

Fractionated exhaled breath condensate collection shows high hydrogen peroxide release in the airways

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Abstract

Background: Exhaled breath condensate (EBC) allows non-invasive monitoring of inflammation in the lung. Activation of inflammatory cells results in an increased production of reactive oxygen species, leading to the formation of hydrogen peroxide (H_2O_2). In addition cigarette smoking causes an influx of inflammatory cells and higher levels of H_2O_2 have been found in EBC of smokers. However there are still unresolved issues reflected by large variations in exhaled H_2O_2 and uncertainties about the origin of H_2O_2 release in the lung.

Methods: We collected exhaled breath condensate as fractionated samples from the airways and from the lung periphery in ten non-smokers, eight asymptomatic smokers and in eight COPD patients, and H_2O_2 concentration and acidity (pH) were analyzed in the airway and the alveolar fraction.

Results: In all subjects studied, H_2O_2 was 2.6 times higher in the airway versus the alveolar fraction. Airway H_2O_2 was twofold higher in smokers and fivefold higher in COPD patients compared to non-smokers. In all study groups, there was no significant difference in de-aerated pH between the airway and the alveolar sample.

Conclusions: Exhaled H_2O_2 is released at higher concentrations from the airways of all subjects studied, implying that the airways may be the dominant location of H_2O_2 production. Since many lung diseases cause inflammation at different sites of the lung, fractionated sampling of EBC can reduce variability and maintain an anatomical allocation of the exhaled biomarkers.

Keywords: exhaled breath condensate, fractionated sampling, airways, alveoli, hydrogen peroxide, acidity

Introduction

Cigarette smoking induces an inflammatory response in the airways that may play a key role in the pathogenesis of chronic obstructive pulmonary disease (COPD). Therefore noninvasive markers of inflammation are needed to quantify inflammation in the lung. Inflammatory processes in the lung elicit so called oxidative stress, meaning that the integrity of the lung is jeopardized by oxidants, and the latter process is supposed to play an important role in the development of COPD ^(1, 2). Oxidative stress is known to be increased in both stable and unstable COPD patients, due to either exposure to exogenous oxidants as present in cigarette smoke, in air pollution or as an enhanced endogenous production of hydrogen peroxide (H₂O₂) due to a neutrophilic inflammation and an impaired antioxidant system ^(3, 4). Oxidants are a prerequisite in the antimicrobial and antitumor defense mechanisms of the lung, but excessive production of oxidants may lead to oxidative damage to the tissue ^(1, 5).

Sputum induction has been used for studying airway inflammation ⁽⁶⁾, but because of the risk of aggravating the exacerbation, induced sputum may not be appropriate during exacerbation. Another method of determining the degree of oxidative stress is the collection of exhaled breath condensate (EBC) and the analysis of hydrogen peroxide (H₂O₂) ⁽⁷⁻¹⁰⁾. Although the primary fraction of EBC is water, other volatile and non-volatile substances have been detected in EBC. Besides H₂O₂, biological markers have been identified in EBC, which play a role in inflammatory processes ⁽¹⁰⁻¹³⁾. EBC-H₂O₂ was higher during exacerbation than during stable disease in COPD patients ⁽³⁾ and inhaled antioxidants and corticosteroids can reduce the level of exhaled H₂O₂ ^(14, 15). In addition EBC-pH has provided data supporting the important role of acidic stress in respiratory diseases ⁽¹⁶⁾.

Although there are recommendations for the collection of EBC ⁽¹⁷⁾, the concentration of the biomarkers and of H₂O₂ in exhaled breath condensate in healthy and disease shows great variation among subjects and among laboratories. Besides different breathing pattern, this

variability may depend on the fact that some biomarkers have their origin in specific compartments of the respiratory tract, such as the airways versus the lung periphery. Uncontrolled collection of EBC from both regions can cause an undefined dilution of the marker released from the affected region by the sample from the unaffected region.

