Original article

Scand J Work Environ Health 2006;32(2):145-153

doi:10.5271/sjweh.990

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Corrections

See 2007;33(2):160 for a correction.

The following article refers to this text: 2009;35(5):376-383

Key terms: airway inflammation; bronchial responsiveness; exposure; gas filter; gases; particle filter; pig confinement building; respirator

This article in PubMed: www.ncbi.nlm.nih.gov/pubmed/16680385

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Effect of respirators equipped with particle or particle-and-gas filters during exposure in a pig confinement building

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Sundblad B-M, Sahlander K, Ek A, Kumlin M, Olsson M, Larsson K, Palmberg. Effect of respirators equipped with particle or particle-and-gas filters during exposure in a pig confinement building. *Scand J Work Environ Health* 2006;32(2):145–153.

Objectives This study compared the protective effect of two respiratory protection devices during exposure in a pig confinement building.

Methods Thirty-six healthy persons were exposed for 3 hours in the building, 12 without any protection, 12 with a particle-filter mask, and 12 with a mask filtering both particles and gases. Symptoms, body temperature, nasal lavage fluid, exhaled nitric oxide, and bronchial responsiveness to methacholine were assessed before and after the exposure. Pre- and postexposure urine and blood samples were collected.

Results After the exposure, the participants with respirators reported fewer symptoms than those without. Wearing a mask also reduced the inflammatory response assessed with nasal lavage (cell concentration, interleukins 6 and 8) and peripheral blood (cell number). Lung function was significantly impaired only in the unprotected group; postexposure vital capacity and forced expiratory volume in 1 second showed a decrease of 3–4% from the preexposure levels (P=0.006 and P=0.002, respectively). Bronchial responsiveness (P<0.01) and body temperature (P<0.001) increased similarly in the three groups. Bronchial responsiveness to methacholine increased 2.7, 2.4, and 2.1 doubling concentration steps for those unprotected, those using a particle-filter mask, and those using a mask with particle and gas filters, respectively. The prostaglandin D_2 metabolite, 9α, 11β-PGF_2 increased significantly (P=0.003) only in those unprotected.

Conclusions Wearing a respirator in a pig confinement building reduces the inflammatory reaction but does not influence the increase in bronchial responsiveness, with no difference between the use of a particle-filter mask or a mask with a particle-gas filter combination.

Key terms airway inflammation; bronchial responsiveness; gases.

Pig confinement workers have an increased prevalence of respiratory symptoms, dominated by cough and phlegm (1). In healthy nonfarmers, exposure in pig confinement facilities causes a massive influx of inflammatory cells (2–5) into the respiratory tract, and about a threefold increase in bronchial responsiveness to methacholine (6, 7). The upper and lower airway inflammatory reaction in healthy, previously unexposed persons is characterized by a multifold increase in inflammatory cells, predominately neutrophilic granulocytes and pro-inflammatory cytokines such as interleukins 6 and 8 (IL-6 and IL-8, respectively) and tumor necrosis factor (TNF) in blood and nasal and bronchoalvolar lavage fluid (4, 5, 8, 9).

When respirators with a particle filter were used during a 3-hour exposure in a pig confinement building the increase in exhaled nitric oxide, as well as the inflammatory response in the nose, were considerably attenuated in a group wearing a mask when compared with an unprotected group (7, 10). Mask usage has undisputable protective effects on airway inflammation and exhaled nitric oxide after exposure; nevertheless, the use of a mask did not influence the increase in bronchial responsiveness. The effects of using a respirator, including a reduction in the increase in bronchial responsiveness to methacholine, the cellular increase and cytokine release, has been shown in a study by Dosman et al (11).

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In piggeries, high levels of gases, including hydrogen sulfide, ammonia, carbon dioxide, and methane, are generated (12). Since particle filters did not influence the increase in bronchial responsiveness after exposure, we hypothesized that gases such as ammonia may be responsible for the effect on bronchial responsiveness. Therefore, we carried out a controlled study of 12 healthy volunteers who were exposed to two different concentrations of ammonia (5 and 25 ppm) in an exposure chamber. In that study, ammonia neither induced upper-airway inflammation nor increased bronchial responsiveness after 3 hours of exposure (13).

Thus ammonia by itself does not seem to be responsible for the increased bronchial responsiveness observed after exposure in a pig confinement building. The purpose of this study was to determine whether gases other than ammonia are of importance in this respect. For this purpose, we monitored health effects after pig house exposure among participants using respirators with only particle filters, both a particle and a gas filter, or no protection during exposure. In addition, we investigated two different methods for measuring ammonia exposure and evaluated the use of two different filters for measuring dust exposure during exposure in a pig confinement building.

