Exhaled Breath Condensate pH and FeNO as Biomarkers of Acute and Chronic Exposure to Hazards at Swine Farms

Anita Ljubičić, MD, PhD, Veda Marija Varnai, MD, PhD, Marija Vučemilo, PhD, Kristina Matković, SciD, Dinka Milić, PhD, and Jelena Macan, MD, PhD

Objective: The noninvasive biomarkers of respiratory impairment were assessed in 15 swine confinement (SC) workers and 9 respiratory healthy, nonsmoking volunteers (HV). **Methods:** Spirometry, fraction of exhaled nitric oxide (FeNO), and exhaled breath condensate (EBC) pH were assessed in SC workers after one working shift and one working week and in HV after 5-hour exposure in SC. **Results:** Half of the respiratory symptoms (in 8 of 15 SC workers) were work-related. Basal FeNO values were 7.5 ppb higher in the SC workers compared with HV. In the SC workers, EBC pH increased for 0.17 at the end of a working week ($P < 0.001$). In HV, 5-hour exposure in SC induced 8% drop in forced expiratory flow at 25% of the pulmonary volume (FEF_{25}) ($P = 0.008$), EBC pH drop for 0.10 units ($P = 0.003$), and FeNO drop by 1.8 ppb ($P = 0.047$). **Conclusions:** EBC pH was suggested as a biomarker of acute airway acidification in HV, whereas the SC workers showed signs of the "healthy worker effect."

Swine confinement (SC) workers are exposed to occupational respiratory hazards related to acute and chronic respiratory disorders.[1,2] The main causative environmental agents in SC are organic dust and ammonia.[3]

Fraction of exhaled nitric oxide (FeNO) and exhaled breath condensate (EBC) pH have recently been recognized as noninvasive biomarkers for early detection of airway inflammation in occupational settings.[4–7] The most published data imply a high correlation between FeNO increase and atopic asthma exacerbation with sputum eosinophilia,[5] whereas decreased EBC pH was shown to be related to both atopic and nonatopic airway inflammation and sputum neutrophilia.[4,6–8] Data from various occupational settings to some point confirm these observations, indicating different changes in FeNO and EBC pH in exposures to different occupational hazards. Published data regarding the influence of acute and chronic exposure to swine dust on the nitric oxide metabolism in airways are scarce and contradictory.[9,10] To our knowledge, there are no published data regarding EBC pH in workers or naive volunteers exposed to SC conditions.

This study aimed to evaluate the role of FeNO and EBC pH in differentiating the respiratory status of SC workers chronically exposed to respiratory hazards and respiratory healthy volunteers acutely experimentally exposed to this environment.

From the Institute for Medical Research and Occupational Health (Drs Ljubičić, Varnai, and Macan) and The Faculty of Veterinary Medicine, University of Zagreb (Prof Vučemilo and Dr Matković); and Swine Farm "Dubravica" (Dr Milić), Croatia.

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We declare that we do not have any conflicts of interest to disclose.

Address correspondence to: Anita Ljubičić, MD, PhD, Institute for Medical Research and Occupational Health, Occupational Health and Environmental Medicine Unit, Ksaverska cesta 2, HR-10001 Zagreb, Croatia (aljubicic@imi.hr).

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STUDY PARTICIPANTS AND DESIGN

This study comprises two groups. The first group included 15 (7 male) SC workers recruited from a swine farm in the vicinity of the city of Zagreb, Croatia, and the second, experimental, group included nine (three male) volunteers previously occupationally nonexposed to respiratory hazards. The examination for both study groups was performed during the winter of 2011 to 2012.

Of the 23 SC workers exposed to respiratory hazards at the SC for more than 20 hours per week, 15 agreed to participate in the study (response rate 65%). They worked from Monday to Friday, 8 hours per day, and had an additional working shift on Saturday or Sunday twice per month. For this group, the respiratory health parameters were assessed after a nonworking weekend on Monday before and after their work shifts and on Friday after their shifts.

The experimental group included only nonsmokers or ex-smokers who had quit smoking at least 1 year ago and were respiratory healthy, that is, without respiratory symptoms, with forced vital capacity (FVC) and forced expiratory volume in the first second (FEV_1) of 80% or more of predicted and FEV_1/FVC ratio of 70% or more.[11] For this group, the examination was performed before and after a 5-hour exposure to environmental hazards in an SC, with no physical activity included. None of the SC workers or volunteers used respiratory protection equipment during work and the experimental exposure. The legislation in this field proscribes the obligatory use of respiratory protection equipment, but unfortunately implementation of this safety measure is often insufficient. The study was approved by an authorized Ethical Committee. Potential participants have been informed regarding the aim of the study, the research methodology, and the implications of the obtained results. The exposed workers and volunteers signed an informed consent form before their inclusion in the study.

