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## Particulate Matter, Endotoxin, and Worker Respiratory Health on Large Californian Dairies

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### Abstract

**Objective:** To assess respiratory exposures and lung function in a cross-sectional study of California dairy workers.

**Methods:** Exposure of 205 dairy and 45 control (vegetable processing) workers to particulate matter and endotoxin was monitored. Pre- and postshift spirometry and interviews were conducted.

**Results:** Geometric mean inhalable and PM<sub>2.5</sub> concentrations were 812 and 35.3 [ $\mu$ ]g/m<sup>3</sup> versus 481.9 and 19.6 [ $\mu$ ]g/m<sup>3</sup>, respectively, for dairy and control workers. Endotoxin concentrations were 329 EU/m<sup>3</sup> or 1122 pmol/m<sup>3</sup> and 13.5 EU/m<sup>3</sup> or 110 pmol/m<sup>3</sup>, respectively, for dairy and control workers. In a mixed-effects model, forced vital capacity decreased across a work shift by 24.5 mL (95% confidence interval, -44.7 to -4.3; P = 0.018) with log<sub>10</sub> (total endotoxin) and by 22.0 mL (95% confidence interval, -43.2 to -0.08; P = 0.042) per hour worked.

**Conclusions:** Modern California dairy endotoxin exposures and shift length were associated with a mild acute decrease in forced vital capacity.

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High ambient concentrations of inhalable particulate matter (PM) increase the occurrence of respiratory disease in the general population.<sup>1–3</sup> The smallest particles with a mean aerodynamic diameter of 2.5 [ $\mu$ ]m or less (PM<sub>2.5</sub>) are also associated with cardiovascular disease and increased death rates.<sup>4,5</sup> In dry western states, dairy workers are exposed to high concentrations of both inorganic and organic particles, but primarily to organic matter.<sup>6–8</sup> Exposures to these bioaerosols, in sufficient concentrations, are known to result in decrements in pulmonary function, both acute (across a work shift) and long-term.<sup>9,10</sup>

Workers on dairy facilities are exposed to endotoxin (lipopolysaccharide contained in the cell walls of gram-negative bacteria) concentrations that are much higher than ambient levels.<sup>6,11–16</sup> High endotoxin exposures are believed to be a major factor in acute and long-term decreases in pulmonary function observed in many farmworkers.<sup>17–19</sup> Inhalation of endotoxin-contaminated air produces inflammation of the airways that can resemble asthma, with reversible airflow obstruction and hyperreactivity, including wheezing.<sup>20</sup> Other acute effects include organic dust toxic syndrome, a nonallergic flu-like illness,<sup>19</sup> and asthma.<sup>8,21</sup> Chronic exposure to endotoxin at lower concentrations may produce a myriad of symptoms, such as nose and throat irritation, chest tightness, cough, shortness of breath, wheezing, irreversible chronic bronchitis, and reduced lung function (with or without obstruction).<sup>7,22</sup>

Dairy workers are exposed to endotoxins and other potential respiratory risk factors, such as gram-positive bacteria, molds, fungi, ammonia, and hydrogen sulfide.<sup>6,8</sup> The mix of all these agents is complex, and interactions/synergies are likely. It remains difficult to set a narrow range or a single maximal concentration of a specific contaminant for worker protection. Historically, studies of dairy facilities have reported concentrations of endotoxins and PM averaging above the postulated threshold for respiratory effects. Investigators have reported thresholds in occupational settings at which health effects occur as 2.5 mg/m<sup>3</sup> for PM and between 90 and 614 EU/m<sup>3</sup> for endotoxin, depending on the type of livestock or other source of endotoxin present.<sup>7,23–27</sup>

This cross-sectional study was conducted to determine whether California dairy workers differ in their respiratory health and exposures compared with nondairy workers of similar socioeconomic status and ethnicity. We also wanted to determine whether the exposures to PM and endotoxin on large, naturally ventilated California dairies were comparable with data published from studies of smaller, conventional dairies in cooler, damper climates.<sup>6,28,29</sup> We evaluated whether particulate exposures on California dairies, including endotoxin, were associated with decrements in pulmonary function.

## METHODS

### Recruitment and Design

This cross-sectional study was conducted in the San Joaquin Valley of California in 2008. Male workers were recruited from 13 large (>1000 lactating cows) California dairies, as previously specified.<sup>30</sup> All eligible male workers were invited to participate for a single workday. Control workers were recruited from a garlic and pepper processing plant that made preserves and ingredients for other manufacturers, and included indoor and outdoor working areas. Their inclusion criteria were identical to the dairy workers, but also included

no contact with cows or respiratory contaminants, such as cleaning chemicals. Of eligible workers, approximately 90% were consented to the study. The total enrolled was 226 dairy workers and 49 controls, of which 16 dairy and 2 controls had unusable pulmonary function tests and an additional 5 dairy and 2 controls were excluded because of insufficient exposure data. As a result, the final numbers of participants were 205 dairy workers and 45 control workers. Before starting work, participants were interviewed, given a pulmonary function test (spirometry), and fitted with their sampling backpack. After completion of their shift, they returned the backpack, were given a postshift pulmonary function test and reinterviewed.