Study population and methods

Study population

Thirty-six (20 women) healthy, nonsmoking (not smoked during the last 6 months prior to the study) persons (table 1) were exposed in a pig confinement building while weighing pigs for 3 hours. The mean age was 25 (range 20–43) years, and they were all previously unexposed or only occasionally exposed in a farming environment. All of the participants denied ongoing respiratory infection and past or present symptoms of allergy and airway diseases. A skin prick test (12 most common allergens in Sweden) was carried out, and the participants underwent a physical examination. All of the participants had a negative skin prick test and were healthy in a physical examination. The participants gave their informed consent and the study was approved by the ethics committee of the Karolinska Institute.

Study design

All of the participants were exposed for 3 hours in a pig confinement building while assisting a farmer weighing pigs. Twelve persons were randomized into a group wearing a half mask with a particle filter and 12 into a group wearing a half mask with both a particle and a gas filter during exposure. As a control group, 12 persons were exposed without protection. Five to seven persons were exposed at each occasion on six different days. To eliminate the risk of different exposure in the groups, participants from all of the groups were exposed on each occasion.

All of the participants underwent spirometry and a bronchial provocation test with methacholine within 2 weeks before the exposure and 7 hours after the beginning of the 3-hour exposure. Exhaled nitric oxide was measured, and nasal lavage and blood sampling were also carried out prior to the metacholine test. Urine was sampled twice during the preexposure day and three times after the exposure in the buildging.

Immediately before and after the exposure, body temperature and peak expiratory flow (PEF) were measured. Body temperature was then repeatedly measured up to 7 hours and PEF up to 5 hours after the start of exposure. In addition, symptoms were recorded before and 4 and 7 hours after the participants entered the pig confinement facility.

Symptoms

Symptoms were registered on a visual analogue scale (VAS), 0–100 mm. The participants were requested to put a cross on the scale where 0 indicated no and 100 unbearable symptoms. Five general symptoms and seven airway-specific symptoms were recorded before and after the exposure.

Lung function and bronchial responsiveness

Vital capacity (VC) and forced expiratory volume in 1 second (FEV_1) were measured using a wedge spirome-

Table 1. Baseline characteristics and lung function values of the participants before the exposure. (VC = vital capacity, FEV_1 = forced expiratory volume in 1 second)

Group	Gender		Age (years)		VC (% of the predicted value)		FEV_1 (% of the predicted value)	
	Male	Female	Mean	Range	Mean	95% CI	Mean	95% CI
Without protection	4	8	27	20–43	93	88–98	97	92–103
Mask with particle filter	6	6	24	20–28	95	88–102	100	92–108
Mask with particle and gas filters	6	6	25	21–39	97	91–103	101	94–108

ter (Vitalograph®, Buckingham, UK) according to the criteria of the American Thoracic Society (14). Local reference values were used (15, 16). Peak expiratory flow (PEF) was measured using a peak flow meter (Mini-Wright, Clement Clarke International Ltd, London, UK).

Bronchial responsiveness was assessed by a methacholine challenge performed with inhalation of diluent followed by increasing doubling concentrations of methacholine, 0.5–32 mg/ml. The method has previously been described in detail (17–19). The result was expressed as the cumulative dose causing a 20% decrease in FEV_1 ($PD_{20}FEV_1$) (20).

Measurement of exhaled nitric oxide

Nitric oxide in exhaled air was measured using a single-breath exhalation with an exhalation flow of 50 ml/s in accordance with recommendations of the American Thoracic Society (21). Exhaled nitric oxide was analyzed with chemiluminescence after reaction with ozone (NIOX®, Aerocrine, Stockholm, Sweden). The mean of three measurements was used for the calculations.

To decrease contamination from the oral cavity, mouthwash with water and sodium bicarbonate (10%) was used during 1 minute prior to the measurement procedure. All of the participants avoided vegetables before the measurements (22).

Nasal lavage

Nasal lavage was performed using a procedure described by Bascom et al (23), with minor modifications (4). Five milliliters of sterile 0.9% sodium chloride 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. The lavage sample was centrifuged, and the numbers of cells were counted in a Bürker chamber. The supernatant was frozen (–70°C) for future analysis.

Blood analyses

Whole peripheral blood was collected in ethylene diamine-tetra-acetic acid (EDTA) vacutainer tubes (BD Bioscience, New Jersey, USA). All of the tests were performed within 2 hours after the sampling.