METHODS

Environmental Monitoring

The capacity of the SC building was approximately 10,000 pigs. All the environmental measurements were performed during 10 days in winter season at the worksites. Each day stationary air sampling was performed on five worksites. The temperature, air humidity, and airflow were measured with a TESTO device (Testo Inc, Lenzkirch, Germany), whereas ammonia and carbon dioxide were determined with a Dräger-Multiwarn II device (Dräger, Darmstadt, Germany). Dust was sampled onto glass fiber filters (Whatman International Ltd, Maidstone, the United Kingdom) with an SKC pump (SKC Ltd, Blandford Forum, the United Kingdom) as described previously.[12] Air samples (Merck MAS-100 device; Merck KgaA, Darmstadt, Germany) for the determination of mold and bacteria counts were collected, and molds and bacteria were grown and counted with a method described previously.[13]

Medical History

A questionnaire based on the "organic dust questionnaire"[14] was completed by a physician, who interviewed each participant. Questionnaire recorded reports on the following symptoms in the past year: symptoms of rhinitis (rhinorrhea, nasal itching, and nasal obstruction, not related to common cold); asthma (wheezing and/or dyspnea); chronic bronchitis (everyday cough with phlegm during at least 3 months per year, for two consecutive years); dry cough; phlegm (not compatible with criteria for chronic bronchitis); and general symptoms (fever and joint pain). Work-related symptoms were defined as symptoms that were experienced only during working hours, or as those that worsened during working hours and improved off work (during weekends or holidays). They included both occupationally induced and workplace exacerbated symptoms because they were not differentiated by the questionnaire. Smoking was expressed as a dichotomous variable (smokers and nonsmokers) and as a smoking index for current smokers (the number of cigarettes smoked per day multiplied by the number of smoking years). Participants who never smoked and ex-smokers who had quit smoking at least 1 year ago and were respiratory healthy were designated as nonsmokers.

Spirometry

Forced vital capacity, FEV_1, FEV_1/FVC ratio (Tiffeneau index), forced expiratory flows from 25% to 75% point of FVC (FEF_{25}, FEF_{50}, FEF_{75}, $FEF_{25–75}$) were determined by a standard method[11] with the spirometer Pneumoscreen II (Jaeger, Wurzburg, Germany). Spirometry measurement was performed after the FeNO measurement and EBC sampling. Ventilatory function parameters were expressed and analyzed as percentage of referent values.

EBC Sampling and pH Measurement

Exhaled breath condensate sampling and pH measurement were performed according to recommendations of ATS/ERS Task Force on Exhaled Breath Condensate[15] and the procedure described previously.[16] Participants provided samples breathing tidally into a commercial condenser (EcoScreen, Jaeger, Germany) for 10 minutes. EBC pH was measured after the 10-minute samples argonization (bubbling through the sample at a flow rate of 350 mL/min). pH was measured within 3 months from sampling (samples were kept at −20°C).

FeNO Measurement

The FeNO measurement was performed with the NioxMino analyzer (Aerocrine, Solna, Sweden) at a mouth flow rate of 50 mL/s over 10 seconds and a pressure of 10 cm H_2O, in accordance with the guideline recommendation.[17] This procedure was performed before lung function testing and EBC sampling. Levels of FeNO lower than 26 ppb were set as a cutoff point for "normal" FeNO values, regarding 95% upper limits found for nonatopic male nonsmokers, according to their height.[17]

Skin Prick Testing

Skin prick testing (SPT) was performed only on the SC workers, using a standard method[18] described previously[8] with a panel of 12 common inhalatory allergens: grass pollen mixture, birch, hazel, weed pollens (*Ambrosia elatior* and *Artemisia vulgaris*), mites (*Dermatophagoides pteronyssinus*, *D. farinae*, and *Lepidoglyphus destructor*), cat, dog, and molds (*Cladosporium herbarum* and *Alternaria alternata* [Allergopharma, Germany]), and additional occupational allergens—swine dander and epithelium (Institute of Immunology, Zagreb, Croatia). The results of SPT were considered positive when mean wheal diameter was larger than the negative control for more than 3 mm.