### Health Assessment

Pre- and postshift questionnaires were used to collect demographic and personal characteristics, health conditions, exposures, and work details. These methods were described previously.<sup>30</sup> Spirometric testing was conducted by National Institute for Occupational Safety and Health certified technicians using EasyOne spirometers (Andover, MA). The following parameters were recorded: forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), ratio of FEV1/FVC, and forced expiratory flow rate between 25% and 75% of FVC (FEF25-75). A pulmonary physician with extensive knowledge and experience with occupational respiratory exposures reviewed any questionable maneuver. The American Thoracic Society recommendation of less than 5% variability between the two best acceptable pulmonary function efforts was used to categorize the validity of individual pre- and postshift spirometry maneuvers. Participants with between 5% and 8% variability were placed in a second category; those with a variability of more than 8% or only one acceptable effort were excluded. Categorization was conducted on FEV1 and FVC separately; when looking at the FEV1/FVC ratio, the component with the most variable category characterized the ratio to determine whether they were included in the analysis. Similarly, when looking at cross-shift changes (postshift value - preshift value), the most variable component defined the category of the cross-shift pulmonary function change.<sup>31</sup> Models were run with and without the more variable categories of pulmonary function. The latter observations were included if they did not alter the model by increasing the P value above 0.05 or changing the value of the estimate by more than 10%.

### Exposure Monitoring

Two samplers were attached to a backpack at shoulder level in the personal breathing zone. An SKC button sampler was used to collect inhalable PM (<100 [ $\mu$ m in aerodynamic diameter) onto a Teflon 25-mm Millipore PTFE filter, with a pore size of 3.0 [ $\mu$ m] (Fisher FSLW02500). A GK2.05SH (KTL) cyclone sampler (BGI Inc., Waltham, MA) was used to collect particles with a cut point of 2.5 [ $\mu$ m] (PM2.5) onto a Teflon filter (Fisher, FHLP03700).<sup>13</sup> A high-flow Leland Legacy personal sampling pump (SKC Inc, Eighty Four, PA) with a noise-dampening cover was attached to the samplers. Both dairy and control workers wore the samplers. They were instructed on breaks to keep the samplers with them and return to the study coordination site if there were any problems. Particle mass was determined by gravimetric analysis using a Cahn 35 microbalance (Thermo Fisher Scientific Inc., Pittsburg, PA).<sup>32</sup>

Samples were analyzed for biologically active endotoxin using the recombinant factor C assay (Lonza Pyrogene, Lonza, Basel, Switzerland), which detects the activation of Factor C by utilizing a fluorogenic substrate. The minimum detection limit was approximately 0.01 EU/mL. “Total” endotoxin was determined as the 3-hydroxy fatty acid (3-OHFA) endotoxin components analyzed using gas chromatography-mass spectroscopy.<sup>33</sup> Both types of endotoxin analyses were conducted in the laboratories of Dr Stephen Reynolds (Colorado State University, Fort Collins, CO). The protocols have been described previously.<sup>34,35</sup> Samples and standard solutions containing carbon chain lengths of C8-C10 and C12-C18 were analyzed at several concentrations between 0 and 500 ng. The limit of detection and the limit of quantification were found to be 0.5 and 1 ng, respectively. Any chain length measurement below the limit of detection was assigned a value of the limit of detection divided by the square root of 2. Results were quantified in picomoles (pmol).

### Exposures Characterized

The following exposure concentrations were investigated in this analysis: (1) inhalable particulates PM (mg/m<sup>3</sup>) with an aerodynamic diameter of 100 [μ]m or less; and (2) fine particulates PM<sub>2.5</sub> ([μ]g/m<sup>3</sup>). “Active” endotoxin in the inhalable range was defined by the rFC analysis in endotoxin units (EU/m<sup>3</sup>) as this represents endotoxin capable of reacting in a biologically active manner. Total endotoxin (calculated by summing the chain lengths) in the inhalable range was defined by gas chromatography-mass spectroscopy analysis in picomoles (pmol/m<sup>3</sup>). Concentrations were log transformed before analysis on the basis of visual and physiological grounds; proportional increases in exposure were associated with a linear change in the pulmonary function outcome.

### Data Analysis

All programming was conducted using SAS 9.3 (Statistical Analysis Systems Institute, Cary, NC).

Preliminary investigation of the data began with examination of univariate distributions of the 250 participants who had sufficient data to be included in the analyses (205 dairy and 45 control workers). Scatter plots of pulmonary function (at baseline and cross-shift changes) were plotted against exposure concentrations and correlations between the variables computed. The dependent variables included FEV<sub>1</sub>, FVC, FEV<sub>1</sub>/FVC, and FEF<sub>25-75</sub>. Both dependent and exposure concentrations were examined for outliers. For dairy and control participants, observations greater than 1.5 times the interquartile range above or below the third and first quartiles of their respective distributions were classed as potential outliers. The comparison of dairy and control workers was age adjusted. General linear models were used to age adjust the results for continuous variables. For categorical variables, logistic regression was used.

Mixed-effects multiple linear regression modeling was used to account for possible clustering by facility. Where baseline (long-term effects) modeling was conducted, covariates included age, height, and smoking status (current vs former/never). Current smoking status was defined as smoking in the last 30 days or on the study day; participants did not have to have smoked 100 or more cigarettes in their life to qualify as a current

smoker. Other covariates tested, but not necessarily kept in the model, included years in agriculture, number of days worked since the last rest day, preshift time (before or after noon), history of personal protective equipment use, and historical proportion of work time in dusty conditions. We investigated whether baseline models were influenced by an abnormal exposure on the day monitored. Workers with abnormal exposures (stated either that they had been working at a job that was unusual and/or their exposure to dust was not normal for the day) were first included and then excluded from the analysis. Covariates considered for cross-shift (acute effects) modeling included the monitoring day's measurements of the following: self-reported time in dust, time at start of shift (AM or PM), work shift length, wearing personal protective equipment, and whether they smoked that day. Covariates were included in multivariate models if they exhibited an association of  $P \leq 0.1$  in the univariate regression.