To determine the absolute count of cells, TruCOUNT™ tubes (BD Bioscience, San Jose, CA, USA), containing a specified number of beads, were used. Fifty microliters (reverse pipetting) of well-mixed whole blood and 20 µl of peridinin chlorophyll protein conjugated anti-CD45 antibody (BD Bioscience, San Jose, CA, USA) were added to the TruCOUNT tube. The tubes were incubated in darkness at room temperature for 15 minutes. To lyse red blood cells, 450 µl 1 × FACS Lysing Solution™ (BD Bioscience) was added and followed by a 10-minute incubation, in darkness, at room temperature. All of the samples were then analyzed on a FACS Calibur™ (BD Bioscience). To determine absolute counts of lymphocytes, monocytes, neutrophils, eosinophils, and basophiles, the samples were analyzed in Attractors ™ (BD Bioscience, San Jose, CA, USA) using the TruCOUNT beads as an internal reference population. The samples were analyzed in an Attractor set made to perform a five-part white blood cell differential.

Cytokines in nasal lavage and blood

IL-6 in peripheral blood and nasal lavage fluid and IL-8 in nasal lavage fluid were determined using an enzyme-linked immunosorbent assay developed at our laboratory using commercially available antibody pairs (R&D systems, Europe, Abingdon, UK) (24). The lower detection limit was 3 ng/l for IL-6 and 50 ng/l for IL-8. For duplicated samples, an intraassay coefficient of variation (CV) of <10% and an interassay CV of <20% was accepted.

Measurement of 9α,11β-PGF_2 in urine

An analysis of 9α, 11β-PGF_2 [9α,11β-(15S)-trihydroxy-prosta-5, 13-dienoic acid] in the urine was carried out without prior purification with enzyme immunoassay (Cayman Chemical, Ann Arbor, MI, USA) using rabbit polyclonal antiserum and an acetylcholine esterase-linked tracer as described previously (25). Creatinine was determined in all of the urine samples using a colorimetric assay (Sigma Chemical Company, St Louis, MO, USA), and the results are expressed as nanograms per millimole of creatinine. The mean value of two samples obtained on the preexposure day was compared with the peak value obtained in one out of three samples collected after exposure on the exposure day.

Mask and filters

A half mask, SR 100 (Sundström Safety, Lagan, Sweden) was used. The mask was equipped with either a particle filter (P3, 210/310, Sundström Safety, Lagan, Sweden) or with a particle filter and a gas filter (297, A1B1E1K1, Sundström Safety, Lagan, Sweden). According to the manufacturer, the particle filter separates 99.997% of the pollution in the air and protects against all types of particles, even bacteria and viruses. The gas filter protects against organic compounds with a boiling point above 65°C, inorganic and acid gases, and vapors. This type of filter also includes ammonia protection.

Exposure measurement

IOM filter cassettes (25 mm) (SKC LTD, Dorset, UK) and plastic cyclones (25 mm) (Casella LTD, London, UK) were used to monitor respiratory and inhalable dust levels, respectively. Particles with a size of < 5μm were defined as respirable dust. We defined inhalable dust as particles ≤10 μm. Large particles (5–10 μm) are trapped in the nose, throat, and upper airways (26). The samplers were placed in the breathing zone of 1–2 persons on each exposure occasion. The cassettes were equipped with teflon filters (Millipore, Sundbyberg, Sweden) or with glass fiber filters. To estimate the personal exposure, nasal air samplers were worn in each nostril for 10 minutes per exposure hour. Intranasal exposure assessment has previously been described in detail (27). After the filters were weighed, these and the nasal samplers were extracted, and the endotoxin concentration was analyzed by the use of a chromogen version of the *Limulus amebocyte* lysate assay (QCL-1000, Endotoxin, BioWittaker, Walkersville, USA, with *Escherichia coli* 0111:B4 as the standard).

Ammonia and hydrogen sulfide exposure was measured in the breathing zone (1.5 m above the floor in the middle of the barn) with Draeger tubes (Svenljunga, Sweden) on three occasions during each exposure. The detection range was 2–30 ppm for ammonia and 0.5–15 ppm for hydrogen sulfide. Ammonia was also continuously measured every 30 seconds during the exposure with a Toxi Ultra device (Biosystems, Middletown, USA) in order to compare the two methods. One of the participants was wearing the Toxi Ultra during the 3-hour exposure on each occasion, and the results were analyzed afterwards.

