TLR4 variants may be more resistant to localized forms of endotoxin-induced inflammation. However, it was also noted that these same individuals may be more susceptible to a systemic inflammatory response.[71] Only two studies incorporating examination of genetic polymorphisms have focused strictly on occupational populations,[72,73] and only Smit et al[73] included exposure assessment. Although the small sample size and large confidence limits are cautionary, our results suggest that agricultural workers with a TLR4 polymorphism may be more susceptible to the adverse effects of endotoxin exposure on cross-shift lung function decline. TLR4 itself is not consistently associated with lung function; however, a recent investigation among a community-based population observed a modifying effect of body mass index on the relationship between TLR4 and several lung function parameters.[66] Our small sample precluded our ability to examine gene by environment by obesity interactions or gene by gene by environment interactions. Given the diversity of the agricultural workforce, future studies should consider the investigation of these complex interactions.

Several limitations should be considered in interpreting the results of this study. First, our sample size is small, which limits our

ability to fully examine effects among potentially susceptible subgroups within the study population and contributes to imprecise effect estimates (ie, wide confidence intervals). It is possible that a few workers contributed disproportionately to the effect estimates. However, detailed exposure assessments were conducted, which increased our ability to accurately quantify exposure variability among participants. Second, many of our susceptibility factors were determined via self-report (eg, smoking status, use of pesticides). Although we do not expect potential misclassifications to result in systematic bias, it is possible that some associations may have been attenuated. Few occupational health studies have incorporated detailed and quantitative exposure and health assessments; results from our study, in conjunction with similarly conducted research efforts among larger populations, are needed to establish accurate dose–response relationships.[2] Another limitation of the study is the low response rate of agricultural workers solicited for participation (~50%). The most common reasons for nonparticipation were fear of the blood draw and immigration status. If more highly exposed workers who experience greater reductions in lung function across typical work shifts were more likely to participate, then our observed results could be affected by such bias. However, we believe that it is unlikely that a worker would have participated on the basis of knowledge of cross-shift changes in quantitatively measured lung function. In addition, we do not have a reason to believe that the loss of exposure information (malfunction of samplers, loss during transportation) was related to the level of exposure experienced by the excluded worker, which would preclude the introduction of a systemic selection bias. The uncertainties associated with these limitations limit, to some degree, the interpretation or representativeness of the results. However, the exposures, the distribution of work, and the cross-shift lung measurements in this population were reasonably consistent with other published studies.

CONCLUSIONS

In summary, this study quantified personal work shift exposures to inhalable dust, endotoxin, and its 3-OHFA constituents among workers in several agricultural settings. To our knowledge, this is the first study to examine the relationships between these quantitatively assessed exposures, cross-shift changes in pulmonary function measures, and potential intrinsic and extrinsic effect modifiers of the exposure–response relationships. The cross-sectional nature of this study did not allow for the evaluation of causal associations between 3-OHFA exposures and adverse respiratory outcomes. Nonetheless, some evidence of larger cross-shift reductions in lung function was observed among those more highly exposed. Our results support previous studies establishing smoking exposure and time on the job as modifiers of the effects of inhalable dusts and dust constituents on respiratory morbidity. In addition, we provide new evidence that suggests that the use of pesticides or herbicides exacerbates the effects of dust inhalation on reductions in lung function. There was also limited evidence of the potential modifying effects of obesity, preexisting respiratory conditions, and the presence of genetic polymorphisms (TLR4), which is biologically plausible and consistent with results among other populations; however, further evaluation among larger populations of agricultural workers is needed. The results suggest that interventions among agricultural workers may need to include more comprehensive wellness programs in addition to exposure reduction strategies.

