Pulmonary inflammation and crystalline silica in respirable coal mine dust: dose-response

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This study describes the quantitative relationships between early pulmonary responses and the estimated lung-burden or cumulative exposure of respirable-quartz or coal mine dust. Data from a previous bronchoalveolar lavage (BAL) study in coal miners ($n = 20$) and nonminers ($n = 16$) were used including cell counts of alveolar macrophages (AMs) and polymorphonuclear leukocytes (PMNs), and the antioxidant superoxide dismutase (SOD) levels. Miners' individual working lifetime particulate exposures were estimated from work histories and mine air sampling data, and quartz lung-burdens were estimated using a lung dosimetry model. Results show that quartz, as either cumulative exposure or estimated lung-burden, was a highly statistically significant predictor of PMN response ($P < 0{\cdot}0001$); however cumulative coal dust exposure did not significantly add to the prediction of PMNs ($P = 0{\cdot}2$) above that predicted by cumulative quartz exposure ($P < 0{\cdot}0001$). Despite the small study size, radiographic category was also significantly related to increasing levels of both PMNs and quartz lung burden (P-values $< 0{\cdot}04$). SOD in BAL fluid rose linearly with quartz lung burden ($P < 0{\cdot}01$), but AM count in BAL fluid did not ($P > 0{\cdot}4$). This study demonstrates dose-response relationships between respirable crystalline silica in coal mine dust and pulmonary inflammation, antioxidant production, and radiographic small opacities.

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1. Introduction

Understanding early pulmonary responses to inhaled particulates may lead to prevention and intervention approaches to help eliminate or mitigate the development of irreversible occupational lung diseases. Several studies have established quantitative relationships between cumulative exposure to respirable coal mine dust and coal workers' pneumoconiosis (CWP) (Hurley and Maclaren 1987; Attfield and Seixas 1995; Kuempel *et al* 1997), crystalline silica and silicosis (Hnizdo and Sluis-Cremer 1993; Steenland and Brown 1995), and crystalline silica and lung cancer (Checkoway *et al* 1997; Rice *et al* 2001). A number of experimental and *in vitro* studies have also described the cellular mediators and molecular events involved in the development of particle-associated fibrosis and lung cancer (Mossman and Churg 1998; Schins and Borm 1999; Castranova 2000). These events, which include oxidative stress, pulmonary inflammation, and upregulation of cytokines and growth factors, have

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Abbreviations used: AM, alveolar macrophage; BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary diseases; CWP, coal workers pneumoconiosis; PMN, polymorphonuclear leukocyte; SOD, superoxide dismutase; WLL, whole lung lavage.

been observed in coal miners and other workers in dusty jobs who have developed pulmonary fibrosis (e.g. CWP, silicosis, asbestosis) (Castranova and Vallyathan 2000; Lapp and Castranova 1993; Rom 1991; Wallaert *et al* 1990), as well as chronic obstructive pulmonary diseases (COPD) (Repine *et al* 1997; Linden *et al* 1993). Other studies have reported changes in blood antioxidant status in persons with occupational respiratory diseases, including elevated antioxidant enzymes [catalase, superoxide dismutase (SOD), and glutathione peroxidase] in the erythrocytes of coal miners with CWP or COPD (Schins *et al* 1997); elevated catalase in miners with higher dust exposures and more severe CWP (Nadif *et al* 1998); and decreased glutathione in miners with simple CWP compared to those without evidence of disease (Engelen *et al* 1990). Those changes may reflect a loss of the body's ability to maintain a homeostatic balance between oxidant production and antioxidant defenses in the lungs.

In this study, we examine the quantitative relationships between occupational exposure to airborne particulates and early pulmonary responses. We used bronchoalveolar lavage (BAL) data from a previous study in nonsmoking coal miners and nonminers (Vallyathan *et al* 2000). We also calculated the miners' individual working lifetime exposures to respirable crystalline silica (as quartz) and coal mine dust, and we used the exposure data to estimate internal quartz dose (i.e. lung burden). The responses evaluated in this study include polymorphonuclear leukocyte (PMN) and alveolar macrophage (AM) cell counts and SOD antioxidant levels in BAL fluid, as well as chest radiographic category of small opacities. Pulmonary inflammation is characterized by the recruitment of PMNs in the lungs (Castranova 2000). Particles are cleared from the gas-exchange region of the lungs by AMs; but AMs also release inflammatory mediators and growth factors involved in the development of pulmonary fibrosis (which may be detected on a chest radiograph). SOD and other antioxidants are produced as a protective response to oxidants generated by inflammatory cells and on particle surfaces. It is uncommon to have detailed exposure and lung response data, such as these, in humans, and examining these dose-response relationships may help us to better understand the particle-elicited disease mechanisms, especially at the relatively low doses and early responses in this study.

