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Revision I

Repeated exposure to organic material alters inflammatory and physiological airway responses

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Short title: Adaptation to organic dust

Abstract

Farmers and smokers are repeatedly exposed to airborne organic material. We hypothesised that farmers and smokers show altered airway responses to inhaled organic, pro-inflammatory agents.

Eleven farmers, 12 smokers and 12 controls underwent lipopolysaccharide (LPS) bronchial challenge and spent 3 hours in a pig barn. Lung function, exhaled nitric oxide and bronchial responsiveness were assessed and we also collected nasal lavage fluid and induced sputum. Symptoms and body temperature were recorded before and after exposures.

Following exposure to the pig barn, bronchial responsiveness, exhaled NO, sputum IL-6, nasal lavage cell count and IL-8 were increased to a greater extent in controls compared to farmers. The sputum IL-6 response was also attenuated in farmers after LPS challenge. The response shown by smokers following exposure to the pig barn was similar to controls regarding measurements of exhaled NO, IL-8 in nasal lavage and IL-6 in sputum, but more similar to farmers concerning bronchial responsiveness and the cell numbers present in nasal lavage. Sputum interleukin-8 showed a greater increase in smokers than in the other groups following LPS challenge.

We conclude that individuals who are repeatedly exposed to organic material develop an adaptation to the effects of acute exposure to inhaled organic material.

Key words: Inflammation, occupational exposure, smoking, pig barns, bronchial responsiveness

Introduction

Pig farmers working in pig barns are exposed to organic material on a daily basis, which leads to a chronic airway inflammation, even in those who do not experience airway symptoms [1]. Farmers also have higher prevalence of airway symptoms and chronic bronchitis than the general population [2, 3] and farmers run an increased risk of developing chronic obstructive pulmonary disease (COPD) [4]. It has been suggested that the acute inflammatory airway response that follows exposure to a pig barn is altered in pig farmers compared with healthy, previously non-exposed control subjects [5, 6]. Previous results therefore indicate that farmers develop an adaptation, which is most likely a consequence of repeated exposure to organic material in the pig barn environment [6-8]. In healthy subjects acute exposure to a pig barn causes an intense airway inflammation, enhanced bronchial responsiveness and increased levels of exhaled nitric oxide, NO [9-11]. Additionally, a near 100-fold increase in neutrophils, a 3 – 4 fold increase in lymphocytes and macrophages and a multifold increase in pro-inflammatory cytokine (IL-1, IL-6, IL-8, TNF) levels have been demonstrated in bronchoalveolar lavage fluid (BAL) following exposure to pig confinement facilities [12, 13]. Exposure to a pig barn also induces an intense inflammatory response in the upper airways, dominated by neutrophil granulocytes [11].

There are several microbial components of organic dust, including bacterial endotoxin (lipopolysaccharide, LPS), that may contribute to the biological effects induced by exposure to a pig barn. However, it is not clear to what extent endotoxin contributes to the biological effects caused by exposure. Repeated exposure to endotoxin induces adaptation to further exposures by down regulating the inflammatory response [14]. Smokers are, like farmers, continuously exposed to organic compounds which also include endotoxin [15]. To our knowledge there is no data as to whether smokers, as a consequence of repeated exposure to tobacco smoke, develop adaptation to further exposure to tobacco smoke or other organic material.

The present study was undertaken to find out whether the response to inhalation of organic dust and endotoxin is altered in individuals who are regularly exposed to organic material on a daily basis (pig farmers and smokers) compared to healthy non-smokers. Our hypothesis was that tolerance has been developed in the continuously exposed groups and that there may be a cross reactivity between different types of exposure. Indicators of airway and systemic inflammatory responses and bronchial responsiveness were therefore assessed before and

after exposure to a pig barn and bronchial LPS-challenge (in random order) in pig farmers, smokers and non-smoking, non-farming healthy control subjects.

Methods

Subjects

Subjects were recruited by advertisement in the daily press and working farmers were directly contacted by mail. Thirty-six subjects in three different groups (n=12); controls, non-smoking farmers and smokers were included in the study. All subjects had normal lung function and had no airway hyper-responsiveness [16]. None had a history of COPD, asthma or allergy (confirmed with negative skin prick tests to a panel of 12 common allergens) and had no other chronic diseases. None had suffered any respiratory tract infection during the two weeks prior to the study. Farmers were included if they had been exposed to the pig barn on a daily basis for the past 6 months, and smokers were included if they had smoked ≥ 10 cigarettes per day during the year prior to the study. All subjects gave informed consent and the study was approved by the Ethics Committee of Karolinska Institutet.

Study design

On two separate days, at least 3 weeks apart, all subjects were exposed to dust in a pig barn and underwent a bronchial challenge with lipopolysaccharide (LPS) in randomized order. Two to six subjects from 2-3 groups were exposed in the pig barn at each occasion while weighing pigs for 3 hours. Measurements of exposure levels were carried out at each occasion. Peak expiratory flow (PEF) was measured both before and 3, 4 and 5 hours after the exposure.

On a separate day 6 breaths of LPS were inhaled (*Escherichia coli* serotype 0111:B4 (SIGMA) dissolved in 0.9% sterile saline, 1.25 mg/ml), corresponding to 53.4 μg LPS [17] using an inhalation dosimeter (SPIRA® Elektro 2, Hameenlina, Finland). Forced expiratory volume in one second (FEV_1) was measured before, 30 and 60 minutes after, and then every hour up to 6 hours after the provocation.