We also checked whether any observations exerted undue influence on model construction using a conservative criterion: Cooks'  $D \geq 0.6,36$  which none of the observations reached. Covariates remained in the model if they were significant ( $P < 0.05$ ) or if they caused more than 15% change in the value of the estimate of interest. Both exposure to PM and endotoxin were included in the same model only if at least one had demonstrated a significant association with the pulmonary function variable in question. Appropriate interactions (eg, age and exposure) were tested in models having both those main effects and were rejected if the  $P$  value of the interaction was more than 0.05.

## Results

### Demographics

The male participants were young, overwhelmingly immigrants, Latino, and Spanish-speaking (Table 1). Dairy workers tended to be taller, lived longer in the United States, earned more, but were less likely to have completed the sixth grade or be comfortable speaking English compared with the control vegetable processing plant workers. Despite these statistically significant differences, the groups were similar in that the vast majority earned less than \$1500 in a 2-week pay period, and were unlikely to have attended school above the eighth grade (results not shown).

### Exposures

Long hours were common for both dairy and control workers (Table 2). No significant differences between the groups were found in their early life residential status or in likelihood of being a night worker. A higher, but not statistically significant, percentage of dairy workers were classed as current smokers. Significant differences between dairy and control workers were found in the number of years they had spent in agriculture (packing or processing products were not classed as agricultural occupations). The dairy workers were more likely to have worked and/or lived on a farm or dairy. Control workers were more likely to have ever worn respiratory protection or to wear it on the day of monitoring. Both groups reported working a similar portion of the day in dusty conditions. Differences between exposures for dairy and control workers were significant ( $P \leq 0.001$ ) for all measures of exposure (Fig. 1). The geometric mean concentration of PM and

PM<sub>2.5</sub> particles (812 [ $\mu$ g/m<sup>3</sup> and 35 [ $\mu$ g/m<sup>3</sup>, respectively) in the dairy workers was almost double of those experienced by control workers (482 [ $\mu$ g/m<sup>3</sup> and 19.6 [ $\mu$ g/m<sup>3</sup>, respectively). Total inhalable endotoxin concentrations were approximately an order of magnitude higher for the dairy workers compared with the control workers (1122 pmol/m<sup>3</sup> vs 110 pmol/m<sup>3</sup>, respectively) and almost 25 times higher for active endotoxin (329 EU/m<sup>3</sup> vs 13.5 EU/m<sup>3</sup>, respectively).

### Univariate Associations Between Exposure and Cross-Shift Pulmonary Function

Mean cross-shift changes in pulmonary function were plotted against increasing quartile of exposure (Fig. 2) for all workers. As inhalable particles and total endotoxin concentrations increased, FEV<sub>1</sub>, FVC, and FEF<sub>25-75</sub> decreased across the work shift, although in a nonsignificant fashion in these unadjusted models (Fig. 2a and 2c). The FEV<sub>1</sub>/FVC ratio did not vary, largely because FEV<sub>1</sub> and FVC changed in the same direction and by a similar amount.

### Multivariate Associations Between Exposure and Pulmonary Function

Preshift pulmonary function was modeled against exposure to determine whether there were any long-term associations (Table 3). We assumed the current exposure was representative of long-term average exposure where workers indicated they had both conducted normal tasks and thought they were exposed to their “usual” concentration of airborne particles. Thirty-seven people reported (self-assessed) unusual exposure on the day of monitoring. We compared models with and without those who self-reported unusual exposure and found no significant differences; therefore, this group was also included in the long-term modeling. We also explored removing observations with high Cook’s D values more than 4/n or 0.02, but the significance of the models did not change. (The models of the association between the concentration of PM<sub>2.5</sub> and FEV<sub>1</sub>, FEF<sub>25-75</sub>, and FEV<sub>1</sub>/FVC all became closer to significance.)

Most of the models exhibited a nonsignificant inverse trend between the concentration of exposure and the preshift pulmonary function measure. As expected, the age of the participant was also significant and inversely related to the pulmonary function.

The modeled effect of the different exposures on acute (cross-shift) pulmonary function revealed a significant inverse association between total endotoxin exposure and cross-shift changes in FVC (Table 4; P = 0.018). In this model, the hours worked were also significant in an inverse fashion. As with the long-term models, a nonsignificant inverse trend between each exposure and the cross-shift pulmonary function assessment was recorded, except for the ratio FEV<sub>1</sub>/FVC.

## Discussion

In a previous article,<sup>31</sup> we found that dairy, but not control workers, experienced a cross-shift decline in FEV<sub>1</sub>, FEV<sub>6</sub>, and FVC (P = 0.02, 0.002, and 0.002, respectively), but not in the FEV<sub>1</sub>/FVC ratio. Marginally lower long-term FEV<sub>1</sub> and FEV<sub>6</sub> were associated with dairy work (P = 0.07 and 0.053, respectively). We concluded that dairy work was associated with a mixed airways effect across the work shift, but the effect was reversible

with nonsignificant baseline (long-term) effects. This study addresses whether an association exists between particulate exposures and pulmonary function regardless of the class of work. The dairy and control populations were demonstrated to be similar demographically, but not with respect to respiratory exposures. Dairies recorded approximately double particle concentrations and at least an order of magnitude higher endotoxin concentrations than the control facility. Other articles from this study have analyzed both area and personal concentrations of dairy particles within and between dairies, and the differences in endotoxin compositions between dairies.<sup>13,32,37</sup>