2. Methods

2.1 *Description of data*

The BAL data used in the present study was previously described (Lapp *et al* 1991; Wilt *et al* 1996; Weber *et al* 1996; Vallyathan *et al* 2000). Data from the latter, the most complete study, were used in the present study. The BAL procedures and complete medical examinations were performed from 1991–1993. All participants were nonsmokers at the time of the study, and all but one were lifelong nonsmokers. One miner was an ex-smoker, having quit cigarette smoking seven years prior to the medical examination. Of the 20 miners in this study, six had early radiographical changes (category 0/1 or 1/0) which are considered suspect pneumoconiosis (ILO 1980), and two had developed simple CWP category 2/2. One of these miners had no radiograph data, and two miners had no PMN data. One miner included by Vallyathan *et al* (2000) was excluded because data were not available for any of the responses evaluated in this study. Among the nonminer controls, all had normal chest radiographs (category 0/0). Two controls included by Vallyathan *et al* (2000) were excluded here because one individual's data were no longer available and the other was a repeated BAL procedure of the same individual. Two additional controls in the earlier study were omitted *a priori* because of recorded occupational exposure to second-hand smoke (although their omission was subsequently evaluated). Of the 16 controls, SOD data were missing for one person, and PMN data were missing for four.

The BAL cell counts and SOD levels reported previously (Vallyathan *et al* 2000) were converted to values per whole lungs for this study. The cell count data in Vallyathan *et al* (2000) were reported as cells per ml, but the units should have been cells per subject (personal communication with V Castranova). Because the cells had been centrifuged and resuspended prior to counting, we adjusted the cell counts by the fraction of BAL fluid recovered in order to estimate the cell concentration in the original BAL fluid (assuming the cell concentrations in the recovered and unrecovered BAL fluid were equivalent; this adjustment was subsequently evaluated). A conversion factor was applied to account for the volume of the right, middle lobe in which BAL was performed relative to the whole lungs (estimated conversion factor: left lung approximately 45% and right lung 55% of whole lung volume; right middle lobe estimated as 25% of right lung from two-dimensional lung tracings provided by V Vallyathan; thus, right middle lobe assumed to be 14% or 1/7th of whole lungs). Since the SOD concentration was measured directly in the original BAL fluid, no adjustment for fraction recovered was necessary. The original SOD data (ng/ml) were thus multiplied by 200 ml (the instilled BAL fluid volume) to estimate total SOD in the right middle lobe, and by 7 to estimate total SOD in the whole lung (assuming equivalent concentration throughout the lung). The form of SOD measured was likely manganese-SOD (mitochondrial) and/or extracellular SOD since these forms are upregulated in response to inhaled particles and inflammation, while

copper-zinc-SOD (cytosolic) is constitutively expressed (Vallyathan *et al* 2000).

2.2 *Exposure estimates*

The metrics of external exposure or internal dose evaluated in this study include duration of work in mining, average quartz concentration, cumulative exposure to quartz and/or coal dust, and quartz lung burden. Miners' individual working lifetime exposures were estimated from their reported work histories and mine air sampling data, as time-weighted average daily concentrations (MSHA 2001). The work histories appeared to be complete, including mining and nonmining job titles by year, presumably from the time of beginning work. For each miner, the airborne concentration data were combined with the job title data to provide yearly average exposures to respirable coal mine dust and respirable crystalline-silica dust. Cumulative exposure was computed as the sum of the products of airborne concentration (mg/m^3) and duration (year) for all mining jobs. Most miners were working at the time of medical examination; four miners had left mining 0·5–3 years earlier (including the two miners with CWP 2/2, one with ILO category 0/1, and one with 1/0).

The dust concentration data were collected by mine operators and processed by the Mine Safety and Health Administration. For respirable coal mine dust, personal data on airborne TWA concentration (identified by social security number) were available from 1970 through 1980 and were used as the primary data source (MSHA 2001). In the absence of personal exposure data, grouped means by job code, year, mine, and region were used. The most specific group mean available was used; however, if the data were missing or unreliable (i.e. too few samples), broader group means were used, or data from jobs with similar exposure patterns were substituted. When more than one job was listed per year, these values were averaged; also, the exposure estimates for the first and last year jobs were each halved (to add up to the total number of years worked). Miners' individual exposures to respirable crystalline silica were estimated using national job-specific quartz percentages and the concentration data for the respirable coal mine dust, derived as described above. Quartz was the primary form of crystalline silica to which the coal miners were exposed (MSHA 2001). Coal mine dust and quartz exposures in controls were assumed to be zero.