Approximately two weeks before the first exposure and 7 hours after the start of LPS and dust exposures, lung function and exhaled NO were measured and a bronchial methacholine challenge and induced sputum performed. Nasal lavage was performed pre- and post-dust exposure but not after LPS. Symptoms and body temperature were recorded before and up to 7 hours after exposure.

Symptoms

General and airway specific symptoms were recorded before and after exposure on a visual analogue scale (VAS), 0 - 100 mm. The subjects were requested to put a cross on a scale where 0 indicated none while 100 indicated unbearable symptoms.

Lung function and bronchial responsiveness

Vital capacity (VC) and FEV_1 were measured before and 7 hours after exposure using a wedge-spirometer (Vitalograph®, Buckingham, UK) according to ATS criteria [18]. Repeated FEV_1 measurements after LPS challenge were measured with a One® Flow tester (Clement Clark, Ltd, London, UK) and peak expiratory flow (PEF) was measured with a mini-Wright® peak flow meter (Clement Clark, Ltd, London, UK). Local lung function reference values were used [19, 20].

Bronchial responsiveness to methacholine was tested as previously described [21]. Inhalation of the diluent was followed by inhalation of doubling concentrations of methacholine up to 32 mg/ml starting at 0.5 mg/ml. The result was expressed as the cumulative dose causing a 20% decrease in FEV_1 ($PD_{20}FEV_1$).

Exhaled NO

Nitric oxide in exhaled air was assessed using a single-breath exhalation with a flow rate of 50 mL/s, according to the ATS recommendations [22]. Exhaled NO was analysed by chemiluminescence after reaction with ozone (NIOX®, Aerocrine, Stockholm, Sweden). To decrease contamination from the oral cavity, mouthwash with water (30 seconds) and 10% sodium bicarbonate (30 seconds) preceded the measurement procedure [23].

Nasal lavage

Nasal lavage was performed as previously described [24] with minor modifications [12]. Five ml of sterile 0.9% NaCl was instilled into one nostril and 10 seconds later expelled and collected. The procedure was repeated in the other nostril and the lavage samples were pooled. After centrifugation cell number was counted in a Bürker chamber. The supernatant was frozen (-70^{o}C) until further analysis.

Sputum induction and processing

Sputum induction and processing was performed as previously described [25] with minor modifications. After inhalation of salbutamol (0.4 mg) sputum was induced by inhalation of saline in increasing concentrations (0.9%, 3.0%, 4.0%, 5.0%), using an ultrasonic nebulizer

(De Vibliss Ultraneb 2000) with an output of 3 ml/min. Each concentration was inhaled for 7 minutes followed by FEV_1 measurement. Subjects were asked to blow their noses and rinse their mouths with water after each concentration, and then to cough deeply and to make an attempt to expectorate sputum. The sample was considered adequate when it macroscopically appeared to be free from saliva and had a weight of at least 1000 mg.

Sputum colour and weight were determined and an equal volume of DTT (dithiothreitol) 0.1% was added to the whole sputum sample and rocked for 15 - 25 minutes in a 37°C waterbath. The sample was centrifuged (10 minutes at 280*g*) and the supernatant stored in aliquots at -70°C until analysis.

The cell pellet was re-suspended in 2 ml PBS and passed through a filter. Total cell count and viability test with Trypan blue was performed. Slides were prepared by cytocentrifuge and stained with May-Grünwald Giemsa stain. Three hundred cells were assessed for differential cell counts. Less than 100 cells were considered too few cells for an accurate differential count. Sputum samples containing more than 80% squamous cells were excluded from the analyses.

Cytokine analysis

Interleukin-6 and IL-8 were measured in nasal lavage fluid and sputum using an in-house ELISA method. Commercially available antibody pairs (R&D systems, Europe, Abingdon, UK) were used as previously described [26]. The detection range for IL-6 and IL-8 was 2.8 - 375 pg/ml and 40 - 3200 pg/ml, respectively. For duplicate samples an intra-assay coefficient (CV) of <10% (nasal lavage) or <15 % (sputum) was accepted.

Exposure measurements

IOM filter cassettes (25 mm) (SKC Ltd, Dorset, UK) and plastic cyclones (25 mm) (Casella Ltd, London, UK) were used to monitor inhalable and respirable dust levels, respectively. The samplers were placed in the breathing zone on two subjects at each exposure occasion. The cassettes were equipped with Teflon filters (1.0µ, Millipore, Sundbyberg, Sweden). After weighing, the filter samplers were extracted and the endotoxin concentration was analysed using a kinetic technique version of *Limulus amebocyte* lysate assay (Limulus Amebocyte lysate, Endosafe® Endochrome-K™ U.S. Lisence No. 1197, Coatech AB, Kungsbacka, Sweden), with *E. coli* 0111:B4 as standard.

Statistical Analysis

Within group comparisons were performed using ANOVA repeated measurements, followed by paired Students t-test (lung function, $logPD_{20}FEV_1$) or Friedmans test, followed by Wilcoxon signed rank sum test as post-hoc test. Between group comparisons in bronchial responsiveness were logarithmically transformed and analysed by means of ANOVA with Fisher´s PLSD as post hoc test. Other between groups comparisons were analysed by the Kruksal-Wallis test using the Mann-Whitney U-test as a post hoc test when appropriate. A value of $p< 0.05$ was considered significant. The results were analysed using StatView version 5.0.1 (SAS Institute Inc., Cary, NC).