The purpose of this study was to collect fractionated samples of EBC in healthy volunteers and in COPD patients and to associate EBC-H₂O₂ and pH to the two main anatomical compartments of the lung, the airways (AW) versus the alveolar (AL) region. The individual Bohr dead space was chosen as the threshold volume for separation between airway and alveolar compartment.

Methods

Subjects

Ten healthy never smokers (NS), eight asymptomatic smokers (S) and eight patients with COPD participated in the study. Respiratory symptoms were obtained using a questionnaire⁽¹⁸⁾ and pulmonary function parameters were measured by spirometry and body plethysmography (Jäger Masterlab, Erich Jaeger GmbH, Höchberg, Germany)⁽¹⁹⁾. Six of the eight COPD patients were ex-smokers. Six of the eight COPD patients were studied at the end of a hospitalization period and received oral steroids of 5-10 mg/day. The protocol was approved by the Ethical Committee of the Medical School of the Ludwig Maximilian University (Munich, Germany), and informed consent was obtained from each subject.

Sampling of exhaled breath condensate and analysis of pH and hydrogen peroxide

Exhaled breath condensate was collected using the ECoScreen-2 (Filt GmbH, Berlin, Germany). Based on the exhaled volume the exhaled air was divided by three balloon valves

(Hans Rudolph Inc., USA) into two discarding fractions (DF) and two sampling fractions (SF) in the sequence DF – SF – DF - SF. The DFs were delivered back to the inhalation channel while the sampling channel valves were closed. DF-1 was set to 50 mL, representing air from the oral cavity, and DF-2 (between airway and alveolar sample) was set to zero. The SFs were delivered to either of two distinct condensation chambers, which were lined with polyelophine bags without additional coatings. Based on threshold volumes (see below) the SFs were adapted to exhaled air from the airways and the alveolar space, respectively. During sampling the temperature of the condensation chambers was between -18 °C and -12 °C. During the eleven minutes sampling period the subjects were asked to make two to three pauses, where they passed away from the device and returned to spontaneous breathing pattern. This pause time was not included into the total sampling period. The inhalation air was filtered and conditioned to > 95 % relative humidity at room temperature. EBC was collected during 11 minutes oral breathing, wearing a nose clip. Smokers refrained from smoking at least one hour before EBC sampling.

Because the ECoScreen-2 device does not store the flow profile of each breath and because we wanted to visualize the breathing on a PC monitor, an additional spirometer device (Spiro Pro, Erich Jaeger GmbH, Höchberg, Germany) was coupled between ECoScreen-2 and the subjects mouth piece. Flow and volume were recorded continuously and analyzed for tidal volume and exhalation flow rates.

The threshold volume for separation between airway and alveolar sample was adapted to the individual Bohr dead space (DS_B) as indicated in Figure 1. The dead space was assessed based on the CO_2 exhalation profile ⁽²⁰⁾. Since there is no gas exchange in the airways, no CO_2 is exhaled from this region; therefore this region is determined the dead space of the lung. The exhaled CO_2 profile was analyzed according to the protocol of Fowler ^(21, 22) and three different threshold volumes were derived, as indicated in Figure 1. The phase-1 dead space (DS_{Ph1}) is specific to the conducting airway volume, which does not contain mixing air

(CO₂) from the lung periphery. The Fowler dead space characterizes the transition regime between DS_{PH1} and the alveolar air, and was determined according to a graphical method proposed by Fowler. The Bohr dead space (DS_B) characterizes the beginning of the alveolar plateau (phase-III dead space) and was used as the threshold volume for separation between airway and alveolar compartment of the lung. The exhaled air sampled beyond this threshold can be considered representing mainly alveolar air, although airway contaminations may occur because of its passage through the airways. The first 50 mL of the exhaled breath (oral cavity) were discarded. The following volume up to DS_B was sampled in the first container (AW-sample), while the remaining exhaled gas up to 1-L tidal volume was sampled into the second container (AL-sample).