Statistics

The results are presented as means and 95% confidence intervals (lung function, symptoms, and temperature) or as median values with 25th–75th percentiles (exhaled nitric oxide, bronchial responsiveness and nasal lavage fluid, and blood and urine results) depending on the distribution of the data. An analysis of variance (ANOVA) and Student's t-test were used for comparisons of the data that were assumed to be normally distributed (lung function, symptoms, and temperature) and for the comparisons between the independent groups. Otherwise, the Kruskal-Wallis test, followed by the Mann-Whitney U-test of individual pre- and postexposure differences were performed for comparisons between the groups. Within-group pre- and postexposure comparisons for exhaled nitric oxide, bronchial responsiveness, and nasal lavage fluid, blood and urine results were calculated with the use of Wilcoxon's signed rank test. Correlations were estimated with the Spearman rank correlation test. A P-value of <0.05 was considered significant. When multiple comparisons were made, a P-value of <0.01 was considered significant.

Results

Symptoms

In the group without a mask, the mean value increased for cough by 22 (95% CI 8–36) mm (P=0.006), that for chest tightness by 19 (95% CI 6–31) mm (P=0.008), and that for shivering by 15 (95% CI 4–26) mm (P=0.01) on the visual analogue scale. Cough and chest tightness were still present in the unprotected group 7 hours after the exposure. The mean for chest tightness increased by 10 (95% CI 2–17) mm (P=0.02) and that for shivering by 20 (95% CI 2–8) mm (P=0.03) 4 hours after the exposure among the participants using a mask with a particle filter, but not in the group using a mask with both a particle and a gas filter. Chest tightness, but not shivering, differed significantly between the two groups with filters (P=0.01).

Body temperature increased significantly in all of the groups (P<0.001), 0.60 (95% CI 0.43–0.77)°C, 0.52 (95% CI 0.27–0.76)°C, and 0.48 (95% CI 0.25–0.72)°C in the group without a mask, with a particle filter, and with a particle and a gas filter, respectively. There were no significant differences between the groups.

Lung function and bronchial responsiveness

The preexposure lung function was normal for all of the participants. The fall in VC was 0.15 (95% CI 0.05–0.25) liters (P=0.006) and that of FEV_1 was 0.17 (95% CI 0.07–0.24) liters (P=0.002) after the exposure in the unprotected group, whereas VC and FEV_1 did not change significantly in the groups wearing masks. There was a significant difference between the groups with a particle filter mask and the unprotected group regarding the exposure-induced fall in FEV_1 (P<0.03) and VC (P<0.03). The lowest PEF values were measured 4 hours after the exposure, and they were significantly reduced at that time only in the group without the use of a mask [39 (95% CI 25–52) l/min (P=0.005)]. For PEF, a significant difference was found between the group without a mask and the group which wore both particle and gas filters (P=0.001).

Bronchial responsiveness to methacholine increased 2.7, 2.4, and 2.1 doubling concentration steps among the participants without protection (P=0.002), those with a particle filter (P=0.002), and those with both a particle and a gas filter (P=0.003), respectively. The increase in bronchial responsiveness did not differ significantly between the groups (figure 1).

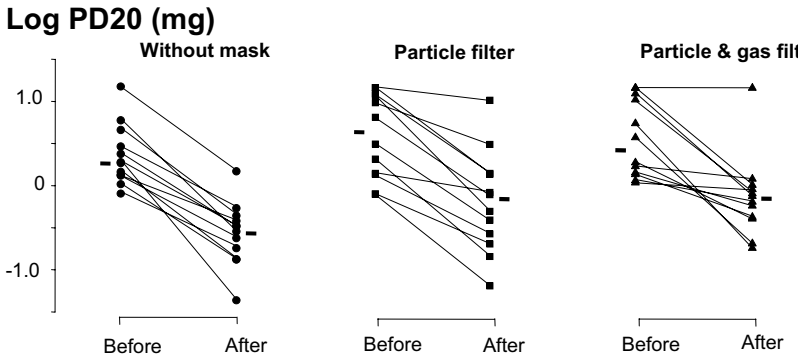

Figure 1. Bronchial responsiveness to methacholine (PD_{20}) increased by 2.7 doubling concentration steps (P=0.002) for the participants without protection (N=12), by 2.4 (P=0.002) for the participants wearing a mask with a particle filter (N=12), and by 2.1 (P=0.003) for those wearing a mask with both a particle and a gas filter (N=12), after exposure in a pig confinement building. The median values are indicated in the figure. The increase in bronchial responsiveness did not differ significantly between the groups.

