

Change in airway inflammatory markers in Danish energy plant workers during a working week

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Abstract

Introduction. It is well known that exposure to organic dust can cause adverse respiratory effect. The pathogen-associated molecular patterns (PAMPS) in the organic dust, such as endotoxin from Gram-negative bacteria cell wall and fungal components, can trigger the release of cytokine (e.g. Interleukin 1 β (IL-1 β)) and chemokine (e.g. Interleukin 8 (IL-8)) from the immune cells in the airways.

Objective. To evaluate the potential inflammatory effects of organic dust exposure in energy plants in Denmark.

Materials and methods. Nasal lavage (NAL) and exhaled breath condensate (EBC) were sampled at Monday morning (referred to as before work) and again at Thursday afternoon (referred to as after work). NAL IL-8, EBC pH, IL-1 β concentration were measured. Personal exposure to endotoxin and dust was calculated from time spent on different tasks and measured average work area exposures.

Results. Before work, workers from biofuel plants had a higher IL-1 β and IL-8 concentration compared to conventional fuel plants (control group). Specifically, the IL-1 β level of moderately and most exposed group, and IL-8 level of the least exposed group were higher compared to the control group.

The changes of IL-1 β , pH and IL-8 during a work week were not significant.

Workers with rhinitis had a lower percentage change of IL-8 compared to healthy workers.

Conclusions. An increased level of EBC IL-1 β in biofuel energy plant workers before work indicated a chronic or sub-chronic inflammation. The percentage change of IL-8 was lower in workers with rhinitis compared to healthy workers.

Key words

energy plant, IL-8, IL-1 β , nasal lavage, exhaled breath condensate

INTRODUCTION

It is well known that exposure to organic dust can cause adverse respiratory effect. Studies on different sources of organic dust, e.g. waste collecting [1, 2], composting [3], swine confinement buildings (SCB) [4, 5] and mould-damaged building [6], have been performed. In these studies, the effect of organic dust exposure was evaluated by respiratory symptoms, lung function and inflammatory markers.

One mechanism of organic dust induced respiratory effect, among others, is pathogen-associated molecular patterns (PAMPs) from microorganisms in organic dust (e.g. endotoxin and fungal components) on active immune cells in the airways. The release of cytokine (e.g. IL-1 β) and chemokine (e.g. IL-8) and the generation of reactive oxygen species (ROS) can lead to acute symptoms, as well as chronic inflammation [7].

Workers from biofuel energy plants also have a potential risk of organic dust exposure. Hence, they were included in this study of inflammatory markers and endotoxin exposure.

Results on respiratory symptoms, spirometry, bronchial metacholine provocation test and skin prick tests have been published previously [8] and showed that working with biofuel

at an energy plant did not generally increase the prevalence of respiratory symptoms, affect lung function or sensitization status. However, the exposure level to endotoxin had an impact on the occurrence of respiratory symptoms among biofuel workers. Associations between fungal exposure and respiratory symptoms were less clear. No associations were seen between lung function and the level of endotoxin or fungal exposure. The current paper focuses on inflammatory markers (EBC pH, IL-1 β , NAL IL-8) in relation to organic dust exposure before and after a work week.

MATERIALS AND METHOD

Population. In 2003, all Danish heat- and power-plants using straw or wood chips were identified and invited to participate. A total of 94 workers from 32 straw plants (participation rate 75%) and 138 workers from 42 wood chips plants (participation rate 74%) were included, 200 of them (80 straw workers, 120 wood chips workers) with clinical examinations. As controls, 11 heat- and power-plants using conventional fuel (mainly fossil oil and gas) we included, a total of 107 control subjects (participation rate 59%). Due to too few female subjects (4.1%), only male workers with endotoxin exposure record (n=283) were included in the analyses.

A modified ECRHS questionnaire [9] with additional questions on asthma, rhinitis and occupational history were handed out at the plants.

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Asthma symptoms were defined if the subject answered 'yes' to at least one group A question (physician-diagnosed asthma, current asthma, ever asthma, ever wheezed) and two or more group B questions (use of asthma drugs, ever chest tightness, ever wake up with chest tightness, wake up wheezing, morning cough, wake up coughing, wheeze when exposed to cold air or pollen or animals, wheeze when exercise) or at least two group A questions [10]. Subjects with asthma symptoms were referred to as the asthma symptom (As) group.