Immediate analysis of the collected condensate regarding the measurement of hydrogen peroxide (H₂O₂) and pH was performed using the EcoCheck device (Filt GmbH, Berlin, Germany). The EcoCheck consists of a biosensor for measuring H₂O₂ concentrations by enzymatic peroxidase reduction. The lower detection limit was 50 nmol/L ⁽²³⁾. EBC acidity (pH) was determined within 5 min after EBC collection. In addition EBC-pH measurement was repeated after de-aeration in Argon gas for 8 minutes.

Data analysis

Data are expressed as mean +/- standard deviation (SD). The Kolmogorov-Smirnov-test showed that none of the parameters significantly differed from normal distribution. Differences among study groups and between airway and alveolar study parameters were assessed by the double sided t-test (Winstat for Microsoft Excel, Version 2005.1, www.winstat.com), using a significance level of $p < 0.05$. Correlation analysis and analysis of variance (ANOVA) was performed to analyze correlations between parameters.

Results

Lung function and dead space data

Table 1 summarizes the mean anthropometric and lung function data of the subjects. Impaired lung function (FEV_1) in COPD patients correlated with increasing cigarette consumption (pack years, PY; coefficient of correlation, $r = -0.74$, $p < 0.01$). The dead spaces were not significantly different among the study groups, but significantly correlated with body height ($r = 0.56$, $p < 0.01$).

EBC sampling parameters

Table 2 shows the EBC sampling parameters in the three study groups. Sampling time and tidal volume were not significantly different among study groups. The coefficient of variation of the tidal volume and the exhalation flow rate was below 0.15 during the sampling period. Both, total gas sampling volume (GV_T) and number of breaths (NB) were smaller in S by tendency and significantly smaller in COPD patients compared to NS ($p < 0.05$). These lower data resulted from lower exhalation flow rates in S by tendency ($p = 0.06$ against NS) and in COPD patients ($p < 0.01$ against NS). GV_T significantly correlated with NB ($r = 0.91$, $p < 0.01$) and the exhalation flow rate, ExFlow ($r = 0.86$, $p < 0.01$). In addition ExFlow and GV_T correlated with lung function parameters (FEV_1 : $r = 0.48$, $p < 0.01$; $FEV_1\%FVC$: $r = 0.51$, $p < 0.01$) and with cumulative cigarette consumption PY ($r = -0.51$, $p < 0.01$).

Airway EBC

During the sampling period about 55 L of exhaled gas and about 0.77 mL of condensate were collected in the airway (AW) fraction of NS. Collected air volume was significantly lower in COPD patients, but there was no significant difference in collected condensate volume among the study groups. Without de-aeration the AW-pH was 7.2 ± 0.3 (mean \pm SD) in NS and there was no significant difference among the study groups. De-aeration of the EBC-AW

samples by Argon gas caused a small but not significant increase in AW-pH. The AW-pH did not show significant correlations with any study parameter.

AW-H₂O₂ in NS was 226±129 nmol/L, as shown in Figure 2. It was 1.9-fold increased in smokers ($p < 0.05$) and 5.2-fold increased in COPD patients ($p < 0.01$). AW-H₂O₂ did not depend on gas or condensate sampling volume. There was a weak, but significant correlation between AW-H₂O₂ and expiratory flow rate ($r = 0.38$, $p = 0.03$). As illustrated in Figure 3, AW-H₂O₂ significantly depended on lung function (FEV₁, Figure 3A, $r = -0.74$, $p < 0.01$) and on cumulative cigarette consumption (PY, Figure 3B, $r = 0.65$, $p < 0.01$).

Alveolar EBC

During the sampling period about 109 L of exhaled gas and about 2.0 mL of condensate were collected in the alveolar fraction of NS. These values were lower in tendency in S and significantly lower in COPD patients ($p < 0.05$), as shown in Table 2. Without de-aeration AL-pH was 6.9±0.5 in NS and there was no significant difference among study groups, although values were lower in tendency in S and in COPD patients. De-aerated AL-pH was 7.6±0.5 in NS and there was no significant difference among study groups, although values were lower in tendency in S and in COPD patients. AL-pH did not show significant correlations with any study parameter.