Statistical Analysis

Statistical analysis was performed with Stata/SE 11.2 for Windows (StataCorp LP, College Station, TX). General characteristics and baseline spirometry, EBC pH, and FeNO in the SC workers and the experimental group were compared by the *t* test or Fisher's exact test. Mixed-effects linear regression was used to test the change in spirometry, EBC pH, and FeNO after one working shift and one working week in the SC workers, with time, age, sex, and smoking status (smokers vs nonsmoker) as fixed effects and study participant as a random effect. The change in analyzed dependent variables was modeled separately for the period of one working shift and one working week. In the experimental group, time (preexposure measurements vs measurements obtained after a 5-hour exposure), age, and sex were introduced in the models as fixed effects and study participant as a random effect. To evaluate the potential effect of age, sex, and smoking on changes in dependent variables during the observed period, the interaction terms time × age, time × sex, and time × smoking status were tested as fixed effects in the regression models. The correlation between basal EBC pH and FeNO, as well as among basal EBC pH, FeNO, and spirometry values, was analyzed by Pearson's correlation, separately in the SC workers and the experimental group. Because there is no standard statistical software that calculates the power for mixed-effects linear regression models, power is calculated for detectable difference between two dependent means with given sample size (15 SC workers and 9 participants in the experimental group), on the basis of average relative SD, significance level (alpha) of 0.05 (two-sided), repeated measurements' correlation, and a power of 80% (by G*Power Version 3.1.5 and Stata/SE 11.2, University Dusseldorf, Germany). It is calculated that the number of participants, both in the SC workers and in the experimental group, allows detecting 5% change in FVC, FEV_1, and FEV_1/FVC spirometric values after exposure to respiratory hazards in SC building, which is a number lower than a drop of 10% set as an indicator value for exercise-induced asthma.[19] Regarding small airways' response (FEF_{25}, FEF_{50}, FEF_{75}, $FEF_{25–75}$), our sample size allows us to detect 7% to 18% change in the SC workers and 8% to 24% change in the experimental group. Taking into account different correlation coefficients between repeated measurements of EBC pH in two study groups, detectable change is 0.16 units in the SC workers and 0.10 units in the experimental group. For FeNO values, detectable change is calculated to be 5 ppb decrease and 7.5 ppb increase from baseline values in the SC workers and 2.5 ppb decrease and 3.5 ppb increase from baseline values in the experimental group. Power calculation for detectable difference between two independent means (the SC workers vs the experimental group) showed that sample size allows us to detect a difference of 12% to 13% in FVC, FEV_1, and FEV_1/FVC values; 30% to 35% in FEF_{25}, FEF_{50}, FEF_{75}, $FEF_{25–75}$ values; 0.20 units of EBC pH; and 7 ppb of FeNO between two study groups.

RESULTS

The environmental monitoring measurements are shown in Table 1. The average values of total dust were under the threshold limit value (TLV) according to the Croatian regulation on TLVs of

TABLE 1. Environmental Monitoring at Swine Confinement During Winter Season (50 Stationary Samples)

	Mean ± SD (Range)	Croatian TLV*
Air temperature, °C	15.5 ± 1.25 (13.5–16.7)	
Relative humidity, %	62.7 ± 1.65 (60.1–64.9)	
Airflow, m/s	0.2 ± 0.02 (0.2–0.3)	
NH_3, ppm	22.5 ± 1.58 (20.0–25.0)	20
CO_2, ppm	3,000 ± 600 (2,000–4,000)	5,000
Total dust, mg/m^3	4.5 ± 0.6 (3.8–5.3)	10
Bacteria, CFU/m^3	$1.1 \times 10^5 \pm 10^4 (1–1.4 \times 10^5)$	. . .
Molds, CFU/m^3	$2.7 \times 10^5 \pm 4.4 \times 10^3 (2.1–3.7 \times 10^5)$	. . .

*Values of total dust were less than the TLV according to the Croatian regulation on TLV of hazardous substances at the workplace and biological exposure limits from 2009 (Croatian Official Gazette No. 13/2009).
TLV, threshold limit value.

hazardous substances at the workplace and biological exposure limits from 2009 (Croatian Official Gazette No. 13/2009), whereas average ammonia concentrations were on average 2 ppm above the TLV. For biological agents (bacteria and molds), there is no proposed TLV in Croatia.

The general characteristics of the study participants are presented in Table 2. Compared with the experimental group, the SC workers were on average 9 years older and had predominantly rural residency and a lower level of education. Eight of 15 SC workers (53%) had at least one respiratory symptom, including rhinitic and lower respiratory symptoms, half of which were work-related (in four of eight symptomatic workers). The most prevalent were cough with phlegm, dry cough, and rhinitic symptoms (Table 3). Two workers reported work-related asthma-like symptoms, and in one participant symptoms fulfilled the criteria for chronic bronchitis, unrelated to work. Two male workers had multiple respiratory symptoms (rhinitic symptoms, dry cough, cough with phlegm, and asthma-like symptoms in one worker; rhinitic symptoms and cough with phlegm in another worker).

Atopy status, which was evaluated in the SC workers by SPT, showed a positive result for at least one of the tested inhalatory allergens in the four workers. Positive reactions included those induced by house dust mites (*D. pteronyssinus* and *D. farinae*), grass, trees (birch and hazel) and weed pollen (*A. elatior*), and cat epithelia. The most prevalent was the sensitization to house dust mite allergens (three of four sensitized workers). There were no positive reactions to swine hair or epithelia. None of the workers who reported asthma-like symptoms had a positive SPT.