Rhinitis was defined as answering 'yes' to at least two nasal symptoms (rhinorrhoea, nasal itching, nasal congestion or sneezing) at least two days a week during the last year. Subjects with rhinitis were referred to as the rhinitis (Rh) group.

Atopic persons were defined by having at least one positive skin prick test (SPT) to a panel of 15 common inhalant allergens (comprising grass, horse, dog, cat, house dust mite, fungi and storage mite). A positive SPT was defined when the mean of the longest diameter and the midpoint orthogonal diameter of the weal was ≥ 3 mm [11]. Atopic subjects were referred to as the atopic (At) group.

Non-atopic subjects without rhinitis and asthma symptoms were referred to as the healthy (He) group. The concordance of subjects in each symptom groups (He, At, Rh and As) are shown in Figure 1. Smokers were defined as present smokers or ex-smokers for less than two years.

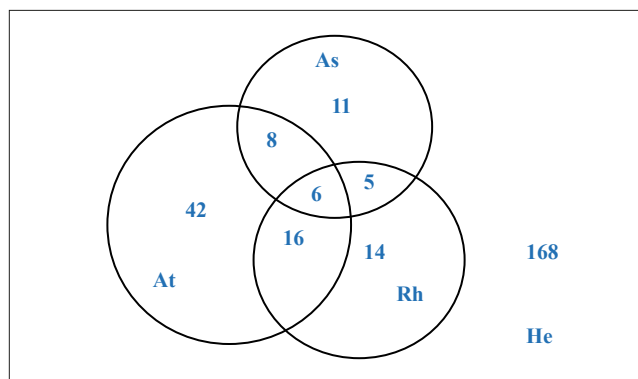


Figure 1. Concordance of subjects in each symptom group. He: healthy group; At: atopic group; Rh: rhinitis group; As: asthma symptom group. Numbers are the number of subjects in each category. There are 13 fewer subjects in the Figure than the total population of the study due to the missing value of symptoms recorded.

Endotoxin and dust exposure. Each participant wrote a diary of time spent on different work tasks or work areas for seven consecutive days. A personal current average exposure level for dust and endotoxin were estimated from 179 stationary work area measurements, and information on time spent on each work task or in each work area. A mean value for each work area was calculated, stratified by type of plant. Subsequently, subjects were divided into three equally-sized groups and denoted least, moderately and most exposed. Subjects from control plants were assigned to a separate category. The number of subjects for each symptom and endotoxin exposure groups is listed in Table 1.

'Total dust' has been defined as the dust collected by a sampler with an entry velocity of 1.25 m/s. 'Total dust' was sampled using 25 mm closed-face cassettes (Millipore holder; Millipore, Bedford, MA, USA), with a 5.6 mm inlet and a flow of 1.9 l/min corresponding to an inlet velocity of 1.25 m/s for approximately six hours. The samplers were fitted with teflon filters, pore size 1.0 μm .

Table 1. Number of subjects in each symptom and endotoxin exposure groups

Endotoxin exposure	Symptom Groups				Total
	He	At	Rh	As	
Control	58	30	18	9	103
Least	40	14	6	9	60
Moderately	35	14	10	4	60
Most	35	14	10	9	60
Total	168	72	44	31	283

He: healthy group; At: atopic group; Rh: rhinitis group; As: asthma symptom group.

Due to subjects with more than one kind of symptom, the total subject number in each exposure group was consistently smaller than the sum of subgroups in the corresponding row.

Endotoxin concentration was determined by the Limulus Amoebocyte Lysate (LAL) test. A standard curve obtained from an *Escherichia coli* O55:B5 reference endotoxin was used to determine the concentrations in terms of endotoxin units (EU) [8].

NAL IL-8 measurement. Nasal lavage was performed using a method first described by the US Environmental Protection Agency [12]. A volume of 5 ml sterile saline at 37°C was injected into each nostril with a 10 ml syringe. After 10 seconds, the sample was blown out into a cup and transferred to test tubes. The material was centrifuged and stored at -80°C until further analysis.

IL-8 was analyzed with a solid phase ELISA technique based on monoclonal antibodies against IL-8 (QuantiGlo[®], R&D Systems). The detection limit of IL-8 was 0.28 pg/ml.