### Dairy Worker Exposure

Extensive reports exist of poultry and swine worker exposures to particles and endotoxin.<sup>7,38</sup> Less data on personal exposures have accumulated from cattle or dairy facilities, and consistently lower particulate concentrations have been found compared with other livestock.<sup>11,25,39,40</sup> Historically, dairy production occurred in areas with cooler climates and enclosed barns; workers in Wisconsin dairies sampled in 1992 to 1993 were found to have personal exposure to inhalable particles and endotoxin with geometric means of 1.78 mg/m<sup>3</sup> and 647 EU/m<sup>3</sup>, respectively.<sup>6</sup> More recently, Colorado and Nebraska dairy workers recorded personal PM and endotoxin with geometric means of 2.4 mg/m<sup>3</sup> and 1357 pmol/m<sup>3</sup>, respectively.<sup>12</sup> Danish cattle facilities <sup>6,11</sup> were found to have 1.0 mg/m<sup>3</sup> PM and mean endotoxin concentrations of 358 EU/m<sup>3</sup>, and Dutch dairy farms <sup>40</sup> were found to have mean endotoxin concentrations of 560 EU/m<sup>3</sup>. In the Netherlands, dairy farms that used compost bedding were found to have inhalable mean PM and endotoxin concentrations of 1.38 mg/m<sup>3</sup> and 895 EU/m<sup>3</sup>, respectively, compared with 0.51 mg/m<sup>3</sup> and 183 EU/m<sup>3</sup> on dairy farms that used sawdust bedding.<sup>3</sup> The California facilities studied here had particulate concentrations at the lower end of these ranges-with geometric means of 0.81 mg/m<sup>3</sup> for inhalable particles, 1122 pmol/m<sup>3</sup> for total endotoxin, and 329 EU/m<sup>3</sup> for active endotoxin. Nevertheless, these concentrations (Fig. 1) were still much higher than exposures in the vegetable processing (the control) facility that had both open air and closed areas but employed workers with similar demographics.

The Occupational Safety and Health Administration has designated the maximal allowed exposure of workers to total particles averaged over an 8-hour workday as 15 mg/m<sup>3</sup> for inhalable particles and 5 mg/m<sup>3</sup> for respirable particles.<sup>41</sup> Agricultural particles are likely of higher toxicity than general dust. Other studies of livestock emissions have determined that concentrations above approximately 2.5 mg/m<sup>3</sup> for total particulates or 614 EU/m<sup>3</sup> endotoxin (depending on the type of livestock) are likely to produce occupational respiratory problems.<sup>24,25</sup> The dairy concentrations found here were lower than both the permissible exposure limits and the suggested thresholds, although higher than the 90 EU/m<sup>3</sup> estimated as a threshold in sensitized workers who carded cotton.<sup>23</sup>

Although PM and endotoxin have been associated with decrements in lung function across shift and over the long-term, a dose-response curve has been difficult to establish (different species of livestock, variable bacterial populations, management practices, differing methodology and sensitivities for assessing endotoxin, and a possible threshold level).<sup>42</sup> Donham et al <sup>25</sup> found that swine workers exhibited a strong decrement in

pulmonary function (FEV1) across shift after 6 years of work exposure. Workers with less than 6 years did not seem to suffer consistent decrements across their work shift. For a 3% pulmonary function decrement across shift, the mean total dust concentration in the swine barns was 0.1 mg/m<sup>3</sup>, and for a 10% pulmonary function decrement, 1.3 mg/m<sup>3</sup> in smokers and 2.8 mg/m<sup>3</sup> in nonsmokers. In this study, only approximately 50% of workers on dairies had worked more than 5 years in any dairy setting. Confining the analyses to those participants did not provide significant statistical evidence for a dose-response curve (n = 102 workers; data not shown).

We looked for both short and long-term decrements in pulmonary function associated with respiratory exposures as it is expected that repeated short-term, reversible cross-shift decrements may accelerate the age-related decline in pulmonary function.<sup>43,44</sup>

### Acute Effects of Particulate Matter on Pulmonary Function

A consistent inverse trend, which did not reach statistical significance, was found between cross-shift FVC and the concentrations of inhalable particulates, PM<sub>2.5</sub>, and biologically active endotoxin. Similarly, FEV1 trended lower in a nonsignificant manner for each of the measured pollutants. The only statistically significant association between air contaminant concentrations and cross-shift changes in pulmonary function was between the total endotoxin concentration and FVC (pmol/m<sup>3</sup>; P = 0.018; Table 4). Eastman et al<sup>31</sup> found a negative association between either cross-shift FEV1 or FVC and dairy work. This analysis indicates that the association between dairy workers and cross-shift FEV1 could not be attributed to their exposure to the air pollutants measured. The association between cross-shift FVC and dairy work could partly be explained by the respiratory exposures measured. In this analysis model, if the total endotoxin concentration increased from the first to third quartiles (from 884 to 3539 pmol/m<sup>3</sup>), cross-shift FVC would decrease by approximately 37 mL. If the work hours increased from the first to third quartiles (8.3 to 10 hours), cross-shift FVC would independently decrease by 38.5 mL, a roughly equal effect. This may indicate some other unmeasured factor added to the effect of endotoxin. We did not measure bacterial counts/gram-positive bacteria (muramic acid) or other compounds with possible links to decreased pulmonary function, such as the [beta]-1,3-glucans (from cell walls of fungi).<sup>6,8,33</sup> We did not see a similar association with the active endotoxin concentrations, perhaps because this method does not assess endotoxin within cell walls, which could be involved in respiratory reactions.<sup>42</sup>

Adding total inhalable particles to the model with total endotoxin did not demonstrate a further significant association with the inhalable particles. Total inhalable particles and total endotoxin were correlated (r = 0.6). Many previous studies have found an acute association between endotoxin and FEV1 and/or FEF<sub>25-75</sub>, but no association with FVC. However, a significant reduction in cross-shift FVC with a nonsignificant reduction in FEV1 is not unique; it has been found in grape workers and other agricultural laborers in California.<sup>45,46</sup> In those studies, the predominant exposures were from crops, and the endotoxin exposures would be expected to be very different than at a dairy.