The retained dose of quartz in the lungs (i.e. lung burden) was estimated using a human lung dosimetry model, which has been described in detail previously (Tran and Buchanan 2000; Kuempel *et al* 2001a,b). Briefly, each miner's individual exposure data (mean airborne quartz concentration each year) were input into the mathematical model, which was implemented in MATLAB (2001, Version 6.1 Release 12.1, The Mathworks, Natick, MA, USA); geometric mean parameter values for alveolar macrophage-mediated quartz clearance and particle transfer to lymph nodes were used (derived from data in UK coal miners exposed to quartz, in Tran and Buchanan 2000) and the model output was the estimated quartz lung burden for each miner at the time of medical examination. To evaluate the model predictions, limited quartz lung burden data were available, which consisted of the quartz mass recovered from the lungs of two individuals who volunteered for whole lung lavage (WLL) subsequent to the BAL procedure (Wilt *et al* 1996).

2.3 *Statistical analyses*

The statistical models used to evaluate these data include linear regression (responses: PMNs, AMs, or SOD); proportional odds logistic regression (response: radiographic category); and cubic B spline (response: PMNs). SAS (2000, Release 8.1 for Windows, SAS Institute Cary, NC, USA) and S-PLUS (1999, S-PLUS 2000, Professional Release 1, Mathsoft, Seattle, Washington, USA) software were used for these analyses. The exposure, or dose metrics (described above), and age at medical examination were evaluated as predictors of the pulmonary responses. PMN, AMs, and SOD were used as either early pulmonary responses or as predictors of radiographic small opacities. The statistical significance of covariates was assessed using likelihood ratio tests.

3. Results

The mean age of miners was 42 years (± 8, standard deviation) and 39 years (± 7) for controls. Among miners, the mean years worked in mining was 16·7 (± 4·8); and the mean concentrations of respirable coal mine dust and respirable crystalline silica (quartz) were 1·1 (± 0·5) mg/m^3 and 0·046 (± 0·029) mg/m^3, respectively. All but one miner had worked in mining only since the enactment of the current US standard for respirable coal mine dust (2 mg/m^3 in 1972; interim standard of 3 mg/m^3 in 1969) (PL 91–173; 30 USC 801–962). The quartz lung burden predicted by the lung dosimetry model for the two individuals with WLL data were consistent with the observed values: 0·46 observed vs 0·66 predicted (using the geometric mean parameter values; or 0·11–0·67 using the 5th and 95th percentiles of the distribution of individual alveolar clearance rate coefficients, which was highly skewed toward slow clearance; Tran and Buchanan 2000); and 0·22 observed vs 0·53 predicted (0·096–0·54, 5th and 95th percentiles).

Results of cumulative exposure to both respirable coal mine dust and quartz were statistically significant pre-

dictors of PMN response when modelled separately; however, in a linear regression model with both covariates, quartz was a very strong predictor, while coal dust no longer contributed to the prediction of PMN count in BAL fluid (table 1). Because of the high correlation between cumulative exposure to coal and quartz (Pearson correlation coefficient: 0·91), the separate effects of these two predictors could not be clearly distinguished. However, quartz had a much greater potency than coal, given that the coefficient for quartz cumulative exposure was more than 20-times larger than the coefficient for coal dust cumulative exposure.

Figure 1 illustrates a statistically significant, nonlinear relationship between estimated quartz lung burden and PMN response in BAL fluid ($R^2 = 0{\cdot}97$; $P < 0{\cdot}0001$). A nearly equivalent model fit was seen with cumulative quartz exposure ($R^2 = 0{\cdot}98$; $P < 0{\cdot}0001$), although the curve was slightly more linear than for lung burden (not shown). In spline models with either predictor variable, the nonlinear and linear components were both statistically significant. As seen in figure 1, the nonlinear dose-response curve is largely determined by the relatively high PMN counts in the two individuals with CWP category 2/2. To evaluate the influence of the two miners with CWP 2/2, we removed them from the data and re-ran the regression models. A statistically significant linear trend ($P = 0{\cdot}01$) was still observed between the estimated quartz lung-burden and the PMN count in BAL fluid when the two miners with CWP 2/2 were omitted from the analysis (figure 2).