Exhaled breath condensate (EBC) IL-1 β and pH measurement. The exhaled breath condensed on the inside of a refrigerated tube [13]. Subjects breathed through the cooled tube for 10 minutes, after which the condensate was collected into tubes and stored at -80°C for later analysis.

The contents of IL-1 β were measured with the same technique as IL-8 (Quantikine[®], R&D Systems). The pH of the condensate was also measured. The detection limit of IL-1 β was 0.1 pg/ml.

Statistical methods. All statistical analyses were performed in STATA (Intercooled STATA 9.2). Measurements below the low detection limit (LOD) were treated as half of the LOD. Non-parametric data, e.g. before work concentration and percentage change of IL-1 β and IL-8, was reported as median (first quartile – third quartile) and analyzed by Mann-Whitney test, Kruskal-Wallis test and Spearman correlation. Normally distributed data, e.g. before work concentration and percentage change of pH, was reported as mean (SEM) and analyzed by student t-test and one-way ANOVA. Multiple linear regression analyses of percentage change of IL-1 β , IL-8 and pH were performed, including endotoxin exposure, respiratory symptoms, age, smoking and body mass index (BMI) as the independent variables. A p value < 0.05 was considered significant in all analyses.

RESULTS

The endotoxin level was highly correlated with the total dust level (Pearson $R^2=0.97$ (categorical variable) and $R^2=0.87$ (continuous variable), $p<0.0001$). It was therefore decided to show the results for endotoxin exposure only, as the results for dust were similar to them and the dust values have been reported previously [8].

There was a significant correlation between the before and after work levels of IL-1 β (Spearman, $p=0.002$), IL-8 (Spearman, $p<0.0001$) and pH (Spearman, $p<0.0001$). Before work, a significant correlation was seen between IL-1 β and pH (Spearman, $p=0.045$, Fig. 2), but not between IL-1 β and IL-8 or between pH and IL-8.

Further analyses were performed between two groups (biofuel and conventional fuel), among four groups (control, least, moderately and most exposed group) or among 16 groups (Tab. 1). Each group in the 16 groups was denoted by combining the exposure and symptom group names, e.g. workers in the least exposed group with asthma symptom were referred to as the least&As group.

For the before work IL-1 β , biofuel plant workers had a higher level compared to controls (Mann-Whitney, $p=0.009$). This was mainly due to the elevated level in moderately (Mann-Whitney, $p=0.009$ vs. control) and most (Mann-Whitney, $p=0.035$ vs. control) exposed groups (Figure 3a).

When taking symptoms into consideration, only the difference between control&As and least&As group was significant (Mann-Whitney, $p=0.041$) (Fig. 3b).

For the before work IL-8, biofuel plant workers also tended to have a higher level compared to controls (Mann-Whitney, $p=0.081$). This difference reached a significant level between the control and the least exposed group (Mann-Whitney, $p=0.008$, Fig. 3c), which is confirmed by the difference between the control&He and least&He group (Mann-Whitney, $p=0.003$, Fig. 3d).

For the before work pH, it did not differ between biofuel and control workers in general. Within biofuel workers, it was lower in the most exposed group compared to least (t-test, $p=0.047$) and moderately (t-test, $p=0.035$) exposed group (Fig. 3e). Within workers with rhinitis, a difference was also found between control&Rh and moderately&Rh group (t-test, $p=0.037$, (Figure 3f).

Before work, smokers tended to have a higher IL-8 level (Mann-Whitney, $p=0.09$) and lower pH level (t-test, $p=0.06$) than non-smokers.

In the light of the heterogenic concentration before work, we used the percentage change to evaluate the variation between before and after work of IL-1 β , IL-8 and pH.

$$\frac{\text{After work concentration} - \text{Before work concentration}}{\text{Before work concentration}} \%$$