Results

Subjects

Twelve non-smoking pig farmers, 12 smokers with no respiratory symptoms according to a questionnaire and 12 non-smoking, non-farming controls participated in the study (table 1). One farmer was pregnant at the first visit and therefore excluded from further participation. Another farmer experienced a migraine headache 5 hours after the LPS challenge and was not included in the analyses of the LPS-provocations.

The pig farmers had worked as farmers for 13 (0.5 - 36) years, spending 3.5 (0.5 - 8) hours per day in pig barns containing 1000 (60 - 3200) pigs. The cumulative smoking exposure in the smoking group was 21 (1.5 - 48) pack years.

Symptoms and body temperature

LPS provocation induced headache and fatigue in all three groups ($p\leq 0.007$) with no significant differences between the groups.

Exposure in the pig barn induced chills, runny nose, cough, and chest tightness in the controls, cough in the smokers and chest tightness in the farmers. Dust exposure induced more cough in controls ($p=0.04$) and smokers ($p=0.002$) than in farmers.

The mean (n=36) increase in body temperature was 0.43°C after LPS and 0.61°C after pig house exposure. Temperature increased significantly in controls ($p=0.001$) and smokers ($p=0.03$) following LPS challenge and post-dust in all groups ($p\leq 0.01$) with no significant differences between the groups after either stimulus.

Lung function and bronchial responsiveness

Baseline FEV_1 measurements were significantly lower in farmers and smokers than in controls (table 1). A small decrease in VC and FEV_1 was observed after LPS and dust exposure in controls and after exposure to dust in smokers and farmers (table 2).

Following LPS challenge, a maximal decrease in FEV_1 was observed at 3 hours ($p<0.001$) with no differences between the groups. Seven hours after LPS challenge VC and FEV_1 were reduced only in the controls. Also, dust exposure induced a slight reduction in FEV_1 ($p\leq 0.033$) with no significant differences between the groups (table 2).

Post-dust PEF fell in controls ($p<0.0001$) and smokers ($p=0.003$) but not in farmers ($p=0.19$), and to a lesser extent in farmers than in the other groups ($F=4.38$; $p=0.021$).

Pre-exposure bronchial responsiveness did not differ significantly between the groups (figure 1). LPS induced an increase in bronchial responsiveness which did not differ between the groups ($F=0.02$; $p=0.98$). Post-dust bronchial responsiveness increased to a greater extent in controls than in farmers ($p<0.001$) and smokers ($p<0.001$), with no difference observed between farmers and smokers ($p=0.57$, figure 1). The absolute level of post-dust $PD_{20}FEV_1$ was similar in all groups ($F=0.26$; $p=0.77$).

Exhaled NO

Pre-exposure levels of exhaled NO were lower in smokers than in the other groups and higher in farmers than in controls and smokers (figure 2 and table 1).

LPS inhalation did not influence exhaled NO levels, whereas dust exposure significantly increased the exhaled NO levels in controls and smokers but not in farmers (figure 2), the levels were significantly less in farmers than in the other groups ($p=0.007$).

There was a negative correlation between the increase in exhaled NO following dust exposure and the cumulative exposure to tobacco smoke ($r = -0.54$; $p = 0.05$).

Nasal lavage

Pre-exposure cell number and cytokine (IL-6, IL-8) levels in nasal lavage fluid were similar in the three groups ($p\geq 0.28$, figure 3). Following exposure, IL-8 in nasal lavage increased to a lesser extent in farmers than in the other groups ($p=0.007$), and nasal lavage cell count

increased significantly more in controls (p=0.003, figure 3). The post-dust IL-6 alteration in nasal lavage fluid did not differ between the groups (p=0.30).

Sputum

Pre-exposure IL-6 in sputum was higher in smokers than in controls (p=0.001). Smokers exhibited the highest sputum IL-6-levels following LPS challenge, whereas farmers showed a lower increase in sputum IL-6 levels than the other groups following dust exposure (figure 4).

Pre-exposure IL-8 in sputum was higher in smokers and farmers than in controls (p=0.01) and LPS induced a greater sputum IL-8 in smokers compared to the other groups (p=0.03, figure 4). Sputum IL-8 increased more in smokers than in farmers following dust exposure (p=0.02).

Pre-exposure sputum cell count was similar in the three groups (figure 4) and LPS-exposure induced a smaller increase in farmers than in the other groups (p=0.04). Dust exposure induced similar changes in cell number in the three groups (p=0.28).

Exposure measurements

The levels of inhalable and respirable dust were 8.3 (6.2 - 9.7) mg/m^3 and 0.32 (0.30 - 0.33) mg/m^3, respectively. The corresponding endotoxin concentrations were 62.8 (48.0 - 85.3) ng/m^3 and 12.9 (3.0 - 26.9) ng/m^3.

The airborne levels of hydrogen sulphide were below the detection limit (<0.05 ppm) on all exposure occasions and the ammonia concentration was 5.0 (3.0 - 6.6) ppm (n=7).