Some studies of the association of endotoxin with pulmonary function have proposed a threshold level below which acute effects have not been seen. A cross-shift study of 257



poultry workers and 150 nonexposed controls 24 divided exposures by quartiles and looked at the percentages of workers experiencing cross-shift FEV1 decrements of 0%, 3%, or 5%. They found a dose response with endotoxin and total (PM) or respirable (PM2.5) dust. The threshold level started with the third quartile of exposure from 614 to 1505 EU/m<sup>3</sup>, with an odds ratio 4.9 (2.1 to 11.3) being predictive of 3% or greater cross-shift decline in FEV1. The median of 614 EU/m<sup>3</sup> was set as the lowest level an effect could be seen, hence declared a threshold. In the California dairies studied here, both general particulate and endotoxin concentrations were much lower than in the majority of earlier studies of dairy facilities; even the 75th percentile of endotoxin distribution in the California dairies did not reach the 614 EU/m<sup>3</sup> threshold concentration. We believe the cross-shift decrease in FVC and nonsignificant decrease in FEV1 and FEF<sub>25-75</sub> indicate a mild mixed effect of acute restriction and trending to mild acute obstruction similar to industrial bronchitis through exposure to irritating air contaminants.<sup>31</sup>

### **Long-Term Effects of Particulate Exposure on Pulmonary Function**

Because of the acute association of the concentration of total endotoxin and FVC, we might expect to see a similar long-term association. No association was found, although a decline in FVC has been associated longitudinally with dairy work in French dairy farmers.<sup>22</sup> Because of the high turnover of dairy workers (the median length of dairy work was 5 years or less), it would be difficult to view a long-term association unless a very large population was followed up for at least 10 years. Possible long-term marginal associations between the log [PM<sub>2.5</sub>] and FEV1, or FEF<sub>25-75</sub> were noticed, but may be due to chance, as the significance did not meet the stated criterion. Another article from this same study <sup>37</sup> determined that PM<sub>2.5</sub> concentrations on these Californian dairies had a large contribution from regional sources. PM<sub>2.5</sub> is generated on these dairies, but the (downwind - upwind) concentrations are negligibly different, although sources are somewhat dispersed before the downwind measurement. The dairy-generated PM<sub>2.5</sub> may have a different composition than the regional PM<sub>2.5</sub>. At present we have not assessed the endotoxin content of the PM<sub>2.5</sub>. Therefore, we are unable to state whether the model, which was marginally significant, indicated a possible association between the pulmonary decrement and PM<sub>2.5</sub> or specifically the endotoxin content of the PM<sub>2.5</sub>. Consequently, any long-term association with PM<sub>2.5</sub> may be attributed to living in a region of low humidity, hot summers, and high dust loads <sup>47</sup> rather than specifically with working on a dairy.

### **Use of Respiratory Protection**

At baseline, respiratory protection was positively associated with higher FEV1 and FVC irrespective of the exposure. P values for ever wearing respiratory protection ranged from 0.014 to 0.039, depending on the model (not shown). Nevertheless, only 13.3% of dairy workers ever wore respiratory protection (approximately half the percentage reported by the control workers) despite being exposed to higher concentrations of environmental particles.

### **LIMITATIONS AND STRENGTHS**

A significant association was revealed between the total concentration of endotoxin and cross-shift FVC, and a weaker, non-significant association was noted for the active

endotoxin. We were limited in determining the strength of these associations by sample size. Similarly, as they were not measured, we were unable to assess the personal concentration of other air contaminants, which may contribute to the models. We assumed the chemical composition of the particles and endotoxins is similar in toxicity between the dairies and the control facility. This is unlikely because animal and plant-based endotoxin differ somewhat. The control endotoxin concentrations were 10 to 25 times lower than the mean for the dairies, so the potential difference in toxicity may not have significantly affected the models. For the long-term models, we assumed the current exposure represented the long-term exposure because we conducted a sensitivity analysis, removing and adding back those with self-reported abnormal exposures, without a change in the significance of the models. Self-reported exposure assessment of inhalable particles has been found reasonably reliable at the population level but not so reliable at the level of the individual.<sup>48</sup> Nevertheless, the worker would not be able to assess endotoxin content of their subjective exposure estimate. On smaller dairies, where workers are less specialized, individual workers experience large variations in day-to-day exposures.<sup>3,11</sup> Although all of the workers in this study worked on large dairies and usually had more specialized jobs, it is reasonable to assume their day-to-day exposures would also vary because of weather conditions and other facility conditions such as shift changes. Such self-assessment is, therefore, likely to result in a random loss of precision between long-term exposure and response associations and bias estimates toward the null. Worker turnover at the dairies was found to be sufficiently high that if a minimum of 5 to 6 years is needed to observe long-term effects associated with exposures, we would need a much larger population or a longitudinal study. Our results may be an underestimate because of a healthy worker effect; those experiencing troubling respiratory symptoms are likely to leave dairy work, thus artificially reducing the true effect of air contaminants on long-term respiratory health.

The study strengths include the use of a random selection of dairies, with high recruitment rates at each facility.<sup>30</sup> Dairies and the control were measured in the same 3-month period, avoiding gross temporal changes that might occur between season and year. Both control and dairy workers were similar demographically and had a similar mix of night and day work shifts. Unlike many other studies, personal exposure was assessed across the total work shift, giving a more accurate daily exposure estimation. Finally, we used a protocol that assessed personal exposure to inhalable particulates, PM<sub>2.5</sub>, and both total and active endotoxin.