AL-H₂O₂ in NS was 94±48 nmol/L, as shown in Figure 2. It was 1.6-fold increased in smokers (n.s.) and 5.0-fold increased in COPD patients ($p < 0.01$). There was no correlation between AL-H₂O₂ and expiratory flow rate and AL-H₂O₂ did not depend on gas or condensate sampling volumes. Similarly, as illustrated for the airway fraction, AL-H₂O₂ significantly correlated with lung function (FEV₁, Figure 3A, $r = -0.62$, $p < 0.01$) and with cumulative cigarette consumption (PY, Figure 3B, $r = 0.66$, $p < 0.01$). (Figure 3).

Comparison between airway and alveolar EBC

In all subjects the non-de-aerated AL-pH was lower compared to the non-de-aerated AW-pH ($p < 0.01$), but there was no significant difference among the study groups. There was a high correlation between non-de-aerated AL and AW EBC-pH ($r = 0.92$, $p < 0.01$). After de-aeration by Argon-gas there was no significant difference between AL and AW pH, although AL-pH was slightly increased. There was a high correlation between de-aerated AL and AW EBC-pH ($r = 0.91$, $p < 0.01$). In all subjects AL-H₂O₂ was significantly lower compared to AW-H₂O₂ ($p < 0.01$), resulting in lower AL-H₂O₂ in all study groups ($p < 0.01$).

Discussion

This study shows that fractionated sampling of EBC allows significant discrimination of non-de-aerated pH and exhaled H₂O₂ between the airway and the alveolar compartment of the lung. H₂O₂ was exhaled at higher concentrations from the airways compared to the lung periphery in all subjects and in all study groups.

EBC sampling parameters

Although the sampling was standardized with respect to tidal volume and sampling time there were differences in some sampling parameters among the study groups. Smokers and COPD patients had lower exhalation flow rates, which resulted in lower numbers of collected breaths and lower total gas sampling volume. Lower exhalation flow rates may result from airway obstructions and correlated with impaired lung function and cumulative cigarette smoke consumption. Breathing for eleven minutes at a tidal volume of 1 L is different from spontaneous breathing conditions at rest (generally one has to consider that breathing at a device may not represent true spontaneous breathing) and for example in healthy non-smokers the totally sampled gas volume of 201 L during 11 minutes results in a minute ventilation of 19 L/min, which can be considered as mild hyperventilation. Therefore we can not exclude that part of our results may be affected by this issue.

The EBC sampling protocol was adapted in order to collect separate fractions from the airway and from the alveolar compartment of the lung, and the Bohr dead space was used as threshold (Figure 1). This threshold may not precisely represent the true anatomical transition between airway and pulmonary region, but it can be easily assessed, even in patients with lung diseases. Each of the three dead space thresholds could be used to separate the exhaled air into an airway and alveolar associated fraction, respectively, and the question arises which of the thresholds provides the sample with lowest influence of the other region. Using the phase-1 dead space, the exhaled air would be closely limited to conducting airways. However, a much smaller amount of condensate would be achieved,

because in this region the inhaled air is warmed up and humidified. In addition the small airways, which are the most interesting target site for inflammatory processes in smokers and COPD patients, might not be included. Using the protocol as proposed here collects the total transition region between airways and gas exchange region to the AW sample. However, there may be oral contaminations, both in the AW and the AL sample while passing the oral cavity, although the fraction originating from the mouth cavity (first 50 mL) was discarded. The alveolar sample should only contain air from the gas exchange region, but because the alveolar exhalate passes the airways and the oral cavity (both during inhalation and during exhalation), there may be contaminations from these regions. EBC collection was performed during oral breathing of conditioned air (filtered and humidified air at room temperature, wearing a nose clip), preventing contaminations of the EBC samples by nasally released biomarkers ⁽²⁴⁾.