PMN count in BAL fluid was a significant predictor of SOD level in BAL fluid ($P = 0{\cdot}0001$; $R^2 = 0{\cdot}43$) (figure 4), and vice versa (table 1), which indicates an association between neutrophilic inflammation and antioxidant production. Estimated quartz lung-burden was a predictor of SOD level ($P < 0{\cdot}01$), but only if miners with CWP 2/2 were included. Estimated quartz lung-burden was a highly statistically significant predictor of radiographic category ($P < 0{\cdot}0001$, table 1), even if the two miners with CWP 2/2 were removed ($P < 0{\cdot}0003$). Both PMN count and estimated quartz lung-burden were predictive of radiographic category in a model with both covariates (table 1). SOD alone was a significant predictor of radiographic category, but not when PMN count was included as a covariate (table 1). In contrast to the strong dose-response relationships seen with PMNs, AM count in BAL fluid was not associated with quartz lung burden ($P > 0{\cdot}4$) (figure 3), nor to PMN count or radiographic category (table 1).

Age at medical examination was a statistically significant predictor of all responses when it was the only predictor variable in the linear regression models (*P*-values

Table 1. Fit of statistical models[a] for either PMN count in BAL fluid or radiographic category of small opacities, using various predictors.

	Response variable		
	PMN count in BAL fluid[b]		Radiographic category[d]
Predictor variable(s)[b]	R^2	*P*-value[c]	*P*-value
Duration exposure (years)	0·72	0·01	0·05
Mean quartz concentration (mg/m^3)		0·0001	0·0002
Cumulative exposure (mg/m^3 × year):			
Coal	0·88	0·2	0·6
Quartz		< 0·0001	0·0003
Quartz lung-burden, estimated (g)	0·79	< 0·0001	< 0·0001
SOD (μg/lungs)	0·41	0·001	0·003
AM count (× 10^6/lungs)	0·003	0·8	0·09
PMN count (× 10^6/lungs)	NA	NA	< 0·0001
PMN count (× 10^6/lungs)	NA	NA	0·04
Quartz lung-burden, estimated (g)			0·04
PMN count (× 10^6/lungs)	NA	NA	0·002
SOD (μg/lungs)			0·8

[a]Linear regression (PMN count) and logistic regression (radiographic category).
[b]SOD, AMs, and PMNs were measured in BAL fluid and estimated for whole lungs (see § 2).
[c]All *P*-values are based on likelihood ratio tests.
[d]Includes ILO (1980) categories 0/0, 0/1, 1/0, and 2/2.
NA, Not applicable (models with PMN count as predictor not used with same variable as response).