Discussion

In the present study it was demonstrated that both symptom-free smokers and farmers - two groups of individuals who are repeatedly exposed to organic material on a daily basis, have signs of an ongoing airway inflammation in the lower, but not in the upper airways. The most noticeable finding was the different response to exposure in a pig barn between the groups. In general, farmers responded to a lesser extent than controls whereas smokers responded similarly to controls with regard to certain parameters, but more like farmers regarding others. Exposure-induced symptoms, physiological outcomes (lung function, bronchial responsiveness) and markers of airway inflammation (exhaled NO, cells and cytokines in sputum and nasal lavage fluid) were attenuated in farmers compared with controls. Our results thus indicate an adaptation to acute exposure in farmers also observed to a certain extent in smokers. Another clear finding was that exposure to dust in the pig barn was a much stronger pro-inflammatory stimulus than the inhalation of pure endotoxin (LPS) even though the doses

of the latter are more than 200 fold (see below) higher than the doses inhaled in pig barns. Inhalation of LPS induced similar alterations in lung function and bronchial responsiveness in the three groups. However, the response as assessed by sputum cell content and IL-6 levels was down regulated in farmers compared to smokers and controls, whereas the IL-8 response was augmented in smokers.

Pig barn exposure induced less of an increase in bronchial responsiveness in smokers and farmers than in controls, the increase in controls being similar to what has previously been shown [10]. The present results are in line with previous findings of an attenuated response in farmers [5] and show that smokers seem to be more similar to farmers than to controls in these respects. Regarding the increased bronchial responsiveness following exposure in the pig barn, we cannot exclude the possibility that this difference was influenced by the small, although non-significant, difference in pre-exposure bronchial responsiveness. We have previously shown that the inter-individual difference in the absolute, post-dust $PD_{20}FEV_1$-value is small, implicating that the exposure-induced increase in bronchial responsiveness is almost totally independent of pre-exposure values [27]. Absolute post-dust $PD_{20}FEV_1$ was similar in the three groups indicating that the exposure-induced enhancement may be due to the non-significant difference in the pre-exposure values.

For most of the outcome measures, exposure in a pig barn was a stronger stimulus than was inhalation of pure LPS. Interestingly, we found no differences between the groups with regard to the enhancement of bronchial responsiveness following LPS challenge, but bronchial responsiveness increased to a greater extent in the controls than in the other groups after dust exposure. This indicates that exposure in a pig barn induces a maximal increase in bronchial responsiveness ($PD_{20}FEV_1$ was similar in all three groups after dust exposure) whereas exposure to endotoxin does not. It has also been shown that mice with defective TLR4 (an important receptor for LPS) have an attenuated inflammatory response in the lung after exposure in a swine stable, but there was no effect on bronchial responsiveness compared to WT mice [28].

Assuming a ventilation rate of 15 – 20 litres per minute during the light work carried out in the pig barn, the total ventilation during three hours would be approximately 3 m^3 leading to a total endotoxin exposure of <200 ng (≈63 ng/m^3) which should be compared with the exposure of 53.4 μg LPS during the LPS-challenge. Furthermore, inhalation of LPS did not influence the level of exhaled NO in either group while dust exposure increased exhaled NO-levels in controls and smokers but not in farmers. In addition, the cell and cytokine (IL-6 and

IL-8) response assessed in sputum was generally stronger after exposure to dust than LPS. The differences between LPS and dust exposure are intriguing considering that the endotoxin dose after LPS challenge is more than 200 times higher than the total endotoxin exposure during three hours work in a pig barn. These findings strongly support the idea that endotoxin is not the most important pro-inflammatory constituent of organic dust from pig barns. Previous results indicate that microbial products from Gram-positive bacteria may be of importance for the biological reaction to exposure in a pig barn [29, 30]. From these data it may also be concluded that the attenuated response observed in farmers and to some extent in smokers, is not due to endotoxin tolerance.

The pre-exposure exhaled NO-levels were higher in farmers than in controls, which may be due to daily exposure to microbial products in the farming environment [31]. We confirmed previous findings of low levels of exhaled NO in smokers in whom down-regulation of NO-synthase has been demonstrated [32]. Exhaled NO was unaffected after LPS exposure in all three groups. This is in conflict with earlier findings of elevated exhaled NO following LPS exposure in a study similar to ours [33]. Exposure in the pig barn induced increased exhaled NO-levels in controls and smokers but not in farmers. The difference in post-exposure increases in NO-levels may be explained by the different pre-exposure levels, as the absolute post-exposure NO-levels are similar in the three groups.

The farmers exhibited an attenuated cell and cytokine response after pig barn exposure compared with smokers and controls, as assessed in nasal lavage fluid and sputum. These exposure-induced differences cannot be explained by different pre-exposure values which were similar in the three groups. Chronic exposure in the farming environment includes exposure to high amounts of bacteria and microbial products, an exposure which in the long run may influence the inflammatory response to irritating stimuli, and pathogen-associated molecular patterns (PAMP). It has been shown that smokers exhibit down regulation of Toll-like receptor 2 (TLR2) on alveolar macrophages, a receptor that binds PAMP [34] and we have recently demonstrated reduced expression of TLR-2 on blood monocytes in farmers [35]. It cannot be excluded that this down-regulation of TLR-2 expression is related to the attenuated inflammatory response [34, 35].