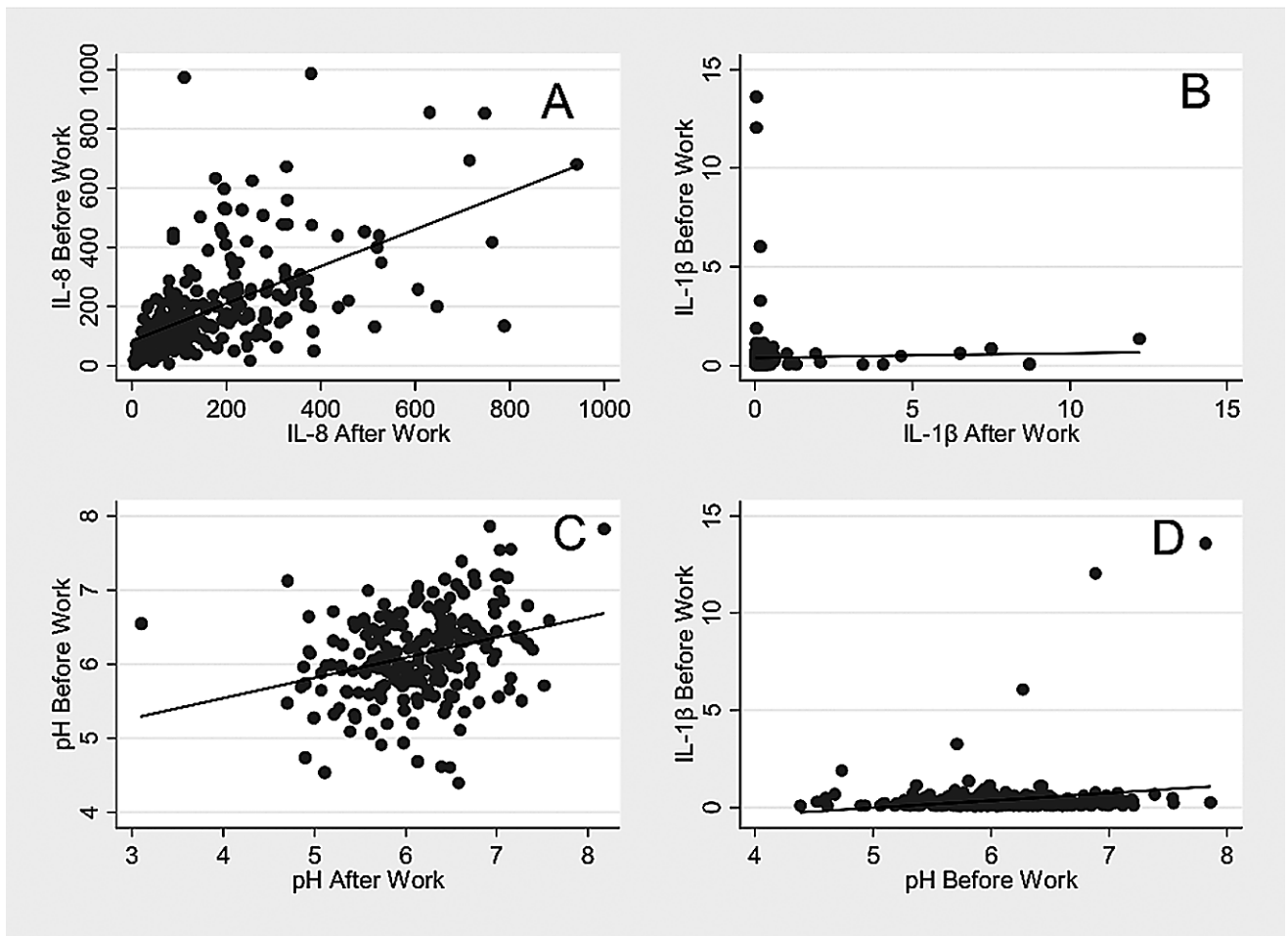


Figure 2. Correlation between before and after work level of IL-1 β , IL-8 and pH and between before work pH and IL-1 β concentration (Spearman). A: $p<0.0001$; $R=0.658$; B: $p=0.002$; $R=0.202$; C: $p<0.0001$; $R=0.294$; D: $p=0.045$; $R=0.132$.