## CONCLUSIONS

Workers on large California dairies are exposed to lower concentrations of particulates than historically found on smaller, conventional, and colder climate dairies. The lower concentrations may result from open, ventilated freestall structures that allow more modern management practices. Nevertheless, workers on dairies in this study were still exposed to much higher concentrations than those in a vegetable processing factory. Across the course of a work shift, this study found a mild acute restriction associated with exposure to the total endotoxin concentration. This association was reversible; we did not observe long-term statistically significant decrements in pulmonary function. The concentration of total endotoxin, not the active (rFC) fraction, was significantly associated with the cross-shift

decrement. The cross-shift decrease in FVC was also associated with total length of the work shift, indicating the presence of some other factor that was not measured (possibly other air contaminants, such as gram-positive bacteria, molds, or fungi). We found an independent protective effect on workers' FEV1 or FVC in long-term models if workers had ever used respiratory protection. Nevertheless, the very low rate of protective equipment use (13.3% ever wore protection) in dairy workers should be a cause of concern as their exposures to particles were approximately double that of control workers and an order of magnitude or more when compared with their endotoxin levels.

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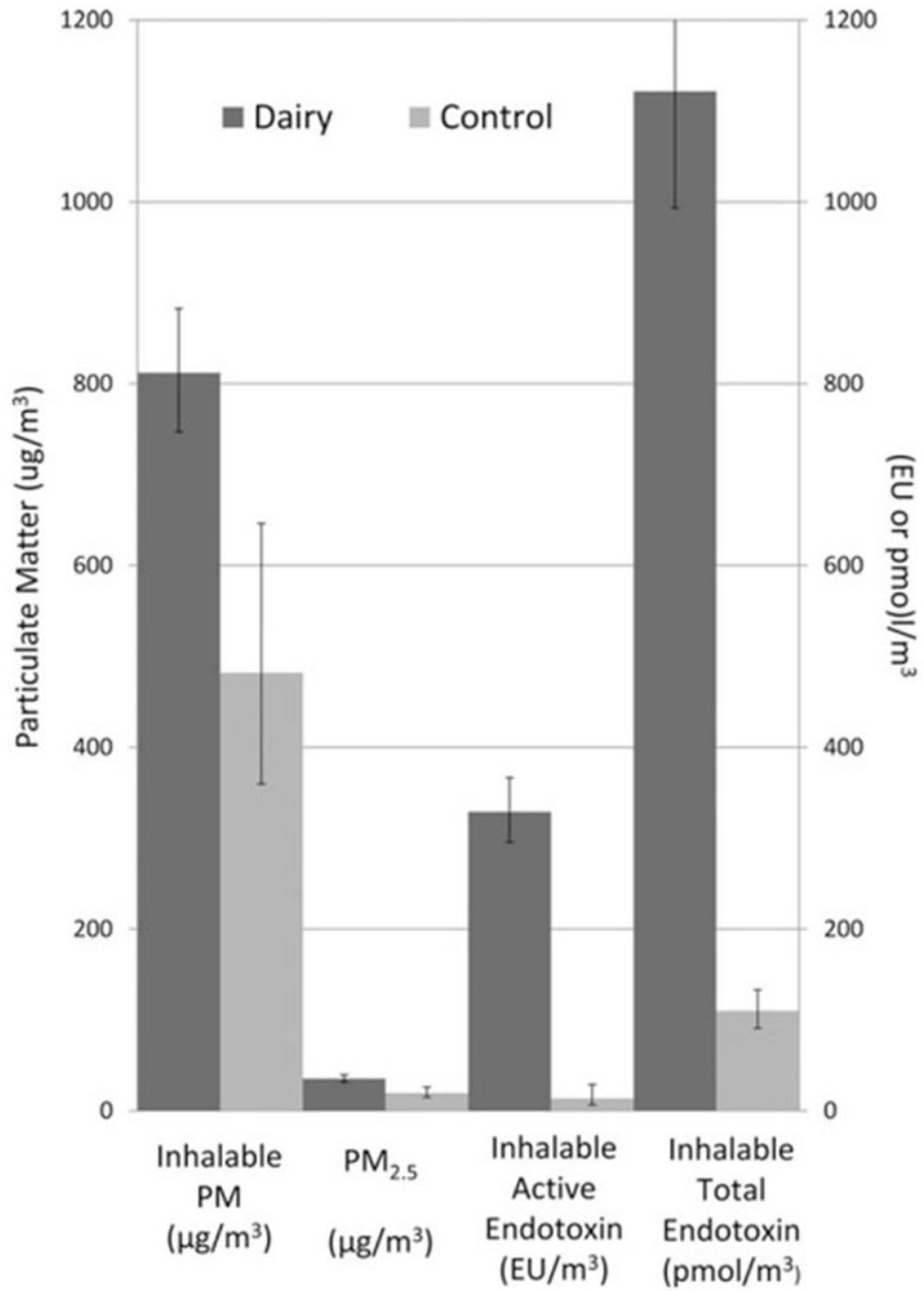
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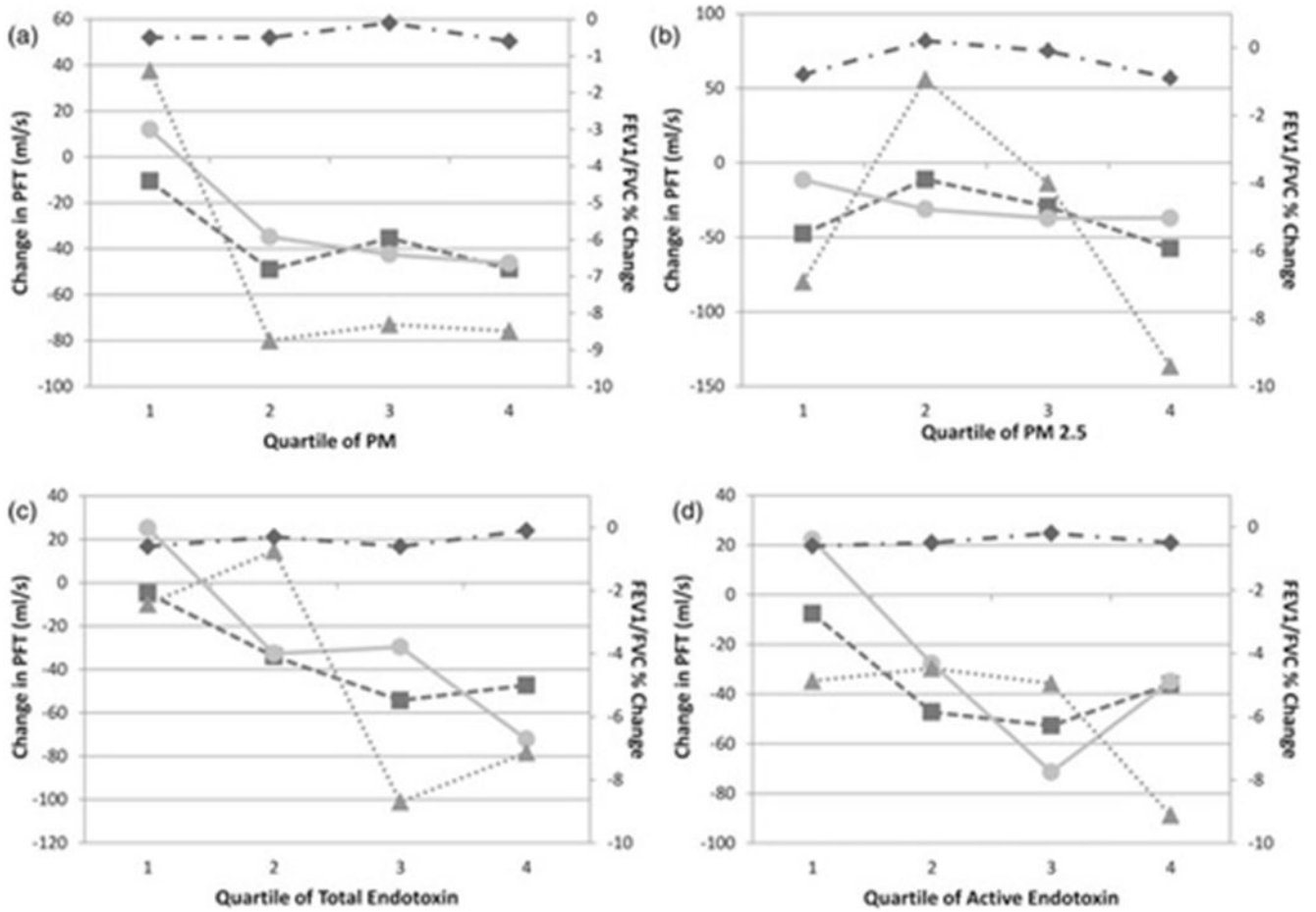
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**FIGURE 1.** Mean geometric exposures in dairy and control facilities. Mean particulate concentrations and 95% confidence intervals.