Basal spirometry and EBC pH values were not significantly different between groups (Table 4), as well as between smoking and nonsmoking SC workers (data not shown). No participant from the experimental group had below-normal FVC, FEV_1, or FEV_1/FVC basal values, but four of nine participants (44%) had one or more below-normal forced expiratory flow value (FEF_{50}, FEF_{75}, or $FEF_{25–75}$). Among the SC workers, one participant had a below-normal FEV_1 value, and 5 of 15 workers (33%) had one or more below-normal forced expiratory flow value (FEF_{25}, FEF_{50}, FEF_{75}, or $FEF_{25–75}$). The prevalence of below-normal spirometry values did not differ between groups. The spirometry results for seven male and eight female SC workers are shown in Table 5. Statistically significant difference was observed in basal values between male and female SC workers ($P < 0.05$; the *t* test); significant increase in FEF_{25} during the week was observed in male SC workers ($P < 0.05$; the paired *t* test); and near significant weekly decline was observed in FEV_1/FVC ratio in women ($t = 2.30$; $P = 0.055$; the paired *t* test). Spirometry values during observational period are shown in Figs. 1A. and 1B. Statistically significant decline was observed for FEF_{25} values in the experimental group ($P < 0.05$; mixed-effects linear regression controlled for age and sex). Basal FeNO values were 7.5 ppb lower in the experimental group than in the SC workers. Two SC workers, one man and one woman, both of whom were nonsmokers and had no symptoms of chronic respiratory disease, had basal FeNO values above 26 ppb (set as a cutoff point for "normal" values), that is, 34 ppb and 35 ppb, respectively. All of the participants from the experimental group had FeNO less than 26 ppb.

TABLE 2. General Characteristics of Study Participants*

	SC Workers ($n = 15$)	Experimental Group ($n = 9$)
Men, *n* (%)	7 (47)	3 (33)
Age, mean ± SD, y	44 ± 7	35 ± 12†
Smoking habit		
Never smokers	8 (53)	7 (78)
Ex-smokers	3 (20)	2 (22)
Current smokers	4 (27)	0
Smoking dose in current smokers		
Cigarettes per day	17.5 (13–20)	NA
Years of smoking	20 (10–28)	NA
Smoking index	349 (170–560)	NA
Seniority (yrs)‡	18 ± 8	NA
Urban/rural residence	3 (20)/12 (80)	9 (100)/0§
Education level§		
≤8 yrs of education	9 (60)	0
12 yrs of education	3 (20)	1 (11)
≥12 yrs of education	3 (20)	8 (89)

*Data are presented as arithmetic mean ± SD, median (range) or number (%).
†Statistically significant difference between groups obtained by the *t* test ($P < 0.05$).
‡Duration of employment at the swine farm.
§Statistically significant difference between groups obtained by Fisher's exact test ($P < 0.001$).
NA, not applicable; Smoking index, number of cigarettes smoked per day × years of smoking.

TABLE 3. Prevalence of Respiratory Symptoms and Atopy Status in SC Workers*

Respiratory Symptoms	All Symptoms†	Work-Related Symptoms
Rhinitis	3 (20)	2 (13)
Dry cough	3 (20)	2 (13)
Cough with phlegm‡	3 (20)	1 (7)
Chronic bronchitis	1 (7)	0
Asthma	2 (13)	2 (13)
Any above-stated symptom	8 (53)	4 (27)
Positive SPT§	**4 (27%)**	

*The results for 15 SC workers are presented as number (%).
†All symptoms include work-related and non–work-related symptoms.
‡Symptoms not compatible with the criteria for chronic bronchitis.
§Positive skin prick testing to at least one tested inhalatory allergen.

TABLE 4. Basal Spirometry, EBC pH, and FeNO in Swine Farm Workers and Experimental Group*

Spirometry	Farm Workers ($n = 15$)	Experimental Group ($n = 9$)
FVC	109.5 ± 11.5	102.7 ± 8.5
FEV_1	104.0 ± 13.0	97.6 ± 11.1
FEV_1/FVC	98.4 ± 8.6	99.0 ± 7.8
FEF_{25}	97.6 ± 16.2	104.0 ± 20.7
FEF_{50}	87.4 ± 29.9	83.3 ± 25.6
FEF_{75}	76.3 ± 34.4	71.5 ± 27.4
$FEF_{25–75}$	83.0 ± 28.8	79.6 ± 25.1
EBC pH	8.10 ± 0.13	8.23 ± 0.20
FeNO	18.5 ± 8.5	11.0 ± 3.4†

*The results are presented as arithmetic mean ± SD. Spirometry values are expressed as percentage of predicted[11] and FeNO as ppb.
†Statistically significant difference between groups obtained by the *t* test ($P < 0.05$).
EBC pH, exhaled breath condensate pH; FEF, forced expiratory flow; FeNO, fraction of exhaled nitric oxide; FEV, forced expiratory volume; FVC, forced vital capacity.