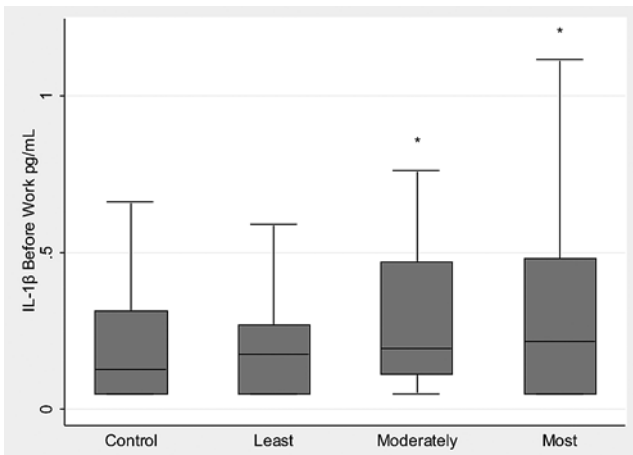


Figure 3a. IL-1 β concentration before work by exposure status. Outliers not shown. * p<0.05 compared to control group (Mann-Whitney)

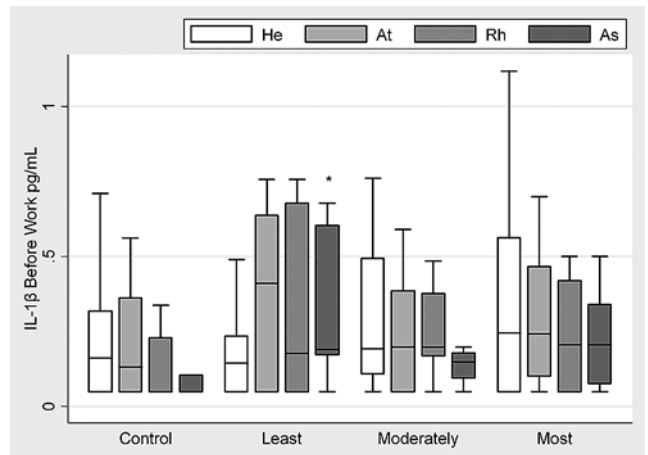


Figure 3b. IL-1 β concentration before work by both exposure and symptom groups. Outliers not shown. He: healthy group; At: atopic group; Rh: rhinitis group; As: asthma symptom group. * p<0.05 compared to control group (Mann-Whitney).

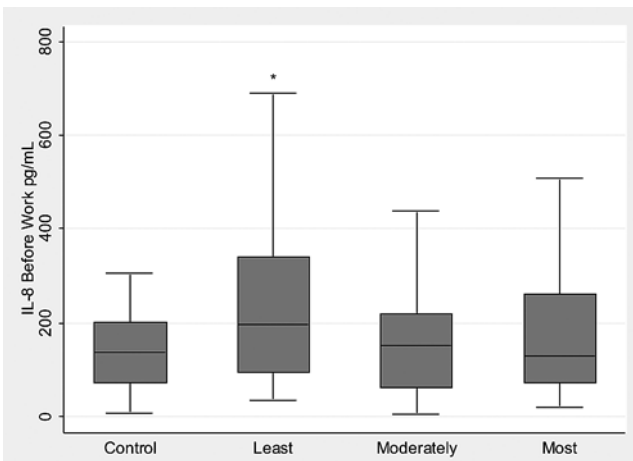


Figure 3c. IL-8 concentration before work by exposure status. Outliers not shown. * p<0.05 compared to control group (Mann-Whitney)

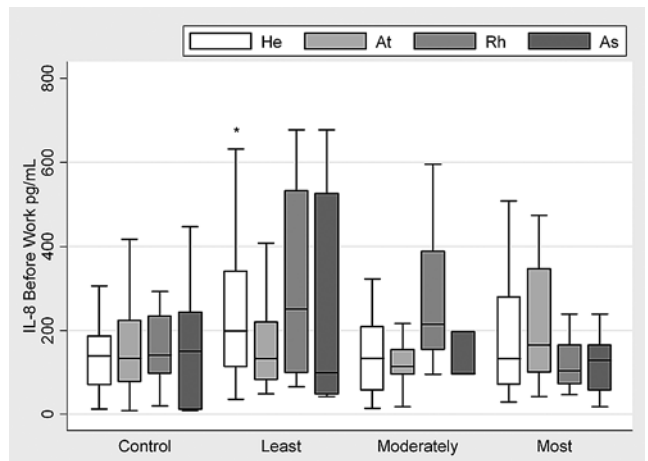


Figure 3d. IL-8 concentration before work by both exposure and symptom groups. Outliers not shown. He: healthy group; At: atopic group; Rh: rhinitis group; As: asthma symptom group. * p<0.05 compared to control group (Mann-Whitney)