**FIGURE 2.** Cross-shift changes in pulmonary function by quartile of exposure concentration. 2a: Inhalable PM/m<sup>3</sup>, 2b: PM<sub>2.5</sub>/m<sup>3</sup>, 2c: Total Endotoxin (pmol/m<sup>3</sup>), 2d: Active Endotoxin (EU/m<sup>3</sup>).

TABLE 1.

## Demographics of Participants With Usable PFT Results

Characteristic	All Subjects, <i>n</i> = 250 Mean (SE) [Median]	Dairy, <i>n</i> = 205 Mean (SE)	Control, <i>n</i> = 45 Mean (SE)	<i>P</i> Value
Age, yrs	33.7 (0.7) [31.5]	33.4 (0.8)	35.1 (1.8)	0.340
Following comparisons age adjusted: least square means and standard errors reported				
Height, cm	168.4 (0.4) [168]	169.3 (0.4)	164.3 (0.9)	<0.0001
BMI	27.7 (0.3) [27.4]	27.9 (0.3)	26.9 (0.6)	0.224
Years in the United States <sup>†</sup>	11.5 (0.6) [10.0]	12.2 (0.4)	8.6 (0.9)	0.005
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	
Latino	226 (90.4)	184 (90.6)	42 (89.4)	0.076
Non-Latino <sup>‡</sup>	13 (5.2)	12 (5.9)	1 (2.1)	
Unknown	11 (4.4)	7 (3.5)	4 (8.5)	
Interviewed in Spanish	240 (96.0)	199 (98.0)	41 (87.2)	0.004
Income (biweekly)				<0.0001
<\$500	3 (1.2)	2 (1.0)	1 (2.1)	
\$501–\$750	25 (10.4)	6 (3.1)	19 (40.4)	
\$751–\$1000	103 (42.7)	84 (43.3)	19 (40.4)	
\$1001–\$1500	95 (39.4)	91 (46.9)	4 (8.5)	
>\$1501	15 (6.2)	11 (5.7)	4 (8.5)	
Education				0.034
Sixth grade or less	132 (52.8)	112 (55.2)	20 (42.6)	
More than sixth grade	118 (47.2)	91 (44.8)	27 (57.5)	

\* Except for age, all variables were age adjusted using least square means and standard errors reported.

<sup>†</sup> Six participants were born in the United States.

<sup>‡</sup> Remainders were Portuguese or white, non-Latino.