Exhaled nitric oxide

The exhaled nitric oxide concentration before and after the exposure was 12.7 (95% CI 9.0–16.6) ppb and 15.5 (95% CI 11.2–18.6) ppb in the unprotected group, 9.3 (95% CI 7.5–11.7) ppb and 9.6 (95% CI 7.8–12.7) ppb in the group using a particle filter, and 13.0 (8.7–15.7) ppb and 9.7 (7.0–13.3) ppb in the group with two filters, respectively. The change in the nitric oxide concentration from before to after the exposure was not significant for any of the groups, and there were no significant differences between the groups.

Nasal lavage analysis

The results for total cells, IL-6, and IL-8 in nasal lavage fluid are given in table 2.

Blood analyses

The total number of leucocytes in peripheral blood increased significantly in all three groups 7 hours after the start of the exposure (P<0.05). The leucocytes in the group without a mask increased 2.1 (95% CI 1.9–2.6) times after the exposure, and this increase was significantly higher in the unprotected group than in the two groups with respiratory protection (P<0.001). The leucocyte increase in blood was mainly due to an increase in the neutrophilic granulocytes (figure 2), and this increase was significantly greater in the group without a mask than in the other two groups (P<0.001). The monocytes increased significantly in all three groups and significantly more in the group without the use of a mask than in both of the protected groups (P<0.05).

The IL-6 concentration in blood increased from <3 ng/l (detection limit) before the exposure to 10.2 (95% CI <3–12.8) ng/l after the exposure in the unprotected group (P=0.01). No significant increase in the IL-6 concentration in blood was found in the other two groups, as was found for the unprotected group—particle filter users (P=0.02) and particle and gas filter users (P=0.002).

Table 2. Nasal lavage analyses of cells and interleukins 6 and 8 (IL 6 and IL-8, respectively). P-values represent comparisons within groups (Wilcoxon's signed rank test). Comparisons between groups were calculated using the differences between the pre- and post-exposure values for each individual (Kruskal–Wallis test followed by Mann -Whitney U test)

	Total cells concentration × 10^9/l [a]		IL-8 (ng/l) [b]		IL-6 (ng/l) [c]	
	Median	25th–75th percentile	Median	25th–75th percentile	Median	25th–75th percentile
Without protection (A)						
Before	5.6	2.9–15.4	5.7	2.9–9.2	5.2	0.9–9.5
After	31.3	14.2–115.0	10.0	4.8–22.1	12.8	7.4–16.9
P-value	0.002		0.14		0.003	
Mask with particle filter (B)						
Before	152	49–270	157	112–275	62	50–137
After	418	303–579	171	141–246	220	131–281
P-value	0.003		0.75		0.002	
Mask with particle and gas filter (C)						
Before	2.9	2.9–3.4	3.7	2.9–7.5	<2.9	<2.9–<2.9
After	36.7	18.3–62.1	7.9	4.2–15.0	7.3	4.5–14.5
P-value	0.02		0.04		0.005	

[a] Between-group comparisons for total-cell concentration: A versus B: P=0.003; A versus C: P=0.03; B versus C: P=0.12.
[b] Between-group comparisons for IL-8: A versus B: P=0.002; A versus C: P=0.04; B versus C: P=0.008.
[c] Between-group comparisons for IL-6: A versus B: P=0.02; A versus C: P=0.03; B versus C: P=0.69.

9α, 11β-PGF_2 in urine

The urinary excretion of 9α, 11β-PGF_2, increased from 27.9 (95% CI 24.3–44.9) to 46.6 (95% CI 31.3–72.9) ng/mmol creatinine after the exposure in the pig confinement building in the unprotected group (P=0.003). Pre- and postexposure urinary concentrations of 9α, 11β-PGF_2 were 27.5 (95% CI 20.9–40.6) and 32.8 (95% CI 24.6–39.5) ng/mmol creatinine (P=0.43) in the group wearing a mask equipped with a particle filter and from 37.5 (95% CI 29.7–44.2) to 42.3 (95% CI 37.5–61.1) ng/mmol creatinine (P=0.06) in the group using a mask with both a particle and gas filter. There were no significant differences between the groups. The outcome of the statistical calculation was the same if the mean of the two values after the exposure was used instead