TABLE 5. Spirometry, EBC pH, and FeNO Values in SC Workers During Observation Period*

	Men			Women		
Spirometry Values	**Basal**	**Monday Post-Shift**	**Friday Post-Shift**	**Basal**	**Monday Post-Shift**	**Friday Post-Shift**
FVC	102.9 ± 9.7	109.8 ± 10.4†	109.1 ± 18.3	115.3 ± 10.0‡	116.2 ± 10.3	116.5 ± 12.7
FEV_1	100.9 ± 12.3	103.5 ± 8.0	106.9 ± 16.9	106.8 ± 13.7	105.8 ± 14.4	103.3 ± 10.0
FEV_1/FVC	98.9 ± 9.6	96.4 ± 8.1	100.4 ± 10.8	98.0 ± 8.3	96.5 ± 8.8	94.7 ± 8.0§
FEF_{25}	99.7 ± 16.5	95.9 ± 14.9	115.0 ± 17.7†	95.8 ± 16.9	98.6 ± 19.0	90.9 ± 20.1
FEF_{50}	89.3 ± 36.8	86.0 ± 27.9	100.2 ± 36.3	85.8 ± 24.9	85.8 ± 28.0	79.5 ± 20.5
FEF_{75}	76.2 ± 41.2	69.2 ± 28.4	78.2 ± 36.0	76.5 ± 30.3	61.6 ± 19.5	64.2 ± 20.5
$FEF_{25–75}$	86.1 ± 35.8	82.3 ± 27.6	96.8 ± 33.6	80.2 ± 23.4	77.4 ± 23.1	73.1 ± 18.0
EBC pH	8.13 ± 0.11	8.11 ± 0.20	8.35 ± 0.09†	8.06 ± 0.16	8.20 ± 0.06†	8.20 ± 0.13†
FeNO	19.7 ± 8.9	17.4 ± 8.2	17.7 ± 10.2	17.4 ± 8.6	14.3 ± 3.8	17.3 ± 11.4

*The results for seven male and eight female SC workers are presented as arithmetic mean ± SD. Spirometry values are expressed as percentage of predicted[11] and FeNO values as ppb.

†Significantly different from basal values of the same sex, obtained by the paired *t* test ($P < 0.05$).

‡Statistically significant difference in basal values between male and female SC workers, obtained by the *t* test ($P < 0.05$).

§Near significant difference compared with basal values of the same sex ($t = 2.30$; $P = 0.055$, obtained by the paired *t* test).

EBC pH, exhaled breath condensate pH; FEF, forced expiratory flow; FeNO, fraction of exhaled nitric oxide; FEV, forced expiratory volume; FVC, forced vital capacity.

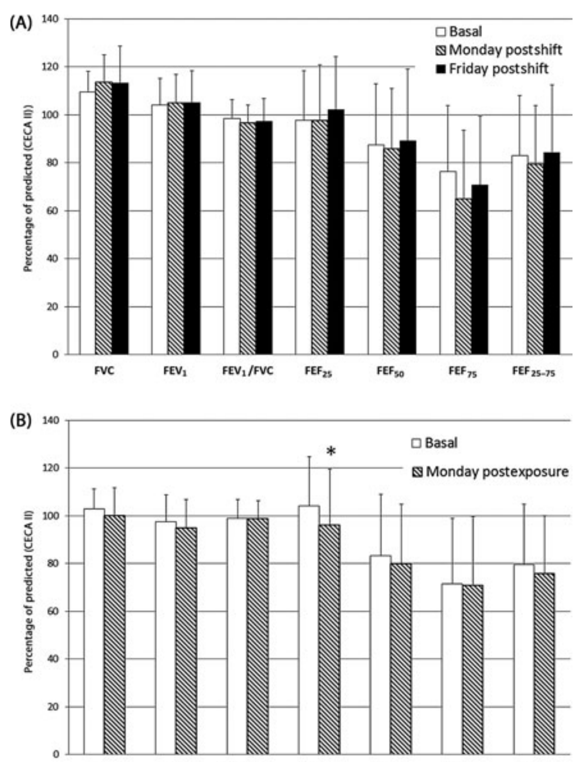

FIGURE 1. (A) Spirometry values in farm workers during observation period. The results for 15 farm workers and 9 participants from the experimental group are presented as arithmetic mean with one SD (error bars). There were no statistically significant differences from the basal values (mixed-effects linear regression controlled for age, sex, and smoking status). (B) Spirometry values in the experimental group during the observation period. The results for 15 farm workers and 9 participants from the experimental group are presented as arithmetic mean with one SD (error bars). *Statistically significant difference from the basal values ($P < 0.05$; obtained by mixed-effects linear regression controlled for age and sex).