Cigarette smoke contains high levels of endotoxin and it has been demonstrated that smoking one cigarette results in the inhalation of 17.4 pmol of endotoxin and that indoor exposure to environmental tobacco smoke leads to the inhalation of 12.1 pmol of LPS/m^3 [15]. Laan et al showed that cigarette smoke extract inhibited LPS-induced production and mRNA expression

of GM-CSF and IL-8 in human bronchial epithelial cells [36]. Their data indicated that cigarette smoke possesses immunosuppressive properties in the airways by down-regulating the pathogen-induced production of neutrophil-mobilizing cytokines. We therefore hypothesized that adaptation to acute exposure to organic dust occurs in smokers as well as in farmers. We found a negative correlation between exhaled NO following dust exposure and smoking habits, possibly indicating an attenuated response in the most heavy smokers. On the other hand, the sputum IL-6 and IL-8 response to LPS exposure was stronger in smokers than in controls and farmers, which is in line with the finding of a more pronounced increase in peripheral blood neutrophils after LPS provocation in smokers compared with controls and farmers (Sahlander et al submitted). There are thus factors which support the idea that smokers may respond more strongly to LPS exposure than non-smokers, thereby contradicting the hypothesis of LPS adaptation in smokers. These observations are supported by the finding by Wesselius et al who showed that the recovery of neutrophils and IL-1β concentration in BAL fluid from smokers exceeded that of non-smokers after LPS inhalation [37].

In conclusion, we have demonstrated that clinical, physiological and inflammatory airway responses to acute pro-inflammatory agents are attenuated in farmers and (to some extent) smokers compared with controls. It is suggested that the adaptation, clearly identified in farmers, is a result of long-term daily exposure to organic material. However, exposure to the farming environment and tobacco smoke do not activate identical adaptive mechanisms. The results strongly indicate that endotoxin is not the most important pro-inflammatory agent in organic dust in a pig barn. It is unclear whether the adaptation to exposure, with down-regulation of inflammatory responses, is a significant factor in the increased prevalence of chronic bronchitis and COPD observed in farmers and smokers.

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Figure legends

Figure 1.

Bronchial responsiveness to methacholine ($PD_{20}FEV_1$) at baseline, after LPS-challenge and after exposure in the pig barn. Horizontal lines indicate medians and brackets indicate within group (pre- to post-exposure) differences. Pre-exposure $PD_{20}FEV_1$ did not differ between the groups (F=2.60; p=0.09, ANOVA; p=0.073, Kruskal-Wallis test). There was no significant difference between the groups with regard to the reaction to LPS (F=0.02; p=0.98). Exposure in the pig barn induced a significantly greater enhancement of bronchial methacholine responsiveness in the controls compared with farmers and smokers (p<0.001).

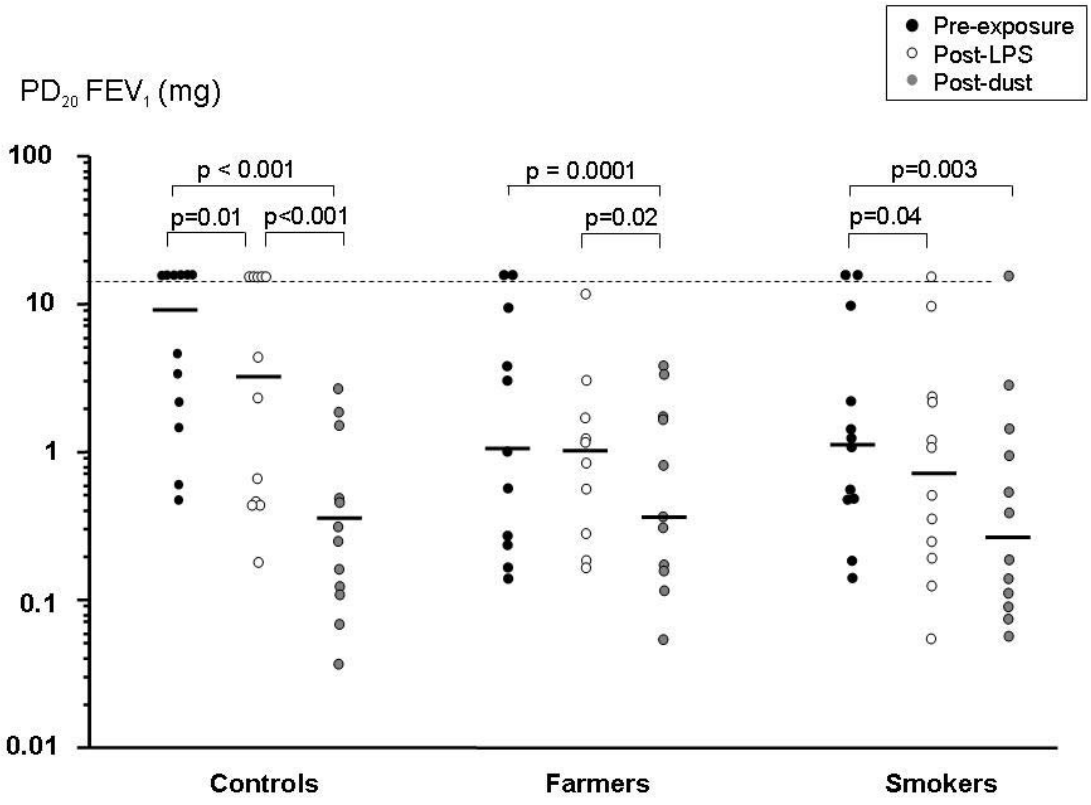

Figure 2.