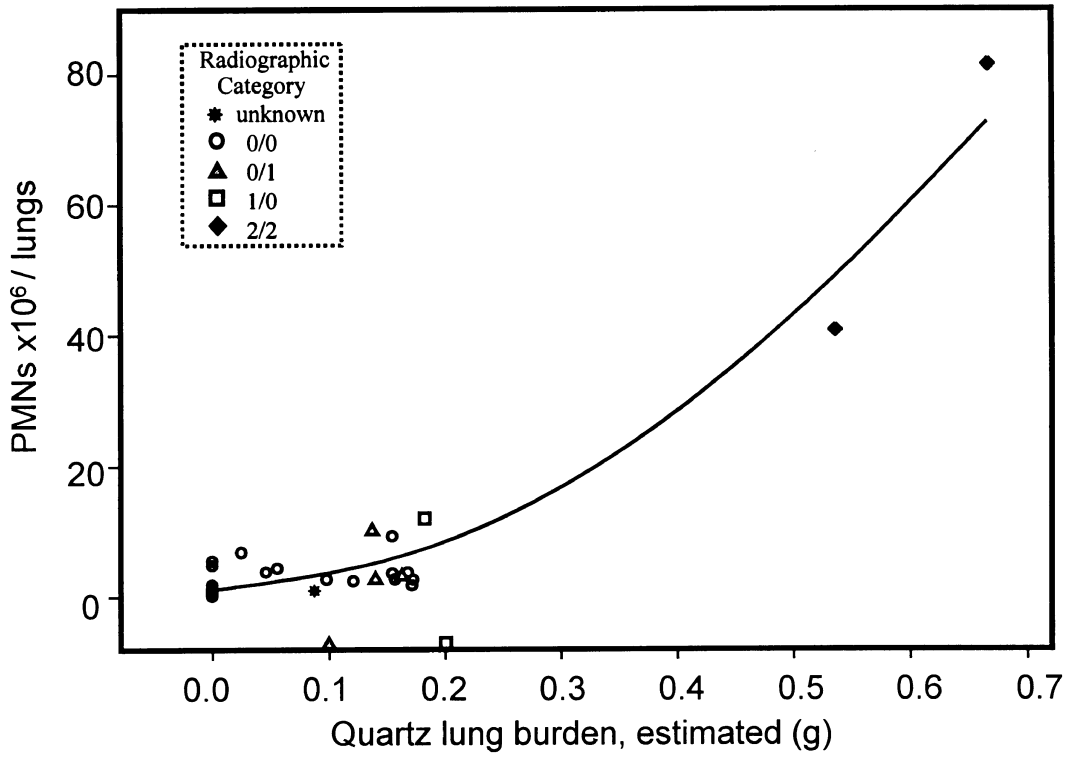

Figure 1. PMNs in BAL fluid and quartz lung burden: spline model fit to data of all individuals (20 miners and 16 controls). *Notes*: PMNs were measured in BAL fluid and estimated for whole lungs; lung burdens were estimated using a dosimetry model (see § 2). The two data points plotted on the x-axis and below zero on the y-axis show the estimated quartz lung-burdens of the two miners without PMN data; these data are plotted to provide additional information regarding the association between quartz lung-burden and radiographic category.

< 0·03); increase in age was associated with increasing PMN count, SOD level, and radiographic category, but with decreasing AM count. However, when estimated quartz lung-burden was included as a covariate, age was no longer a significant predictor of either PMN count ($P = 0{\cdot}3$) or radiographic category ($P = 0{\cdot}2$), and was a marginally significant predictor of SOD ($P = 0{\cdot}06$). AM count in BAL fluid remained significantly and negatively related to age ($P < 0{\cdot}03$) when quartz lung-burden was included in the model. However, age as a covariate did not influence the nonsignificant relationship between AM count and quartz lung-burden ($P = 0{\cdot}07$ with AM count adjusted for BAL fluid recovery fraction; $P = 0{\cdot}3$ with unadjusted AM count).

In a sensitivity analysis to evaluate the influence of having adjusted the PMN counts for BAL fluid recovery volume (i.e. fraction of the original 200 ml instilled), we re-ran the linear regression models using the unadjusted PMN counts as the response variable. The results showed little difference in the R^2 values for models with either the PMN response variable: 0·88 (PMNs adjusted) vs 0·83 (unadjusted) for quartz cumulative exposure as a predictor; and 0·79 (adjusted) vs 0·84 (unadjusted) for quartz lung burden as a predictor (P-values < 0·0001). We also evaluated the influence of the *a priori* omission of the two controls with recorded occupational exposure to second-hand smoke (one had a PMN count close to the mean control value, and the other had a PMN count nearly 10 times higher). The dose-response for quartz lung-burden and PMN count remained highly statistically significant ($P < 0{\cdot}0001$) when those two controls were added to the analysis, although the R^2 in the linear regression model dropped from approx. 0·8 to 0·7.

4. Discussion

In this study we describe the quantitative relationships between the estimated quartz lung burden and several pulmonary responses, including neutrophilic inflammation (i.e. increased PMN count in BAL fluid), antioxidant production (SOD in BAL fluid), and fibrosis (radiographic small opacities). Although very limited data were available to evaluate the dosimetry model predictions, the