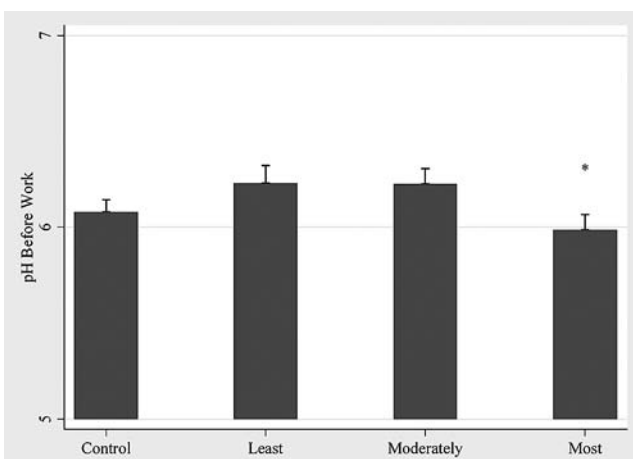


Figure 3e. pH before work by exposure status. * p<0.05 compared to the least and moderately exposed groups (t-test)

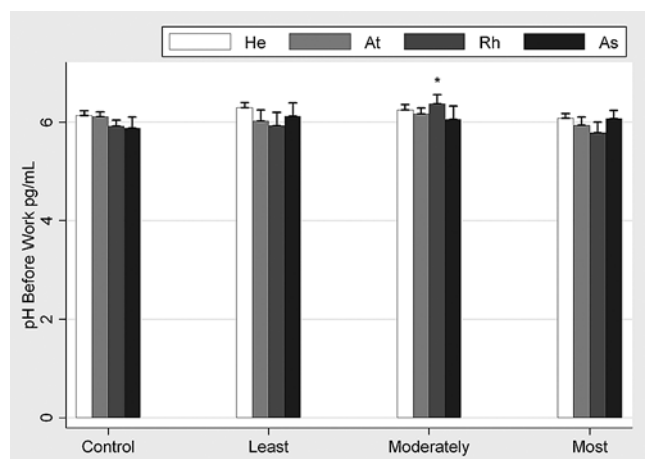


Figure 3f. pH before work by both exposure and symptom groups. He: healthy group; At: atopic group; Rh: rhinitis group; As: asthma symptom group. * p<0.05 compared to the control group (t-test)



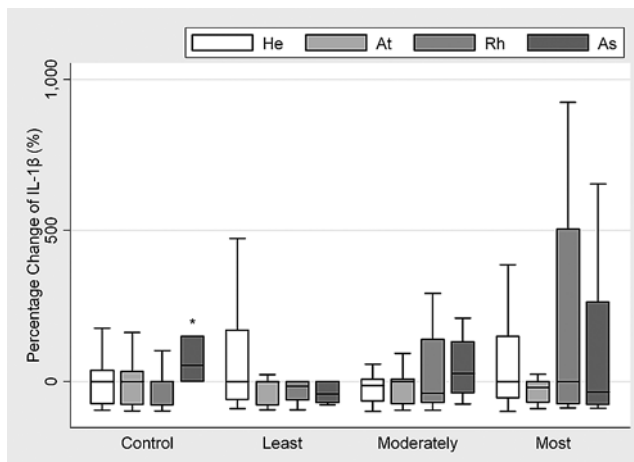


Figure 4a. Percentage change of IL-1 β . Outliers not shown. He: healthy group; At: atopic group; Rh: rhinitis group; As: asthma symptom group. * $p < 0.05$ compared to He group (Mann-Whitney)

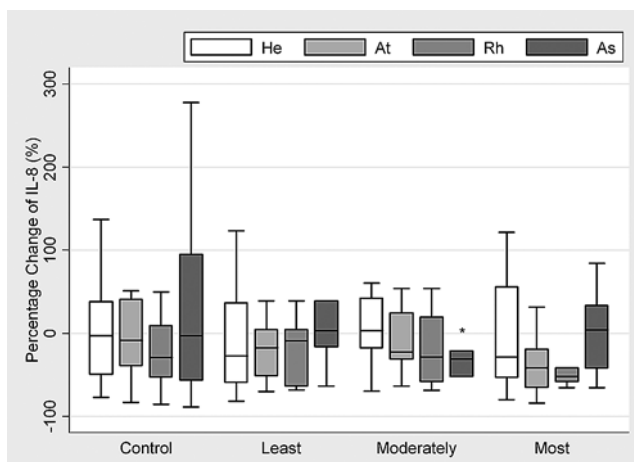


Figure 4b. Percentage change of IL-8. Outliers not shown. He: healthy group; At: atopic group; Rh: rhinitis group; As: asthma symptom group. * $p < 0.05$ compared to He group (Mann-Whitney)