Exhaled nitric oxide at baseline and following LPS and pig barn exposure. Brackets indicate within group (pre- to post-exposure) differences. LPS did not influence exhaled NO-levels in

either group (F=1.11; p=0.34). Exhaled NO increased more in controls (p=0.003) and smokers (p=0.02) than in farmers after pig barn exposure.

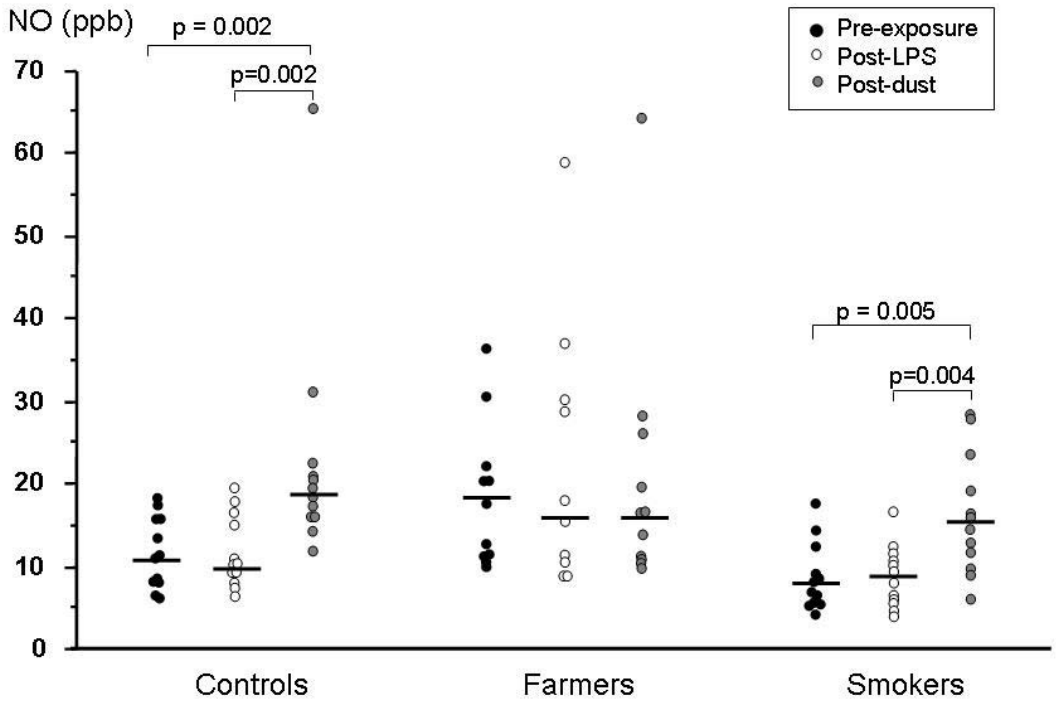

Figure 3.

The concentration of IL-6, IL-8, and total cell count in nasal lavage fluid before and after exposure in a pig barn. Brackets indicate within group (pre- to post-exposure) differences.

Pre-exposure: No differences between the groups with regard to IL-6 (p=0.44), IL-8 (p=0.28) or cell count (p=0.88) in nasal lavage fluid were found.

Post-dust increase in nasal lavage fluid: IL-6 increase: No difference between the groups (p=0.30). IL-8 increase: controls *vs* farmers (p=0.014); smokers *vs* farmers (p=0.010); controls *vs* smokers (p=0.083). Cell count increase: controls *vs* farmers (p=0.002); controls *vs* smokers (p=0.043).