Fractionated EBC acidity (pH)

In all study groups, the acidity (pH) without de-aeration and the hydrogen peroxide concentration (H_2O_2) were significantly different between the airway and the alveolar fraction ($p < 0.01$). Although the difference in non-de-aerated pH is not very large between the AW and the AL samples, all subjects studied showed higher pH in the AW compared to the AL fraction, implying stronger acidity in the alveolar region. Since the alveolar region is the source of exhaled CO_2 , this may determine a lower pH in this compartment. In previous studies using full breath EBC sampling it was shown, that the CO_2 in the exhaled air significantly determines the pH measurements because de-aeration by argon caused a significant increase in pH ^(25, 26). Interestingly this de-aeration did not change the relation between subjects or study groups. In our study removal of dissolved CO_2 from the EBC samples after de-aeration by Argon gas did not significantly influence AW-pH, but significantly increased AL-pH, as shown in Table 2. Interestingly the difference between AW and AL pH vanished after de-aeration. This confirms the significant influence on exhaled CO_2 on pH-determination in EBC samples. Using the EBC fractionation protocol as suggested in

our study suggests, that the CO₂ influence on EBC acidity can be neglected in the airway sample, since CO₂ is not released in this compartment.

Previous studies showed lower pH (increased acidity) in EBC samples of asthmatic patients⁽²⁷⁾ and the authors concluded that the airways may be the main sources of lower EBC-pH. Our study does not show effects of smoking or disease on EBC-pH, although pH values may be lower in tendency in COPD patients. In addition the source of EBC-pH can not be adjusted to either of the compartments selected in our study since there was no significant difference between AW and AL-pH after de-aeration.

Fractionated EBC hydrogen peroxide concentration

There was a significant (2.6-fold) difference in H₂O₂ between the airways and lung periphery in all study groups. This suggests that the major site of H₂O₂ production and release in the lung are the central airways. Previous studies using full breath EBC sampling have shown that H₂O₂ depended on the exhalation flow rate⁽²⁸⁾. Lower exhalation flow rates were associated with higher H₂O₂ concentrations in EBC. When the exhaled H₂O₂ is primarily released in the airways, then higher exhalation flow rates will dilute the constantly released H₂O₂. Our study using fractionated sampling confirms this assumption. However, lower exhalation flow rates in S and in COPD patients might have further contributed to increased H₂O₂ concentrations in these study groups, even during fractionated sampling. This is supported by a weak correlation of AW-H₂O₂, but not AL-H₂O₂ and exhalation flow rate.

H₂O₂ and airway inflammation

Since inflammatory cells, such as macrophages or neutrophils, are the main sources of H₂O₂ production and release in the lung, these cells must be present and active more frequently in the airways, even in healthy non-smokers. The higher hydrogen peroxide production in the airways of smokers and COPD patients is evident because this anatomical site is the primary

target site of cigarette smoke particle deposition, resulting in increased numbers of inflammatory cells and early and chronic inflammation ⁽⁶⁾. Increased levels of exhaled hydrogen peroxide in EBC of smoker and COPD patients was reported previously using full breath condensate sampling ^(3, 4, 29, 30). The increase of exhaled hydrogen peroxide in smokers and in COPD patients correlates with impaired lung function and with increasing cumulative cigarette consumption, as demonstrated in Figure 3. A similar correlation between impaired FEV₁ and increased levels of H₂O₂ concentration in full breath EBC has been shown before ⁽³¹⁾.

Six of the eight COPD patients were studied at the end of a hospitalization period and received oral steroids, therefore they may not show conditions of stable COPD. A previous study showed that EBC-H₂O₂ was higher during exacerbation than during stable disease in COPD patients ⁽³⁾. In addition it has been shown that steroids diminish the severity of inflammation processed in the lung and thereby may lower the level of H₂O₂ production, as has been confirmed in previous studies ^(14, 15). Nevertheless the limited number of COPD patients in our study show significantly elevated H₂O₂ exhalation profiles, as was shown previously in full breath EBC collection studies ^(3, 31), confirming the increased level of inflammation in the lung of these patients, even during medication by oral steroids.