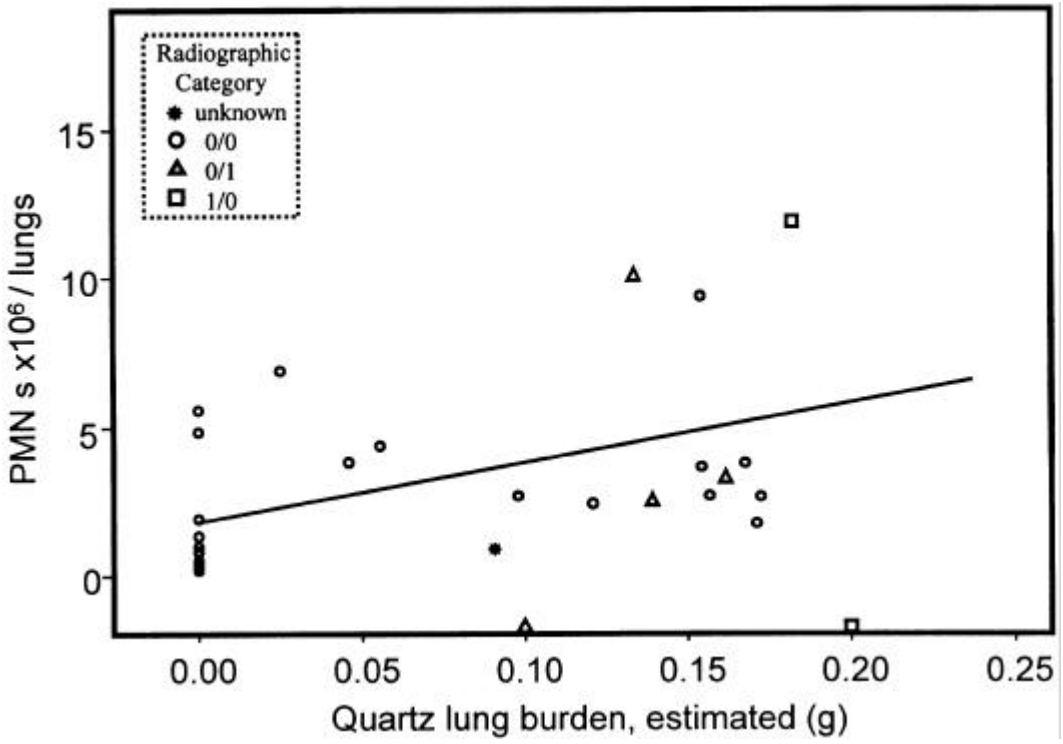

Figure 2. PMNs in BAL fluid and quartz lung-burden: linear regression model fit to data excluding the two individuals with CWP category 2/2 (i.e. 18 miners and 16 controls). See notes to figure 1.

predicted quartz lung burdens were similar to the quartz lung burdens measured in two individuals by WLL. Since quartz may be sequestered in the lungs (e.g. in granulomous lesions in the alveolar lumen or in the interstitium), the total lung burden is likely to be higher than that lavageable by WLL. This is consistent with the somewhat higher burdens predicted by the model. The dosimetry model assumes very slow alveolar clearance, such that nearly all of the quartz deposited in the deep lungs is predicted to be retained either in the alveoli, or in the interstitium, or transferred to the lung-associated lymph nodes (Tran and Buchanan 2000). Particle deposition and clearance may vary among individuals; thus the actual and predicted lung burdens would differ to the extent that an individual's values vary from the average values assumed in the model. Finally, any uncertainty in the exposure estimates would be reflected in the predicted lung burdens. Given these considerations, the dosimetry model appears to have provided adequate predictions of the quartz lung burdens in these miners.

Despite the small size of the study population, a highly statistically significant relationship was observed between the estimated quartz lung burden and PMN response. This relationship remained strong in several sensitivity analyses–whether or not PMN counts were adjusted for BAL fluid recovery fraction; with or without the two controls with recorded occupational exposure to second-hand smoke; and whether or not the two miners with CWP 2/2 were included in the analysis. Unlike PMNs, AM count in BAL fluid was not associated with the estimated quartz lung-burden. This finding is consistent with the earlier analysis by Vallyathan *et al* (2000), who suggested that the lavage recovery of AMs may be influenced by the extent of cellular activation, which may cause AMs to adhere more tightly to alveolar walls and make them more difficult to lavage. The AM counts were also quite variable, even among controls with assumed zero exposure to quartz. This scatter was due in part to the statistically significant (negative) association between age at medical examination and AM count in BAL fluid in both miners and controls. An age association could be caused by a selection effect if the healthy, older population in this study had a different distribution of AM counts relative to the healthy, younger individuals. However, age was not an important factor in the relationship between quartz lung burden and AM count (i.e. dose-response was not statistically significant whether or not age was included as a covariate).