BMI, body mass index; PFT, pulmonary function test.



**TABLE 2.**

## Work and Historical/Residential Exposures

Exposure	All Subjects, <i>n</i> = 250 Mean (SD)	Dairy, <i>n</i> = 205 Mean (SD)	Control, <i>n</i> = 45 Mean (SD)	<i>P</i> Value
Hours worked	9.2 (1.2)	9.3 (0.08)	9.0 (0.17)	0.126
Hours in dust	2.9 (3.7)	3.1 (0.3)	2.0 (0.5)	0.118
Pack-years (current and ex-smokers only)	6.2 (10.7)	6.4 (1.1)	5.1 (2.6)	0.887
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	
Smoking status				0.056
Never	139 (55.6)	108 (53.2)	31 (66.0)	
Ex	50 (20.0)	40 (19.7)	10 (21.3)	
Current	61 (24.4)	55 (27.1)	6 (12.8)	
First day back from break	26 (10.4)	26 (12.8)	0 (0.0)	0.068
Ever wear respirator or mask	41 (16.4)	28 (13.8)	13 (27.7)	0.028
Wore respirator or mask that day	10 (4.0)	3 (1.5)	7 (14.9)	0.0006
Late/night shift	76 (30.4)	66 (32.5)	10 (21.3)	0.158
Years in agriculture				<0.0001
0–10	99 (39.9)	57 (28.4)	42 (89.4)	
11–20	70 (28.2)	66 (32.8)	4 (8.5)	
> 20	79 (31.9)	78 (38.8)	1 (2.1)	
Early life exposure				0.741
Farm/ranch/rural	113 (45.4)	99 (49.0)	14 (29.8)	
Small town	93 (37.4)	73 (36.1)	20 (42.6)	
City: inner or suburb	43 (17.3)	30 (14.9)	13 (27.7)	
Currently live on a farm or dairy	51 (20.4)	49 (24.1)	2 (4.3)	0.007

\* All variables were adjusted for age. Hours in dust were self-reported. Work in agriculture could include livestock or crop cultivation, but did not include packing of products.

SD, standard deviation.

**TABLE 3.**

## Long-Term Exposure Associated With Baseline Pulmonary Function

Pulmonary Function	PM <sub>2.5</sub>		Inhalable PM		Active Endotoxin		Total Endotoxin	
	Estimate (95% CI)	P Value	Estimate (95% CI)	P Value	Estimate (95% CI)	P Value	Estimate (95% CI)	P Value
FEV <sub>1</sub> , mL/s	-48.9 (-116.3 to 8.8)	0.115	-23.7 (-96.7 to 49.3)	0.523	-12.0 (-42.1 to 18.1)	0.434	-15.4 (-58.6 to 27.8)	0.453
FEV <sub>1</sub> /FVC, %	-0.72 (-1.76 to 0.32)	0.174	-0.47 (-1.5 to 0.51)	0.343	-0.12 (-0.43 to 0.67)	0.661	-0.47 (-1.29 to 0.36)	0.264
FVC, mL	-24.1 (-110.4 to 62.1)	0.579	-6.35 (-89.4 to 47.6)	0.896	-8.4 (-54.4 to 37.6)	0.711	18.4 (-48.9 to 85.7)	0.577
FEF <sub>25-75</sub> , mL/s	-154.7 (-338.8 to 129.3)	0.098	-43.8 (-2250.1 to 162.6)	0.676	-5.0 (-101.2 to 91.2)	0.917	-47.8 (-192.6 to 97.0)	0.505

\* All mixed effect models were adjusted for age, height, smoking status, and whether they ever used respiratory protection.

CI, confidence interval, FEF<sub>25-75</sub>, forced respiratory flow rate between 25% and 75% of FVC; FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced vital capacity; PM, particulate matter.

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**TABLE 4.**

## Acute Exposure Associated With Cross-Shift Pulmonary Function

Pulmonary Function	PM <sub>2.5</sub>		Inhalable PM		Active Endotoxin		Total Endotoxin	
	Estimate (95% CI)	P Value	Estimate (95% CI)	P Value	Estimate (95% CI)	P Value	Estimate (95% CI)	P value
FEV <sub>1</sub> , mL/s	-0.05 (-27.76 to 27.66)	0.997	-8.1 (-40.06 to 23.79)	0.620	-6.24 (-18.34 to 5.87)	0.311	-10.55 (-28.46 to 7.35)	0.247
FEV <sub>1</sub> /FVC, %	-0.004 (-0.38 to 0.37)	0.983	-0.015 (-0.41 to 0.44)	0.946	0.039 (-0.11 to 0.36)	0.638	0.125 (-0.13 to 0.20)	0.301
FVC, mL	-6.78 (-39.67 to 26.11)	0.685	-13.71 (-51.73 to 24.31)	0.231	-11.97 (-26.06 to 2.11)	0.096	-24.46 (-44.65 to -4.27)	0.018
							Hours worked	0.042
							-22.0 (-43.24 to -0.075)	
FEF <sub>25-75</sub> , mL/s	6.08 (-73.6 to 85.8)	0.881	-42.03 (-132.1 to 48.1)	0.359	-18.39 (-51.4 to 17.0)	0.286	-29.13 (-78.6 to 20.3)	0.247

\* Mixed models for FEV<sub>1</sub>/FVC and FEF<sub>25-75</sub> were adjusted for age and shift time (morning/afternoon). FEV<sub>1</sub> models were adjusted for the average number of hours worked per day where dust is visible in the air. Models for FVC were adjusted for hours worked.

CI, confidence interval; FEF<sub>25-75</sub>, forced respiratory flow rate between 25% and 75% of FVC; FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced vital capacity; PM, particulate matter.