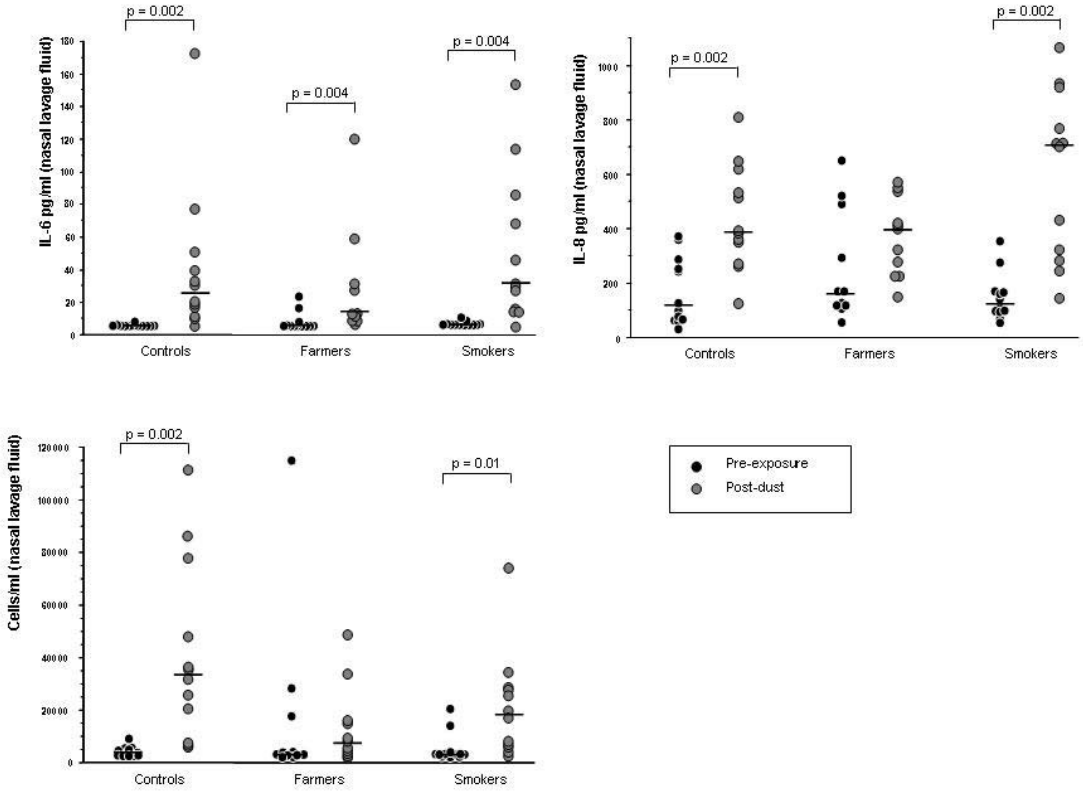

Figure 4.

The concentration of IL-6, IL-8 and total cell count in sputum before and 7 hours after LPS-challenge and exposure in a pig house. Brackets indicate within group differences.

Pre-exposure: IL-6: controls *vs* smokers (p=0.001). IL-8: controls *vs* farmers (p=0.007); controls *vs* smokers (p=0.015). Cell count: no difference between the groups (p=0.76).

Post-LPS increase: IL-6: controls *vs* farmers (p=0.010); controls *vs* smokers (p=0.05) and smokers *vs* farmers (p=0.002). IL-8: smokers *vs* controls (p=0.013); smokers *vs* farmers (p=0.018). Total cell number: controls *vs* farmers (p=0.047); smokers *vs* farmers (p=0.019).

Post-dust increase: IL-6: controls *vs* farmers (p=0.049); smokers *vs* farmers (p=0.016). IL-8: smokers *vs* farmers (p=0.023). Total cell count: no difference (p=0.92).

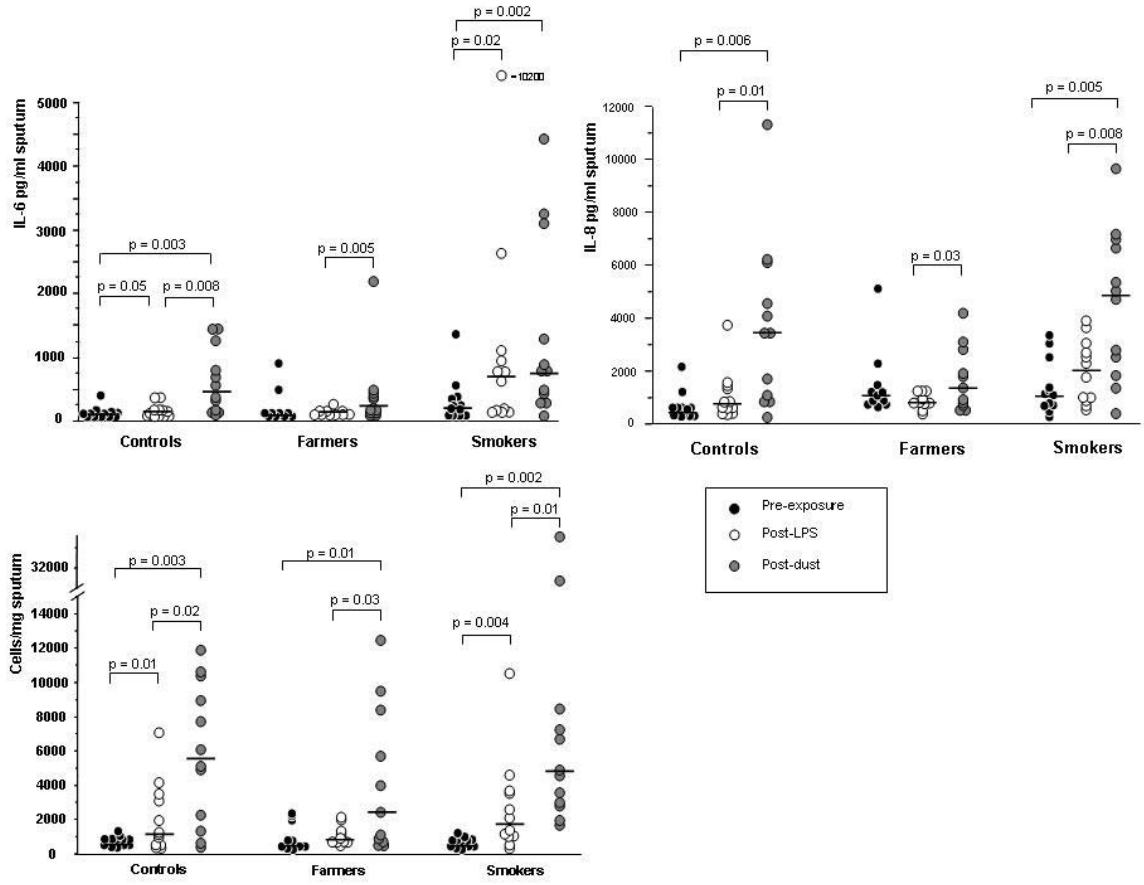
IL-6 pg/ml sputum
p = 0.002
p = 0.02
10200
5000
4000
3000
2000
1000
0
p = 0.003
p = 0.05
p = 0.008
p = 0.005
Controls
Farmers
Smokers
IL-8 pg/ml sputum
p = 0.006
p = 0.01
12000
10000
8000
6000
4000
2000
0
p = 0.005
p = 0.008
p = 0.03
Controls
Farmers
Smokers
Cells/mg sputum
p = 0.002
p = 0.01
32000
14000
12000
10000
8000
6000
4000
2000
0
p = 0.003
p = 0.02
p = 0.01
p = 0.01
p = 0.03
p = 0.004
Controls
Farmers
Smokers
Pre-exposure
Post-LPS
Post-dust