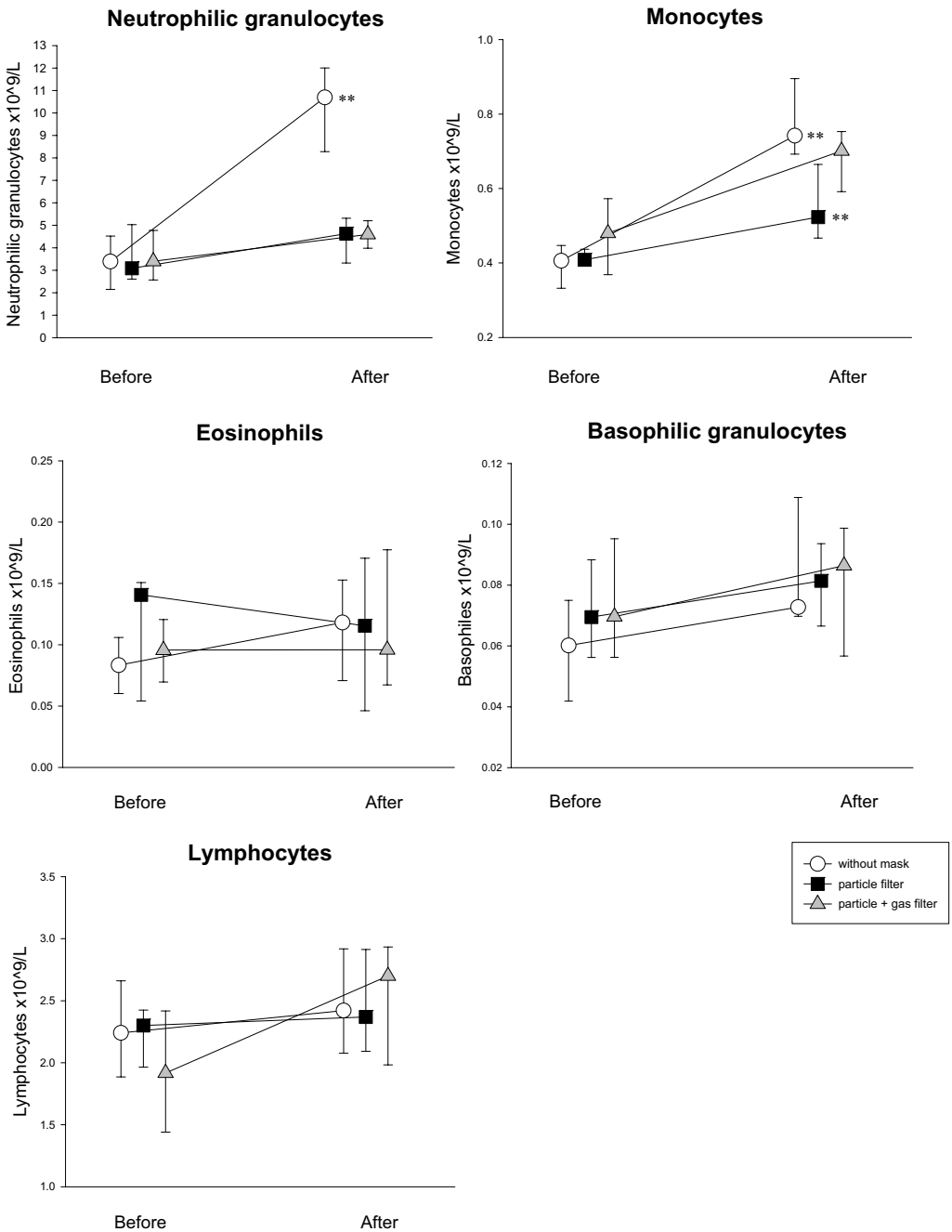

Figure 2. Absolute number of cells in peripheral blood before and 7 hours after the exposure started. There was a significantly greater increase in the neutrophilic granulocytes in the group without a mask than in the two groups with respiratory protection (P<0.001). The increase in monocytes in the unprotected group was significantly higher than in the two groups with respiratory protection (P<0.05). The values are expressed as the median (25th and 75th percentiles). Because of multiple within-group comparisons, P<0.01 was considered significant. (** indicates P<0.01 when pre- and postexposure within the group values are compared)

of the peak value. There was no correlation between the change in 9α, 11β-PGF_2 and the measure of bronchial responsiveness to methacholine (r=0.04).

Exposure measurements

The level of airborne inhalable and respirable dust was similar to that measured in previous studies carried out in the same type of conditions (28) (table 3). There were no significant differences in inhalable or respirable dust levels measured with teflon or glass fiber filters (P=0.16). Furthermore, we confirmed the previous finding that air sampling with glass fiber filters may show higher levels of endotoxin than sampling with teflon filters (P<0.01). With the use of intranasal samplers, endotoxin exposure was reduced by 93% when the mask

Table 3. Exposure levels (median, 25th–75th percentiles) measured with IOM filter cassettes (inhalable dust), plastic cyclones (respirable dust), and nasal samplers (personal exposure).