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REFERENCES

1. Eduard W, Pearce N, Douwes J. Chronic bronchitis, COPD, and lung function in farmers. *Chest.* 2009;136:716–725.
2. Omland O. Exposure and respiratory health in farming in temperate zones–a review of the literature. *Ann Agric Environ Med*. 2002;9:119–136.
3. Linaker C, Smedley J. Respiratory illness in agricultural workers. *Occup Med.* 2002;52:451–459.
4. Spurzem JR, Romberger D, Von Essen S. Agricultural lung disease. *Clin Chest Med.* 2002;23:795–810.
5. Rushton L. Occupational causes of chronic obstructive pulmonary disease. *Rev Environ Health.* 2007;22:195–212.
6. Rylander R. Endotoxin and occupational airway disease. *Curr Opin Allergy Clin Immunol.* 2006;6:62–6.
7. Seifert SA, Von Essen S, Jacobitz K, Crouch R, Lintner CP. Organic dust toxic syndrome: a review. *J Toxicol Clin Toxicol.* 2003;41:185–93.
8. Gutsmann T, Schromm AB, Brandenburg K. The physicochemistry of endotoxins in relation to bioactivity. *Int J Med Microbiol.* 2007;297:341–52.
9. Thorn J. The inflammatory response in humans after inhalation of bacterial endotoxin: a review. *Inflamm Res.* 2001;50:254–261.
10. Liebers V, Raulf-Heimsoth M, Brüning T. Health effects due to endotoxin inhalation (review). *Arch Toxicol.* 2008;82:203–210.
11. Reynolds SJ, Thorne PS, Donham KJ, et al. Comparison of endotoxin assays using agricultural dusts. *AIHA J*. 2002;63:430–438.
12. Spaan S, Heederik DJ, Thorne PS, Wouters IM. Optimization of airborne endotoxin exposure assessment: effects of filter type, transport conditions, extraction solutions, and storage of samples and extracts. *Appl Environ Microbiol.* 2007;73:6134–6143.
13. Poole JA, Dooley GP, Saito R, et al. Muramic acid, endotoxin, 3-hydroxy fatty acids, and ergosterol content explain monocyte and epithelial cell inflammatory responses to agricultural dusts. *J Toxicol Environ Health A*. 2010;73:684–700.
14. Burch JB, Svendsen E, Siegel PD, et al. Endotoxin exposure and inflammation markers among agricultural workers in Colorado and Nebraska. *J Toxicol Environ Health A.* 2010;73:5–22.
15. Elfman L, Riihimaki M, Pringle J, Walinder R. Influence of horse stable environment on human airways. *J Occup Med Toxicol*. 2009;4:10.
16. Donham KJ, Reynolds SJ, Whitten P, Merchant JA, Burmeister L, Popendorf WJ. Respiratory dysfunction in swine production facility workers: dose-response relationships of environmental exposures and pulmonary function. *Am J Ind Med.* 1995;27:405–418.
17. Reynolds SJ, Donham KJ, Whitten P, Merchant JA, Burmeister LF, Popendorf WJ. Longitudinal evaluation of dose-response relationships for environmental exposures and pulmonary function in swine production workers. *Am J Ind Med.* 1996;29:33–40.
18. Kleeberger SR, Ohtsuka Y. Gene-particulate matter-health interactions. *Toxicol Appl Pharmacol.* 2005;207:276–281.
19. Kleeberger SR. Genetic aspects of pulmonary responses to inhaled pollutants. *Exp Toxicol Pathol.* 2005;57(Supplement 1):147–153.
20. Sacks JD, Stanek LW, Luben TJ, et al. Particulate matter-induced health effects: who is susceptible? *Environ Health Perspect*. 2011;119:446–454.
21. Dosman JA, Fukushima Y, Senthilselvan A, et al. Respiratory response to endotoxin and dust predicts evidence of inflammatory response in volunteers in a swine barn. *Am J Ind Med.* 2006;49:761–6.
22. Castellan RM, Olenchock SA, Kinsley KB, Hankinson JL. Inhaled endotoxin and decreased spirometric values. An exposure-response relation for cotton dust. *N Engl J Med.* 1987;317:605–610.
23. Schwartz DA. TLR4 and LPS hyporesponsiveness in humans. *Int J Hyg Environ Health.* 2002;205:221–227.
24. Kline JN, Cowden JD, Hunninghake GW, et al. Variable airway responsiveness to inhaled lipopolysaccharide. *Am J Respir Crit Care Med.* 1999;160:297–303.
25. Israël-Assayag E, Cormier Y. Adaptation to organic dust exposure: a potential role of l-selectin shedding? *Eur Respir J.* 2002;19:833–837.
26. Von Essen S, Romberger D. The respiratory inflammatory response to the swine confinement building environment: the adaptation to respiratory exposures in the chronically exposed worker. *J Agric Saf Health.* 2003;9:185–196.
27. Loh LC. Tolerance to repeat exposure of inhaled endotoxin: an observation in healthy humans. *Eur Respir J.* 2005;26:363–364.
28. Thorne PS, Perry SS, Saito R, et al. Evaluation of the Limulus amebocyte lysate and recombinant factor C assays for assessment of airborne endotoxin. *Appl Environ Microbiol.* 2010;76:4988–4995.