Significantly lower basal FeNO values were observed in women in comparison with men (the *t* test; $P < 0.05$) (Table 6). Basal EBC pH and FeNO values did not correlate both in the SC workers (Pearson's $r = -0.10$; $P = 0.750$) and in the experimental group (Pearson's $r = 0.44$; $P = 0.232$). In the experimental group, basal EBC pH values were inversely correlated with FEV_1/FVC ratio (Pearson's $r = -0.86$; $P = 0.003$), FEF_{50} ($r = -0.71$; $P = 0.031$), FEF_{75} ($r = -0.90$; $P = 0.001$), and $FEF_{25–75}$ ($r = -0.77$; $P = 0.015$) values, and FeNO values were near-significantly inversely correlated with FEF_{25} values ($r = -0.65$; $P = 0.058$). Basal spirometry values were not correlated with EBC pH and FeNO values in the SC workers (either in all participants or in each sex separately). Nevertheless, in the SC workers with no reported asthma-like or chronic bronchitis symptoms and negative atopic status, EBC pH values were positively correlated with FEF_{75} ($r = 0.68$; $P = 0.044$) and $FEF_{25–75}$ ($r = 0.69$; $P = 0.041$) values. FeNO values did not correlate with spirometry in this subgroup of the SC workers.

Mixed-effects regression models adjusted for age, sex, and smoking status showed that the spirometry values in the SC workers did not significantly change during the period of observation (after one working shift and after one working week). In the experimental group, only FEF_{25} significantly dropped (by approximately 8%) after a 5-hour exposure to respiratory hazards in a swine house (coefficient time −7.9; 95% confidence interval [CI], −13.7 to −2.1; $P = 0.008$; adjusted for age and sex).

The EBC pH values in the SC workers did not significantly change after one working shift but did increase by 0.17 units after a 5-day exposure period (coefficient time 0.17; 95% CI, 0.10 to 0.25; $P < 0.001$; adjusted for age, sex, and smoking status) (Fig. 2A). In the experimental group, EBC pH dropped 0.10 units after a 5-hour exposure period (coefficient time −0.10; 95% CI, −0.16 to −0.03; $P = 0.003$; adjusted for age and sex) (Fig. 2A). The FeNO values in the SC workers did not change significantly after one working shift or after a 5-day exposure period (Fig. 2B). In the experimental group, FeNO values dropped by 1.8 ppb after a 5-hour exposure period (coefficient time −1.78; 95% CI, −3.53 to −0.03; $P = 0.047$; adjusted for age and sex) (Fig. 2B). Age, sex, and smoking status did not influence pattern of change in EBC pH and FeNO values during both observational periods, and the results of regression models were similar when workers who reported asthma-like or chronic bronchitis symptoms and those with positive atopic status were excluded from the analyses.

TABLE 6. Spirometry, EBC pH, and FeNO Values in the Experimental Group During Observation Period*

	Men		Women	
Spirometry Values	**Basal**	**Monday Post-Shift**	**Basal**	**Monday Post-Shift**
FVC	103.4 ± 10.0	102.2 ± 12.4	102.4 ± 8.7	99.0 ± 12.1
FEV_1	94.3 ± 11.6	96.0 ± 11.0	99.2 ± 11.5	94.3 ± 13.4
FEV_1/FVC	92.5 ± 3.4	94.9 ± 3.8	102.3 ± 7.3	100.7 ± 8.5
FEF_{25}	90.7 ± 21.0	85.9 ± 16.3	110.7 ± 18.6	101.3 ± 25.9
FEF_{50}	74.4 ± 18.3	80.2 ± 17.9	87.8 ± 29.0	79.7 ± 29.5
FEF_{75}	60.2 ± 11.9	68.3 ± 17.9	77.2 ± 32.1	72.0 ± 34.5
$FEF_{25–75}$	69.8 ± 15.4	73.6 ± 16.4	84.5 ± 28.7	76.8 ± 28.8
EBC pH	8.32 ± 0.05	8.27 ± 0.05	8.18 ± 0.23	8.06 ± 0.24‡
FeNO	15.0 ± 1.7	12.7 ± 3.2	9.0 ± 1.7†	7.5 ± 2.1

*The results for three male and six female participants from the experimental group are presented as arithmetic mean ± SD. Spirometry values are expressed as percentage of predicted[11] and FeNO values as ppb.

†Statistically significant difference in basal values between male and female SC workers, obtained by the *t* test ($P < 0.05$).

‡Significantly different from basal values of the same sex, obtained by the paired *t* test ($P < 0.05$).

EBC pH, exhaled breath condensate pH; FEF, forced expiratory flow; FeNO, fraction of exhaled nitric oxide; FEV, forced expiratory volume; FVC, forced vital capacity.