SOD was the only antioxidant enzyme evaluated in this study; however, in the earlier study of these same individuals, Vallyathan *et al* (2000) found a similar pattern of response for SOD, catalase, and glutathione peroxidase. The levels of these antioxidants were similar among controls and "asymptomatic" miners (with radiographic cate-

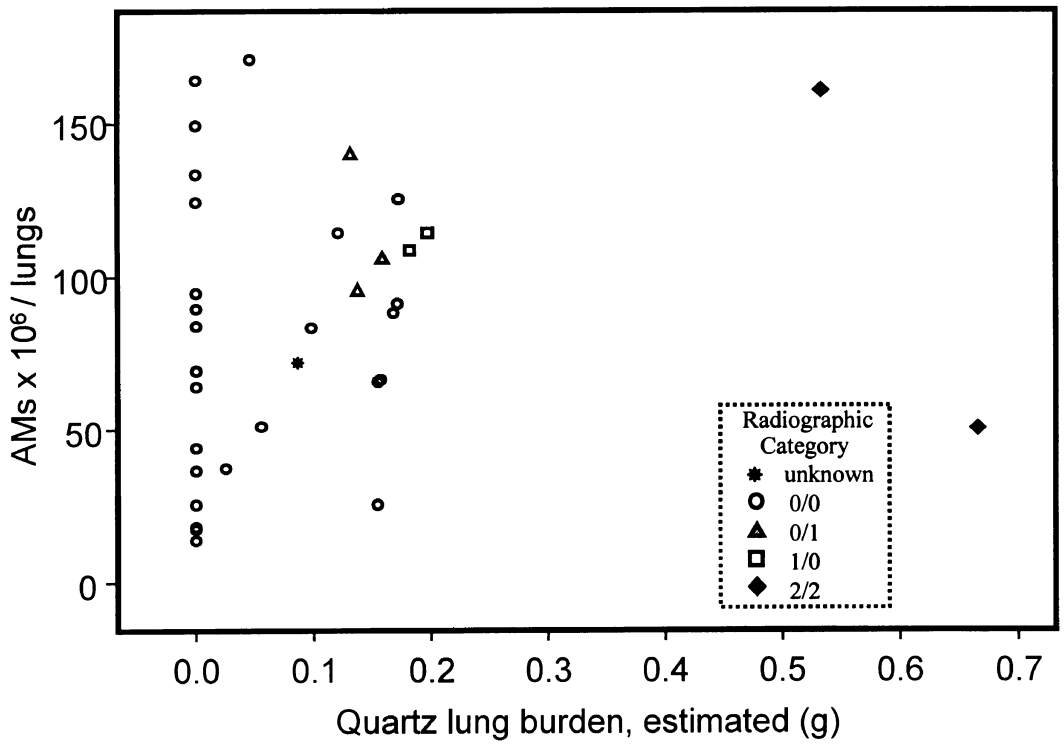

Figure 3. AMs in BAL fluid and quartz lung-burden: scatter plot of all individuals (one miner had missing AM data; thus, data are plotted for 19 miners and 16 controls). AMs were measured in BAL fluid and estimated for whole lungs; lung-burdens were estimated using a dosimetry model (see § 2).

gory 1/0 or lower), but were markedly elevated in the two "symptomatic" miners (with CWP 2/2). Other responses that were elevated only in symptomatic miners included: proinflammatory and fibrogenic cytokines (tumour necrosis factor-α, interleukin-1, and interleukin-6), cell growth factors (transforming growth factor-β and fibronectin), and antiprotease enzyme (α_1-antitrypsin) (Vallyathan *et al* 2000). Among both symptomatic and asymptomatic miners, increased oxidant-generating potential and oxidant damage were observed compared to the controls (Vallyathan *et al* 2000). These collective findings – statistically significant relationships between estimated quartz lung-burden, PMN count, SOD, and radiographic category, as seen in this study, combined with the earlier evidence of oxidative, proinflammatory, and fibrogenic events in miners' lungs – support a role for oxidative stress and inflammation in the development of particle-elicited pulmonary fibrosis (i.e. pneumoconiosis).

The statistically significant relationship between estimated quartz lung-burden and early radiographic categories (including 0/1 and 1/0) suggests that these changes represent early stages of pneumoconiosis. Radiographic small opacities can also be observed in individuals without occupational dust exposures, especially at older ages (Castellan *et al* 1985). Yet, among miners, the risk of developing complicated CWP has been shown to increase once small opacities – including category 0/1 or 1/0 – have been detected (McLintock *et al* 1971). Moreover, CWP may not be detected on a chest radiograph until 'moderate to severe' pathological abnormality has already occurred (Attfield *et al* 1994). Given the progressive and irreversible nature of pneumoconiosis (Wilt *et al* 1996), it may be necessary to prevent these early adverse pulmonary changes in order to ensure that CWP does develop.