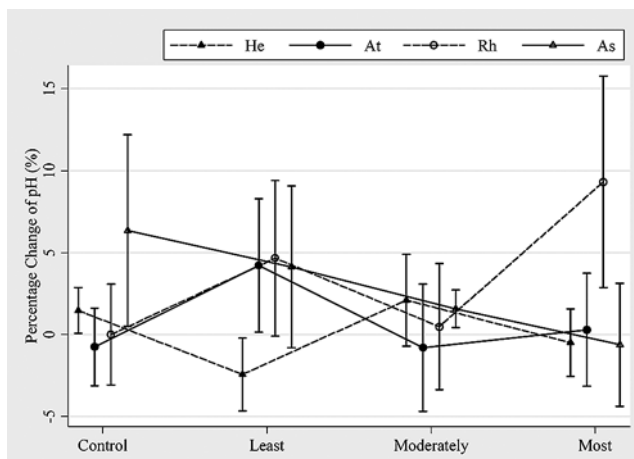


Figure 4c. Percentage change of pH. He: healthy group; At: atopic group; Rh: rhinitis group; As: asthma symptom group.

For the percentage change of IL-1 β , it was higher in control&As compared to control&He group (Mann-Whitney, $p = 0.044$, Fig. 4a).

For the percentage change of IL-8, workers with rhinitis had a significant lower level compared to health workers in general (Mann-Whitney, $p = 0.014$). Although this difference

was significant within none of exposure groups, it reached a borderline significant level between moderately&Rh and moderately&He group (Mann-Whitney, $p = 0.062$) and between most&Rh and most&He group (Mann-Whitney, $p = 0.092$). The percentage change of IL-8 of moderately&As group was lower compared to moderately&He group (Mann-Whitney, $p = 0.045$, Fig. 4b).

The percentage change of pH did not differ among groups. Only the most&Rh group tended to have a higher level compared to the most&He group (t-test, $p = 0.082$, Fig. 4c).

A multiple linear regression model including endotoxin exposure, smoking, rhinitis, asthma symptoms, age and BMI as the independent variables failed to reach a significant level for the percentage change of neither IL-1 β nor pH. The fitness of the model for percentage change of IL-8 was significant ($p = 0.008$). However, a low R^2 value (0.085) indicated that the percentage change of IL-8 was poorly explained by these variables. The result of this regression analysis confirmed that workers with rhinitis had a lower percentage change of IL-8 ($p = 0.003$) compared to healthy workers. While the percentage change was higher in workers with asthma symptom compared to health workers ($p = 0.001$).

DISCUSSION

Before work, biofuel workers had a higher IL-1 β and IL-8 level compared to conventional fuel workers. A higher before work concentration has also been reported in other studies of organic dust exposed workers. Wouters et al. [1, 2] found a higher IL-8 level in NAL from waste workers than among controls on the Monday before work. They suggested this phenomenon indicated a chronic or sub-chronic effect of working in an occupational environment with exposure to organic dust. Thus, even after a weekend at rest, exposed workers still had a higher level of inflammatory markers in their airways. However, we were not able to identify any clear exposure-response relationship for the changes during the work week. In the waste worker studies [1, 2], the after work cytokine concentrations were higher than before work. The increases of cytokine concentration were confirmed by experimental studies exposing healthy subjects to occupational environments [14, 15, 16], but this increase was not seen in the presented study. In general, no significant change of IL-1 β , pH and IL-8 concentration was observed across the work-shift in energy plant workers. This was in agreement with our previous report of symptoms and lung function analyses [8]. This lack of change could be due to the relative low level of endotoxin and total dust level in the energy plants, compared to those found in other organic dust exposed occupational environments. The mean (range) dust and endotoxin exposure in the presented study was 0.05 (0–0.33) mg/m³ and 3.5 (0–294) EU/m³ (12.0 EU \approx 1 ng) [17] which was 100 – 1,000-fold lower than in swine confinement buildings, and approximately 10-fold lower than the exposure of waste workers. In an investigation in mould-damaged Danish schools which had a comparable endotoxin and dust concentration to the energy plant, Ebbehøj et al. found that the NAL IL-8 concentration was not related to mould exposure [18].