29. Saito R, Cranmer BK, Tessari JD, et al. Recombinant factor C (rFC) assay and gas chromatography/mass spectrometry (GC/MS) analysis of endotoxin variability in four agricultural dusts. *Ann Occup Hyg.* 2009;53:713–722.
30. Alwis KU, Milton DK. Recombinant factor C assay for measuring endotoxin in house dust: comparison with LAL, and (1→3)-β-D-glucans. *Am J Ind Med.* 2006;49:296–300.
31. Ferris BG. Epidemiology Standardization Project (American Thoracic Society). *Am Rev Respir Dis.* 1978;118:1–120.
32. Rylander R, Peterson Y, Donham K. Questionnaire evaluating organic dust exposure. *Am J Ind Med.* 1990;17:121–126.
33. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med*. 1999;159:179–187.
34. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. *Eur Respir J.* 2005;26:948–968.
35. Donham KJ, Cumro D, Reynolds SJ, Merchant JA. Dose-response relationships between occupational aerosol exposures and cross-shift declines of lung function in poultry workers: recommendations for exposure limits. *J Occup Environ Med.* 2000;42:260–269.
36. Dutch Expert Committee on Occupational Safety. *Endotoxins: Health-Based Recommended Occupational Exposure Limit*. Den Haag, the Netherlands: Health Council of the Netherlands; 2010.
37. Senthilselvan A, Beach J, Feddes J, Cherry N, Wenger I. A prospective evaluation of air quality and workers' health in broiler and layer operations. *Occup Environ Med.* 2011;68:102–107.
38. Oldenburg M, Latza U, Baur X. Exposure–response relationship between endotoxin exposure and lung function impairment in cotton textile workers. *Int Arch Occup Environ Health.* 2007;80:388–395.
39. Viet SM, Buchan R, Stallones L. Acute respiratory effects and endotoxin exposure during wheat harvest in Northeastern Colorado. *Appl Occup Environ Hyg.* 2001;16:685–697.
40. Radon K, Danuser B, Iversen M, et al. Respiratory symptoms in European animal farmers. *Eur Respir J.* 2001;17:747–754.
41. Chan-Yeung M, Schulzer M, MacLean L, et al. A follow-up study of the grain elevator workers in the Port of Vancouver. *Arch Environ Health.* 1981;36:75–81.
42. Reynolds SJ. Occupational and environmental health in Uzbekistan and Turkmenistan. *Am Ind Hyg Assoc J.* 1996;57:1096–1102.
43. Sigsgaard T, Jensen LD, Abell A, Würtz H, Thomsen G. Endotoxins isolated from the air of a Danish paper mill and the relation to change in lung function: An 11-year follow-up. *Am J Ind Med.* 2004;46:327–332.
44. Wang X-R, Pan L-D, Zhang H-X, Sun B-X, Dai H-l, Christiani DC. Follow-up study of respiratory health of newly-hired female cotton textile workers. *Am J Ind Med.* 2002;41:111–118.
45. Schenker MB. Farming and asthma.*Occup Environ Med.* 2005;62:211–212.
46. Hoppin JA, Umbach DM, London SJ, Alavanja MCR, Sandler DP. Animal production and wheeze in the Agricultural Health Study: interactions with atopy, asthma, and smoking. *Occup Environ Med.* 2003;60:e3.
47. Chen Y, Horne SL, McDuffie HH, Dosman JA. Combined effect of grain farming and smoking on lung function and the prevalence of chronic bronchitis. *Int J Epidemiol.* 1991;20:416–423.
48. Iversen M, Dahl R. Specific antigens in dust from swine confinement buildings. *Am J Ind Med.* 1994;25:49–51.
49. Cotton DJ, Graham BL, Li KYR, Froh F, Barnett GD, Dosman JA. Effects of grain dust exposure and smoking on respiratory symptoms and lung function. *J Occup Environ Med.* 1983;25:131–141.
50. Mustajbegovic JMD, Zuskin EMD, Schachter ENMD, et al. Respiratory findings in livestock farmworkers. *J Occup Environ Med.* 2001;43:576–584.
51. Chan-Yeung M, Dimich-Ward H, Enarson DA, Kennedy SM. Five cross-sectional studies of grain elevator workers. *Am J Epidemiol.* 1992;136:1269–1279.