The miners in this study had relatively low working lifetime dust exposures, having worked on average 17 years and almost entirely under the current US exposure limits for coal and quartz. Even under these conditions, some miners showed evidence of early pulmonary responses to the inhaled particles, including antioxidant production, neutrophilic inflammation, and radiographic small opacities. The findings of this study confirm the high toxicity of quartz; even at the relatively low concentrations of quartz mixed with respirable coal mine dust, quartz (as either cumulative exposure or estimated lung-burden) was the strongest predictor of both PMN count in BAL fluid and radiographic category. Coal dust cumulative exposure alone was also predictive of these responses; but given the high correlation between cumulative exposures of coal and quartz, the small size of this study, and

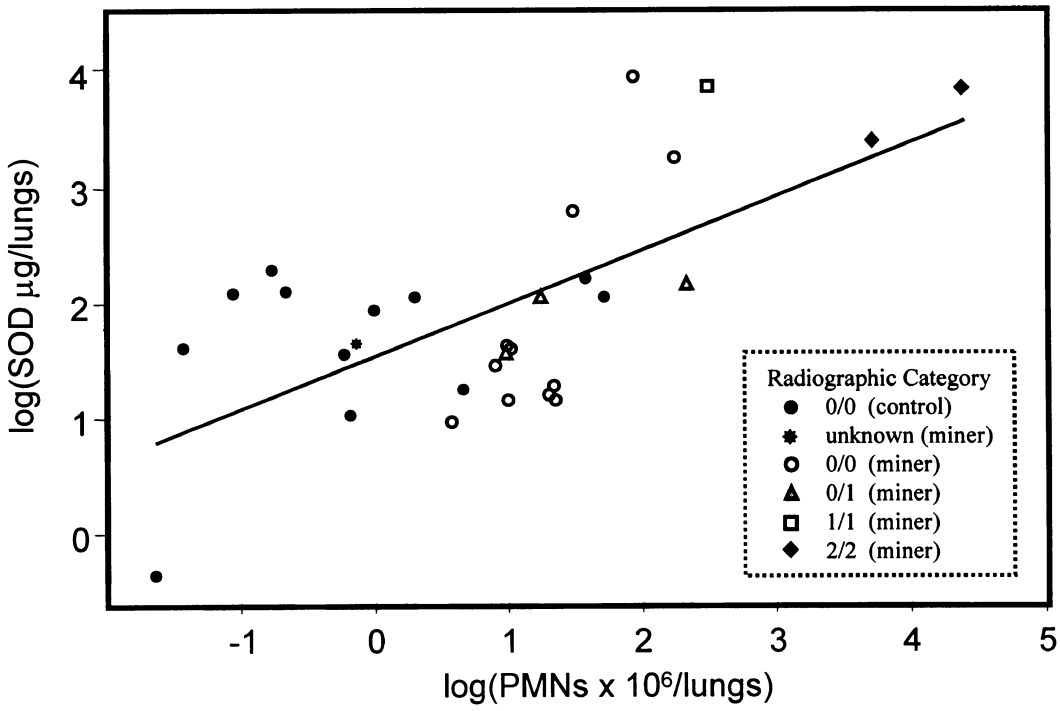

Figure 4. PMNs and SOD in BAL fluid: linear regression model fit to data of all individuals (two miners and four controls had missing PMN data; thus, data are plotted for 18 miners and 12 controls). PMNs and SOD were measured in BAL fluid and estimated for whole lungs (see § 2).

the lower toxicity of coal dust, there may have been insufficient power to detect an added effect of coal dust. These findings do suggest that reducing exposures to quartz in respirable coal mine dust is an important factor in preventing both pulmonary inflammation and fibrosis among coal miners.

The findings in this study in humans are also consistent with the pattern of pulmonary responses observed in rats exposed to respirable crystalline silica and other particles (Castranova 2000; Porter *et al* 2001, 2002a,b; Castranova *et al* 2002). In this human study and in the rat studies, the lung responses at lower particle burdens were relatively mild, suggesting the lungs were still able to handle the presence of the particles; however, at higher lung burdens, neutrophilic inflammation and oxidative damage increased substantially, suggesting that the protective and repair capacities of the lungs had been exceeded and pathological changes were occurring. To further explore these mechanisms, we are currently using these data in a quantitative comparison of dose-response in humans and rats exposed to quartz.

4. Conclusions

A statistically significant relationship was observed between respirable crystalline silica in coal mine dust and pulmonary inflammation (measured as PMN count in BAL fluid). Dose-response relationships were also observed with radiographic category of small opacities and with the level of the antioxidant SOD in BAL fluid. These findings suggest that relatively low occupational exposures to crystalline silica in mixed dust are associated with early pulmonary responses, including antioxidant production, inflammation, and fibrosis.

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