DISCUSSION

In this experimental study, we evaluated influence of respiratory hazards in the SC workers and previously nonexposed respiratory healthy volunteers, using EBC pH and FeNO. Environmental monitoring in the SC showed that the concentration of ammonia exceeded the TLV by 2 ppm on average, and bacteria and molds concentrations were higher than threshold levels related to the respiratory impairments (>10,000 CFU/m^3),[8,20] whereas total dust concentrations were less than upper limit of exposure. It is noteworthy that, in case of exposure to organic dust, total dust concentration is not always a good measure of environmental safety. Namely, total dust concentrations under TLV do not always imply safe concentrations of common constituents of organic dust, such as molds, bacteria, or endotoxin.[8]

The prevalence of respiratory symptoms in the SC workers was 53%, which is in line with other studies showing 25% to 50% prevalence of respiratory symptoms among the SC workers, depending on the level of environmental hazards.[3,21] Nevertheless, "healthy worker effect" could be suggested for the SC workers in this study. Average seniority of the SC workers was 18 years, and only a minority of them (26%) reported work-related respiratory symptoms and were atopic, with no sensitization to specific occupational allergens (swine dander and epithelia). In addition, there were no differences in basal lung function parameters between the SC workers and volunteers, and FEV_1 and/or FVC decline, which could be expected in the SC workers during the working shift or working week,[22] were absent in this study, supporting the indication of "healthy worker effect." Data from the published studies suggested a sex difference in the prevalence of asthma with increased risk for female farmers,[23] which is in line with our finding of the different sex respiratory response among the SC workers. Sex differences could also be an explanation for significantly lower FeNO values in female experimental group members. Namely, estrogens phase of menstrual cycles is related to the lower FeNO values in healthy women.[24]

In this study, only two workers reported work-related asthma-like symptoms, both without signs of atopy (positive SPT), indicating the primarily irritative nature of the detected respiratory symptoms. A recently published review emphasized the importance of considerably underreported irritant-induced occupational asthma and especially occupational chronic obstructive pulmonary disease.[1] It was noted that even multiple below TLV exposures to occupational respiratory irritants could evolve in irritant occupational asthma with a less sudden onset.[1,25]

The hazardous nature of exposure to organic dust and ammonia in the SC from this study was confirmed in both study groups, but in different ways. For respiratory healthy volunteers, an acute 5-hour exposure to an SC environment resulted in the obstruction of small airways and airway acidification. It should be noted, however, that all the volunteers were not exposed to any physical activity that could potentially enhance development of respiratory symptoms and airway obstruction due to the increase in minute ventilation and higher concentration of the inhaled harmful particles. Clinically, latent airway inflammation manifested in the SC workers in basal conditions after the nonworking weekend through the higher basal FeNO and lower basal EBC pH in comparison with the experimental group. A sign of adaptation to chronic exposure was observed with a tolerance-like airway response followed by a significant increase of EBC pH during the working week in the SC workers. Animal studies on airway epithelial cells and human genetic studies have shown that these adaptation mechanisms could be related to the upregulation of the Toll-Like Receptor 2 (TLR2) and consequent decrease in pro-inflammatory cytokine production[26–28] because of the chronic exposure to gram-positive bacteria in SC dust or the TLR2 gene polymorphism described in Canadian SC workers. Namely, significantly greater mean values of lung function parameters were found in workers with TLR2-16933T/A polymorphism (AA) than in workers with wild-type genotypes (AT+TT).[29]

So far, a cross-shift decrease of EBC pH was found in grain workers,[30] and a decrease of EBC pH throughout the working week was found in a group of sawmill workers exposed to high mold concentrations.[8] To our knowledge, this study is the first to report a follow-up of EBC pH in workers exposed to a mixture of organic dust and chemical irritants. In the naive volunteers, acidification of airways expectedly increased after 5 hours of exposure to an SC environment, similarly as it was described in the study on grain workers.[30] On the contrary, EBC pH in the SC workers increased after one working week, reflecting different pathophysiological mechanism, similar to those previously published in this study on acute and chronic effects of smoking on EBC pH.[16] Basal EBC pH in smoking participants (after at least 10 hours' abstinence of smoking) was significantly lower in comparison with nonsmoking controls, and a significant EBC pH increase was observed 15 minutes after