52. Senthilselvan A, McDuffie HH, Dosman J. Association of asthma with use of pesticides: results of a cross-sectional survey of farmers. *Am Rev Respir Dis.* 1992;146:884–887.
53. Sprince NL, Lewis MQ, Whitten PS, Reynolds SJ, Zwerling C. Respiratory symptoms: associations with pesticides, silos, and animal confinement in the Iowa Farm Family Health and Hazard Surveillance Project. *Am J Ind Med.* 2000;38:455–462.
54. Hoppin JA, Umbach DM, Kullman GJ, et al. Pesticides and other agricultural factors associated with self-reported farmer's lung among farm residents in the Agricultural Health Study. *Occup Environ Med.* 2007;64:334–341.
55. Hoppin JA, Valcin M, Henneberger PK, et al. Pesticide use and chronic bronchitis among farmers in the agricultural health study. *Am J Ind Med.* 2007;50:969–979.
56. Hoppin JA, Umbach DM, London SJ, et al. Pesticide use and adult-onset asthma among male farmers in the Agricultural Health Study. *Eur Respir J.* 2009;34:1296–1303.
57. Beseler CL, Stallones L. Pesticide poisoning and respiratory disorders in Colorado farm residents. *J Agric Saf Health.* 2009;15:327–334.
58. Schenker MB, Farrar JA, Mitchell DC, et al. Agricultural Dust Exposure and Respiratory Symptoms Among California Farm Operators. *J Occup Environ Med.* 2005;47:1157–1166.
59. Curwin B, Hein M, Sanderson W, et al. Farm family take-home pesticide exposure study. Dallas, TX: American Industrial Hygiene Conference; 2003.
60. Curwin BD, Hein MJ, Sanderson W, et al. Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in Iowa. *Ann Occup Hyg.* 2007;51:53–65.
61. Zock JP, Heederik D, Doekes G. Evaluation of chronic respiratory effects in the potato processing industry: indications of a healthy worker effect? *Occup Environ Med*. 1998;55:823–827.
62. Sahlander K, Larsson K, Palmberg L. Altered innate immune response in farmers and smokers. *Innate Immun.* 2010;16:27–38.
63. Broad A, Jones DEJ, Kirby JA. Toll-like receptor (TLR) response tolerance: a key physiological 'damage limitation' effect and an important potential opportunity for therapy. *Curr Med Chem.* 2006;13:2487–2502.
64. Pahwa P, Nakagawa K, Koehncke N, McDuffie HH. Effects of polymorphic variations in tumor necrosis factor alpha and occupational exposure to grain dust on longitudinal decline in pulmonary function. *J Agromed.* 2009;14:215–221.
65. Bennett WD, Zeman KL. Effect of body size on breathing pattern and fine-particle deposition in children. *J ApplPhysiol.* 2004;97:821–826.
66. Pahwa P, Karunanayake C, Rennie D, Chen Y, Schwartz D, Dosman J. Association of the TLR4 Asp299Gly polymorphism with lung function in relation to body mass index. *BMC Pulm Med.* 2009;9:46.
67. Smit LAM, Zuurbier M, Doekes G, Wouters IM, Heederik D, Douwes J. Hay fever and asthma symptoms in conventional and organic farmers in The Netherlands. *Occup Environ Med.* 2007;64:101–107.
68. Eduard W, Omenaas E, Bakke PS, Douwes J, Heederik D. Atopic and non-atopic asthma in a farming and a general population.*Am J Ind Med.* 2004;46:396–399.
69. Sepulveda MJ, Castellan RM, Hankinson JL, Cocke JB. Acute lung function response to cotton dust in atopic and non-atopic individuals. *Br J Ind Med*. 1984;41:487–491.
70. London SJ, Romieu I. Gene by environment interaction in asthma. *Annu Rev Public Health.* 2009;30:55–80.
71. Arbour NC, Lorenz E, Schutte BC, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet.* 2000;25:187–191.
72. LeVan TD, Von Essen S, Romberger DJ, et al. Polymorphisms in the CD14 gene associated with pulmonary function in farmers. *Am J Respir Crit Care Med.* 2005;171:773–779.
73. Smit LAM, Heederik D, Doekes G, et al. Endotoxin exposure, CD14 and wheeze among farmers: a gene–environment interaction. *Occup Environ Med.* 2011;68:826–831.