	Inhalable dust				Respirable dust				Nasal sampler	
	Dust level (mg/m³)		Endotoxin (ng/m³)		Dust level (mg/m³)		Endotoxin (ng/ m³)		Endotoxin (ng/30 min)	
	Median	25th–75th percentile	Median	25th–75th percentile	Median	25th–75th percentile	Median	25th–75th percentile	Median	25th–75th percentile
Teflon filter	7.2	6.0–8.9	91.2	73.3–113	0.47	0.39–0.51	8.0	5.6–8.3	·	·
Glass fiber filter	6.3	5.1–7.9	193	152–215	0.51	0.46–0.54	15.6	8.9–22.9	·	·
Without mask	·	·	·	·	·	·	·	·	101	61.5–407
Mask with particle filter	·	·	·	·	·	·	·	·	7.4	0.9–65.9
Mask with particle and gas filter	·	·	·	·	·	·	·	·	0.9	0.2–1.7

with a particle filter was used (P<0.0001) (compared with the unprotected group) and by 99% when the mask equipped with the particle and gas filters (P<0.0001) was used.

The airborne levels of hydrogen sulfide were below the detection limit (<0.05 ppm, the threshold limit in Sweden being 10 ppm) on all of the exposure occasions. The median value of the ammonia concentration on six exposure occasions was 7.0 (95% CI 5.1–8.5) ppm when measured with Draeger tubes and 5.2 (95% CI 3.6–6.8) ppm when measured with the Toxi Ultra device. There was a good correlation (r=0.88) between the two methods, and the outcome with the use of the Draeger tubes was approximately 10% higher than with the Toxi Ultra method.

Discussion

The present study confirms our previous findings that the use of respiratory protection during exposure in swine confinement buildings attenuates the airway inflammatory response (10, 11). We also showed that the use of protection devices reduces symptoms induced by exposure, although the participants using respirators with only a particle filter still reported symptoms, for example, chest tightness and shivering, while the participants using both particle and gas filters reported no significant symptoms. However, the increases in body temperature and bronchial responsiveness were similar in all three groups despite the fact that the use of respirators reduced the endotoxin exposure by 94% with the particle filter and 99% with the combined gas and particle filters.

In this study, we used a design in which the effects of exposure were assessed in different compartments—bronchial responsiveness and inflammatory markers in nasal lavage fluid, blood, and urine. In a previous study, we found that bronchial responsiveness increased significantly both in those who used a mask and in those without a mask, although less among the participants wearing masks with particle filters (10). In our present study, we anticipated a protective effect on the increase in bronchial responsiveness when gas exposure was eliminated by the use of a gas filter together with a particle filter. However, the increase in bronchial responsiveness after the exposure was similar in the three groups. The data shown in figure 1 may indicate a slightly attenuated response in the group that used the combined particle and gas filters when compared with the other two groups, but this difference was not statistically significant. The FEV_1 decreased only in the unprotected group, but the increase in bronchial responsiveness was similar in all three groups, this finding indicating that the change in airway caliber (reflected by the effect on the FEV_1) may not be the cause of the increase in bronchial responsiveness after the exposure.

Previous studies have suggested a relationship between increased bronchial responsiveness and mast-cell mediator release (25). After exposure in the pig confinement building, the PGD_2 metabolite 9α, 11β-PGF_2 increased in the urine of the participants without a mask. This result confirms previous findings that indicated that mast cells are activated by this exposure. However, there were no significant differences between the groups with regard to urinary 9α, 11β-PGF_2 levels. Thus there was probably no causal relationship between PGD_2 release and the increased bronchial responsiveness.

In a previous study, we showed that exposure in a pig confinement building is associated with increased levels of exhaled nitric oxide (7), this increase was avoided by the use of a protective mask. In our present study, there was no significant increase in the exhaled nitric oxide among the participants who did not use a mask. The lack of a significant increase in exhaled nitric oxide can probably be explained by lower levels of airborne inhalable dust in the present study than in the previous study (7). Another possible explanation may be that the preexposure levels of nitric oxide were higher in the unprotected group than in the other two groups.