However, in NS, higher release of H₂O₂ in the airways compared to the alveolar space is not similarly evident. It may depend on the inhalation of environmental particles with preferred deposition in the airways. Because of the much smaller surface area of the airways compared to the alveolar space, the airways have an up to 10-fold higher density of deposited particles ^(32, 33). These particles, although cleared by more efficient mechanisms ^(34, 35), can stimulate defense cells to cause low level oxidative stress and the release of H₂O₂ ⁽³⁶⁾.

Although lower in the alveolar compared to the airway EBC, hydrogen peroxide was also elevated in the alveolar EBC of smokers and of COPD patients. Since the alveolar and the airway hydrogen peroxide show a high correlation, part of the H₂O₂ detected in the alveolar sample may result from H₂O₂ released in the airways during the passage of the alveolar gas.

Conclusions

Both in the airway and in the alveolar fraction, hydrogen peroxide and pH did not depend on the condensate volume or on the sampling gas volume. In part this may be a consequence of standardizing the tidal volume. The fractionated sampling according to the approach suggested in this study highlights the possibility of biomarker assignment to an anatomical location (airways versus alveolar space) including disease states in these locations. If the site of the release of a biomarker is in any of these compartments, then the uncontrolled addition of sample from the other, not affected compartment will dilute the biomarker in an unknown manner. Using this fractionated sampling technique future studies may be useful to discriminate COPD patients with and without emphysema, or the severity of disease progression in the respective compartments. In addition, besides disease monitoring, selective drug monitoring may be possible after targeted delivery of drugs to the lung, such as after aerosol bolus inhalation.

Author Disclosures Statement

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Table and Figure legends

Table 1: Anthropometric data, lung function and dead spaces of non-smokers (NS), smokers (S) and of patients with chronic obstructive pulmonary disease (COPD).. All values are mean +/- standard deviation. *: $p < 0.05$ and **: $p < 0.01$ against NS.

Table 2: Results of fractionated exhaled breath condensate sampling from the airway (AW) and the alveolar (AL) compartment of non-smokers (NS), smokers (S), and of patients with chronic obstructive pulmonary disease (COPD). All values are mean +/- standard deviation. *: $p < 0.05$ and **: $p < 0.01$ against NS; ++: $p < 0.01$ against AW parameters.

Figure 1: Exhaled CO_2 profile of one subject and determination of the phase-1 dead space (DS_{Ph1}), the Fowler dead space (DS_{F}) and the Bohr dead space (DS_{B}). DS_{B} was used as threshold volume for airway and alveolar condensate sampling separation. In addition the first 50 mL of the exhaled air were discarded.

Figure 2: Box plot of hydrogen peroxide (H_2O_2) concentration in the airway (AW) and in the alveolar (AL) fraction of exhaled breath condensate in non-smokers (NS), asymptomatic smokers (S) and in patients with chronic obstructive pulmonary disease (COPD). *: $p < 0.05$ against NS, **: $p < 0.01$ against NS; ++: $p < 0.01$ against AW.

Figure 3: Airway (AW, closed symbols) and alveolar (AL, open symbols) hydrogen peroxide concentration with respect to lung function (FEV_1 , A) and cumulative cigarette smoking (pack years, B; r = coefficient of correlation).

Tables

Table 1: Anthropometric data, lung function and dead spaces of non-smokers (NS), smokers (S) and of patients with chronic obstructive pulmonary disease (COPD). All values are mean +/- standard deviation. *: $p < 0.05$ and **: $p < 0.01$ against NS.