Due to the EBC processing procedure, the EBC pH value was lower in general in the current study compared to others reported [19, 20, 21]. The reason for a lower pH in the most

exposed group compared to the least and moderately exposed groups could be that asthma symptoms and work-related respiratory symptoms (not taken into consideration in the current study) increased with endotoxin exposure [8]. Maestrelli et al. found no effect of personal PM₁₀ and PM_{2.5} and other pollutants exposure on EBC pH in 32 asthmatic patients who were studied 2 years [19]. Corradi and colleagues reported that EBC pH was higher in 40 cleaners who were exposed to cleaning detergents than in 40 unexposed controls [21]. A few studies suggested that subjects with chronic obstructive pulmonary disease (COPD) [20] or asthma [22] had lower EBC pH compared to normal subjects.

The reason for the lack of correlation between before work concentration of IL-1 β and IL-8 could be the different sampling media (i.e. NAL and EBC). For IL-1 β and pH both of which were measured in EBC, there was a significant correlation with the before work level.

In the multiple linear regression analyses, the percentage change of IL-1 β and pH did not differ significantly among exposure groups or symptom groups. For the percentage change of IL-8, it was lower in workers with rhinitis compared to healthy workers. Although the Rh and As group were correlated (Chi-square, $p < 0.001$), workers with asthma symptoms had a higher level of percentage change of IL-8 compared to healthy workers. However, the difference between As and He group was less persuasive than the difference between Rh and He group due to an interaction between asthma symptom and exposure (multiple linear regression model, $p = 0.07$ and 0.038 for most and moderately exposed group, respectively). The percentage change of IL-8 in most&As group was at the similar level as in most&He group. The percentage change of IL-8 in moderately&As group was even significantly lower than in moderately&He group, but this could be a chance finding because of the small number of subjects in moderately&As group ($n = 3$). On the other hand, the difference between Rh and He group was more consistent through all exposure groups. IL-8 change caused by organic dust or endotoxin exposure in subjects with rhinitis or asthma has also been investigated in other studies. Nightingale et al. [23] reported no difference in IL-8 concentration between normal and asthma subjects before and 24 hours post-LPS challenge, but at six hours post-challenge, IL-8 concentration of normal subjects elevated faster than among asthma subjects. Castano et al. [24] observed no change of IL-8 after suspected agent challenge in subjects with occupational rhinitis. Gosset et al. [25] concluded that IL-8 increased significantly after allergen challenge in subjects with allergic rhinitis, but IL-8 was not the main chemotactic factor. Eldridge et al. [26] suggested an augmented effect of allergen pre-challenge to endotoxin exposure in subjects with asthma, but did not observe a significant change of IL-8 concentration in NAL. However, it must keep in mind that, when we discuss the percentage change of inflammatory markers in the presented study, the change of pH, IL-1 β and IL-8 was not significant during a work week, and the multiple linear regression model poorly explained the percentage change of inflammatory markers, as indicated by the low R^2 value.

No significant difference was observed in the presented study between smokers and non-smokers. However, the IL-8 tended to be higher and pH tended to be lower in smokers, while IL-1 β level was more similar between the two groups. Garey et al. reported [27], in EBC, the concentrations of total

protein, nitrite and neutrophil chemotactic activity were significantly higher in 11 smokers compared to nine non-smokers ($p < 0.05$). The concentration of IL-1 β was similar in non-smokers and smokers. Gessner et al. [28] found that both IL-1 β and IL-8 were higher in 21 healthy smokers than 24 healthy non-smokers in EBC.

In conclusion, an increased level of EBC IL-1 β and NAL IL-8 in biofuel energy plant workers before work indicated a chronic or sub-chronic inflammation caused by the organic dust exposure, but the exposure had negligible effect on the percentage change of all three markers investigated. The percentage change of IL-8 was lower in workers with rhinitis compared to healthy workers. To clarify the difference of percentage change of inflammatory markers among subjects with different exposure levels and different symptoms, further investigations are needed.

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