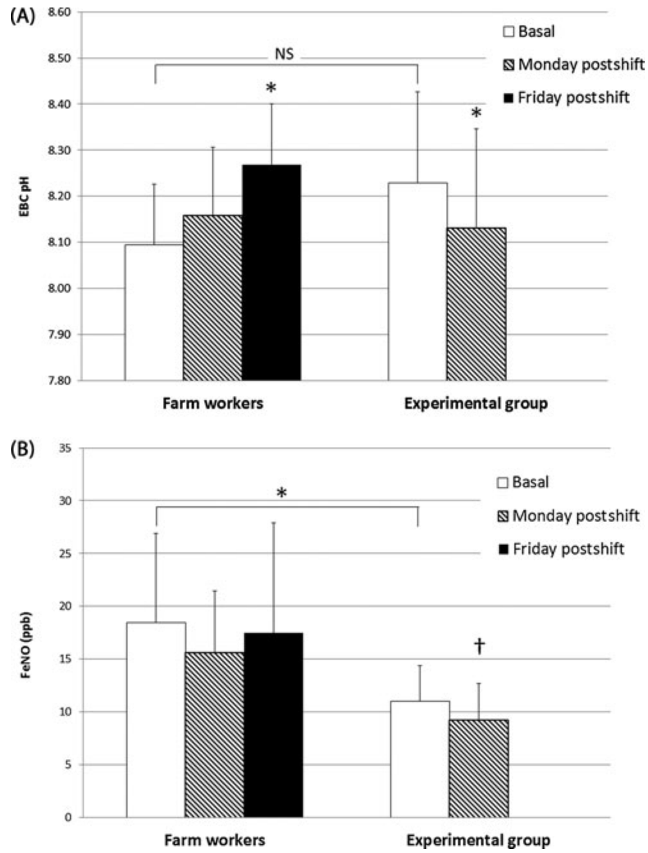

FIGURE 2. (A) EBC pH in farm workers and the experimental group during the observation period. The results for 15 farm workers and 9 participants from the experimental group are presented as arithmetic mean with one SD (error bars). NS, not significant difference in basal EBC pH values between farm workers and the experimental group (the *t* test). *Statistically significant differences from the basal values ($P < 0.05$; obtained by mixed-effects linear regression controlled for age, sex, and smoking status). (B) FeNO in farm workers and the experimental group during observation period. The results for 15 farm workers and 9 participants from the experimental group are presented as arithmetic mean with one SD (error bars). *Statistically significant difference in basal FeNO values between farm workers and the experimental group ($P < 0.05$; obtained by the *t* test); †Statistically significant difference from the basal values ($P < 0.05$; obtained by mixed-effects linear regression controlled for age and sex).

smoking a cigarette, lasting for 2-hour observation period. Ammonium compounds are common additives in cigarettes, and it was shown that ammonia, due to its high buffer capacity, prevents acidification of the airway lining fluid.[31] Furthermore, a similar trend in EBC pH increase was also suggested in sensitized and nonsensitized workers exposed to isocyanates.[32] Therefore, exposure to environmental ammonia can be an explanation for the EBC pH increase in the SC workers during their working week, apart from the previously described tolerance-like effect in airways. No correlation between FeNO and EBC pH was found, underlining the different pathophysiological mechanisms that can be detected with this method. In the experimental group, negative correlations were found between EBC pH and spirometric parameters (FEV_1/FVC ratio, FEF_{50}, FEF_{75}, and $FEF_{25–75}$), and positive correlations between EBC pH and spirometry values (FEF_{75} and $FEF_{25–75}$) were observed only in respiratory asymptomatic nonatopic SC workers. Nevertheless, we do not consider these correlations clinically relevant because all basal EBC pH values in the experimental group were in the alkaline range (7.79 to 8.41), and this group included only participants without respiratory symptoms. In addition, positive correlations between EBC pH and spirometry values were observed in respiratory asymptomatic nonatopic SC workers.

FeNO did not change during the working shift and working week in the SC workers and dropped after acute exposure in healthy volunteers, thus confirming previous observations about the unsubstantial importance of FeNO in the detection of nonspecific, nonatopic airway inflammation.[32] Similarly, an experimental study with an acute 3-hour exposure to high swine dust concentration (inhalable dust 31.4 mg/m^3) in previously nonexposed nonsmoking volunteers induced a significant FEV_1 and FVC decline but without any change in FeNO immediately after and 24 hours after exposure.[9] Conversely, a study on 33 healthy nonatopic volunteers showed an increase of FeNO in 22 volunteers for 5.9 ppb accompanied with a significant decrease of FEV_1 after 3 hours of exposure to swine dust in borderline concentrations regarding TLV (median 9.9 mg/m^3) but with hazardous endotoxin levels of 370 to 430 EU/m^3.[10]

This study has several limitations. Because of its experimental design, only a small number of study participants were involved. Environmental monitoring was performed with stationary samplers only during the winter season, and other, possibly present, environmental hazards such as endotoxin, peptidoglycans, and hydrogen sulfide were not evaluated. Also, gram-negative and gram-positive bacteria were not differentiated in environmental samples.

CONCLUSIONS

This is the first study evaluating EBC pH of the SC workers and respiratory healthy volunteers exposed to the environmental hazardous levels of organic dust constituents and chemical irritants. The volunteers reacted to the acute 5-hour exposure with bronchoconstriction and airway acidification, whereas the SC workers chronically exposed to the hazardous environment showed signs of the "healthy worker effect," without pathological spirometric changes, without signs of sensitization to occupational allergens, and with primarily irritative work-related respiratory symptoms. EBC pH was suggested as a valuable method for detecting acute airway acidification in naive volunteers, as well as chronic latent airway inflammation presented with lower EBC pH in the SC workers in basal conditions in comparison with volunteers.

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