Table 1. Baseline (pre-exposure) characteristics of the three groups. Between group comparisons were assessed by ANOVA and Fisher´s PLSD for lung function and Kruskal-Wallis and Mann-Whitney U-tests for exhaled NO. C I = confidence interval.

	Controls	**Farmers**	**Smokers**	
Subjects, n	12	11	12	
Females/males	2/10	1/10	1/11	
Age, years Mean (range)	33 (25-54)	41 (25-58)	41 (22-61)	
Height, cm Mean (SD)	181 (9)	178 (7)	180 (6)	
Weight, kg Mean (SD)	77 (3)	77 (10)	81 (14)	
Pack years Mean (SD)	-	-	21 (13)	
Cough, %	0	18	58	
Wheeze, %	0	9	42	
Breathlessness, %	0	9	8	
FEV_1, L % predicted value Mean (95% C I)	4.64 (4.06-5.21) 102 (94-110)	3.76 (3.13-4.39) 92 (80-103)	3.82 (3.35-4.29) 94 (85-104)	F=3.78;p=0.03 C *vs* F p=0.02 C *vs* S p=0.03 F *vs* S p=0.86
VC, L % predicted value Mean (95% C I)	5.56 (4.91-6.22) 96 (88-104)	4.73 (4.04-5.43) 88 (76-99)	4.96 (4.45-5.47) 92 (83-100)	F=2.32; p=0.11
FEV1/VC, % Mean (95% C I)	83 (80-87)	79 (75-83)	77 (72-82)	F=3.09; p=0.06
NO, ppb Mean (95% C I)	11.8 (8.9-14.6)	18.7(12.8-24.5)	8.4(5.6-11.1)	p=0.001 C *vs* F p=0.04 C *vs* S p=0.04 F *vs* S p=0.001

Table 2. Changes in lung function in healthy non-smoking, non-farming controls, smokers and farmers. Differences between pre-exposure and 7 hours after LPS-challenge and exposure in a pig barn. Mean and 95% confidence interval. The influence of LPS and pig barn exposure did not differ significantly between the three groups.

	ΔVC c LPS	**p**	**ΔVC (L)** Dust	**p**	**FEV_1 (L)** LPS	**p**	**FEV_1 (L)** Dust	**p**
Controls	-0.12 -0.21 – -0.04	**0.008**	-0.22 -0.32 – -0.13	**0.0004**	-0.10 -0.18 – -0.02	**0.02**	-0.26 -0.35 – -0.17	**<0.0001**
Farmers	-0.01 -0.13 – 0.10	0.8	-0.10 -0.17 – -0.03	**0.01**	-0.05 -0.18 – 0.08	0.4	-0.14 -0.26 – -0.03	**0.02**
Smokers	-0.09 -0.23 – 0.05	0.2	-0.21 -0.39 – -0.03	**0.02**	0.02 -0.11 – 0.15	0.8	-0.12 -0.25 – 0.003	0.06

Tabel 3. Cell differential counts and squamous cell content in induced sputum samples in healthy non-smoking, non-farming controls, smokers and farmers before and 7 hours after exposure in a pig barn and a LPS-challenge. Values are given as medians and 25^{th}-75^{th} percentiles. No eosinophils were observed in the sputum samples.

	Controls			**Farmers**			**Smokers**		
	Pre-exposure n=11	Post-LPS n=10	Post-dust n=12	Pre-exposure n=9	Post-LPS n=7	Post-dust n=10	Pre-exposure n=11	Post-LPS n=12	Post-dust n=12
Macrophages %	91.5 77.6-93.4	42.5 33.5-50	6.5 4.5-12.8	62.8 29-88.5	30 18-62.2	7.5 4.8-12-8	43 24.6-63.5	18.2 7.5-42.2	4.2 2.8-5.8
Neutrophils %	5.5 4.1-16.4	52.2 47.5-59.5	90.5 84.5-94.5	36.5 8-67.5	67 33.9-81.2	87.5 83.8-92.5	52 33.7-74.2	75.2 52.8-88.2	94.5 92.5-95.8
Lymphocytes %	3 1.6-5.4	3 2-5.5	2 1.2-4	2.2 0.5-3.2	2.5 0.1-3.5	2 1-4.9	1.5 0.2-2.9	4 1.8-7	1.5 0.8-1.8
Squamous cells %	25 12.2-33	12.5 3-20	4 1.5-8.5	13 7.8-26	25 10.2-29.8	12.5 3-21	11 6.8-16.8	10.5 4.5-23.5	5 2-10.5