Using a mask with a particle filter (class P3) that protects from all kinds of particles, such as dust, smoke, bacteria, virus, etc, reduced signs of inflammation above

all in the nose and blood. The cell numbers in the nasal lavage fluid were significantly lower in the participants who used this type of mask than in the unprotected participants. This result has also been previously found in a study with a similar design but with another type of respiratory protection device (class P2) (10) and in a study using class P3 filters during the cleaning of stables (29).

The IL-6 in nasal lavage increased in all of the groups, but the increase in the unprotected group was significantly higher than in the groups using masks. Accordingly, the IL-6 conccentration in serum only increased in the unprotected group.

The gas filter protects against ammonia, carbon dioxide, methane, and hydrogen sulfide, gases that are present in pig confinement buildings. The addition of a gas filter to the protection device did not improve the protection against the biological effects after exposure, with one exception, the feeling of chest tightness. Thus gases do not seem to have an effect in addition to that mediated by the particle fraction of the dust in the pig confinement building. In addition, we have not been able to show any effect on the airways after exposure to ammonia in an exposure chamber study (13).

The nasal sampling showed that wearing an airway protection device equipped with a class P3 particle filter reduced the intranasal exposure to endotoxin by 93%, and, with both a particle filter and a gas filter, by 99%. As assessed by nasal air sampling, the endotoxin exposure of the unprotected participants was 101 ng/30 min, corresponding to ~600 ng during 3 hours of exposure. The exposure measurement with traditional IOM samplers gave similar results. The endotoxin exposure level was 91 ng/m^3 with a teflon filter and 193 ng/m^3 with a glass fiber filter. Assuming a ventilation of 16 l/min during light work in the pig confinement building, the total endotoxin exposure would be 262 ng and 555 ng, respectively. Thus nasal air sampling and sampling with a glass fiber filter result in similar estimates of endotoxin levels, and these levels are probably close to the true exposure.

Inhalation of 30–40 μg lipopolysaccharide seems to be a threshold level for inducing clinical symptoms and lung function changes, and the threshold for the induction of a rise in blood neutrophils seems to be less than 0.5 μg lipopolysaccharide in healthy participants (30). In our study, in which the endotoxin exposure was less than 0.6 μg, neutrophilic granululocytes increased in peripheral blood, and the participants reported symptoms. These effects were attenuated after protection with masks that reduced the endotoxin exposure by up to 99%. However, the variables that showed equal results for all three groups (eg, bronchial responsiveness) did not seem to be affected by the endotoxin exposure in this study).

The failure to inhibit or even attenuate the increase in bronchial responsiveness with a combination of these two types of filters may indicate that neither particles nor gas fractions caused the increase in bronchial responsiveness. It cannot be excluded that ultrafine particles (<0.1 μm) passing through the filters may induce biological effects, since these effects are observed irrespective of the use of the protection device. Bronchial responsiveness and other effects could also increase as a result of immunological effects mediated by dermal exposure (31). Furthermore, we cannot exclude leakage of particles and gases for very short periods of time during the exposure, especially during the application of the nasal samplers, which took place three times during the exposure. This finding is in line with those of two other studies with pig farmers; the studies showed that complete protection is not offered by these types of respiratory devices (32, 33). We conclude that an increase in bronchial responsiveness is caused by very low exposure levels in swine confinement buildings.

As an additional aim of this study, we measured ammonia with two different methods, with Dreager tubes (3 times/exposure occasion) and with online measurement with Toxi Ultra (every 30 seconds/exposure occasion). The values measured with Draeger tubes were generally higher, but they correlated significantly with the Toxi Ultra method. We also conclude that the Toxi Ultra registers the short peak values of exposure that may be missed with only a few measurements using the Draeger tubes.

In conclusion, exposure in a swine confinement building results in acute upper-airway inflammation and increased bronchial responsiveness to methacholine. Wearing a respirator equipped with both particle and gas filters during the exposure reduces the inflammatory reaction but not the increase in bronchial responsiveness. Gases or endotoxins do not seem to be the main agent responsible for the increase in bronchial responsiveness. Instead ultrafine particles or possibly dermal exposure may cause these health effects after exposure in a swine confinement building.

Acknowledgments

We wish to thank Dr Karin Strandberg for the physical examination of the volunteers. We also wish to thank Fernando Acevedo, Ingrid Delin, and Inger Ericson at the Institute of Environmental Medicine, Karolinska Institutet for their excellent technical assistance.

The study was supported by grants from the Swedish Heart-Lung Foundation, The Swedish Farmer Foundation for Agricultural Research, and the Karolinska Institutet.

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Received for publication: 23 March 2005