	NS	S	COPD
N	10	8	8
Age , years	47+/-18	50+/-10	64+/-10*
Height, cm	173+/-9	170+/-9	169+/-5
Weight, kg	67+/-12	75+/-12	82+/-17
Pack years	0	38+/-11	51+/-21
<i>Lung function</i>			
FEV ₁ , %pred.	113+/-11	102+/-16	50+/-16**
FEV ₁ %FVC	80+/-9	77+/-5	56+/-13**
TLC, %pred.	104+/-14	105+/-9	116+/-10*
RV, %pred.	87+/-14	108+/-36	189+/-38**
<i>Dead space</i>			
DS _{Ph1} , mL	157+/-29	132+/-12	144+/-22
DS _B , mL	378+/-79	314+/-50	383+/-52

Abbreviations: FEV₁ - forced expiratory volume within 1 second; FVC - forced vital capacity; TLC - total lung capacity; RV - residual volume; DS_{Ph1} - phase-1 dead space; DS_B - Bohr dead space.

Table 2: Results of fractionated exhaled breath condensate sampling from the airway (AW) and the alveolar (AL) compartment of non-smokers (NS), smokers (S) and of patients with chronic obstructive pulmonary disease (COPD). All values are mean +/- standard deviation.

*: $p < 0.05$ and **: $p < 0.01$ against NS; ++: $p < 0.01$ against AW parameters.

	NS	S	COPD
S-Time, sec	651+/-24	632+/-17	633+/-28
GV _T , L	201+/-30	173+/-18*	156+/-30**
NB	180+/-24	163+/-19	147+/-24*
TV, L	1.12+/-0.09	1.06+/-0.09	1.06+/-0.08
ExFlow, L/s	0.67+/-0.17	0.53+/-0.10	0.45+/-0.12**
<i>Airways EBC</i>			
GV _{AW} , L	55+/-7	53+/-6	50+/-5*
CV _{AW} , mL	0.77+/-0.20	0.84+/-0.15	0.71+/-0.18
pH _{AW}	7.2+/-0.3	7.1+/-0.8	7.1+/-0.4
DA-pH _{AW}	7.4+/-0.3	7.3+/-0.8	7.3+/-0.3
H ₂ O _{2AW} , nmol/L	226+/-129	436+/-199*	1166+/-603**
<i>Alveolar EBC</i>			
GV _{AL} , L	109+/-17	97+/-14	92+/-14*
CV _{AL} , mL	2.0+/-0.4	1.8+/-0.3	1.5+/-0.3*
pH _{AL}	6.9+/-0.5**	6.8+/-1.0**	6.7+/-0.5**
DA-pH _{AL}	7.6+/-0.5	7.5+/-1.0	7.3+/-0.6
H ₂ O _{2AL} , nmol/L	94+/-48**	153+/-145**	468+/-328**,**

Abbreviations: S-Time – EBC sampling time; GV_T – total gas sampling volume; NB – number of breaths sampled; TV – tidal volume; ExFlow – mean exhalation flow rate; GV_{AW} – airway fraction gas sampling volume (L); CV_{AW} – airway fraction condensate volume; pH_{AW} – airway acidity (pH); DA-pH_{AW} – airway acidity (pH) after de-aeration by Argon gas; H₂O_{2AW} – airway hydrogen peroxide concentration (nmol/L); parameters similar for the alveolar (AL) sample.

Figures

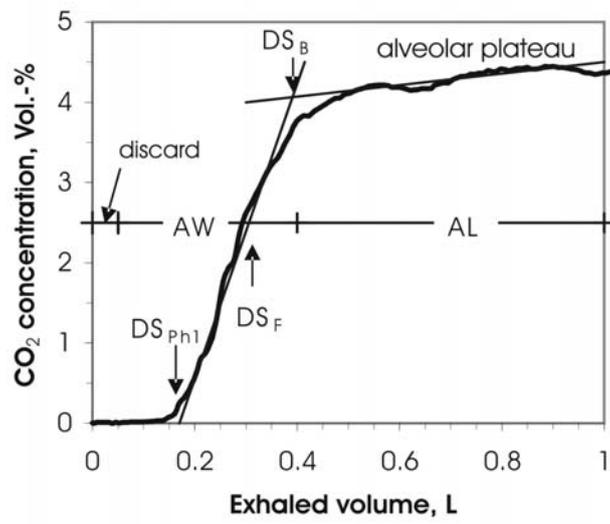


Figure 1

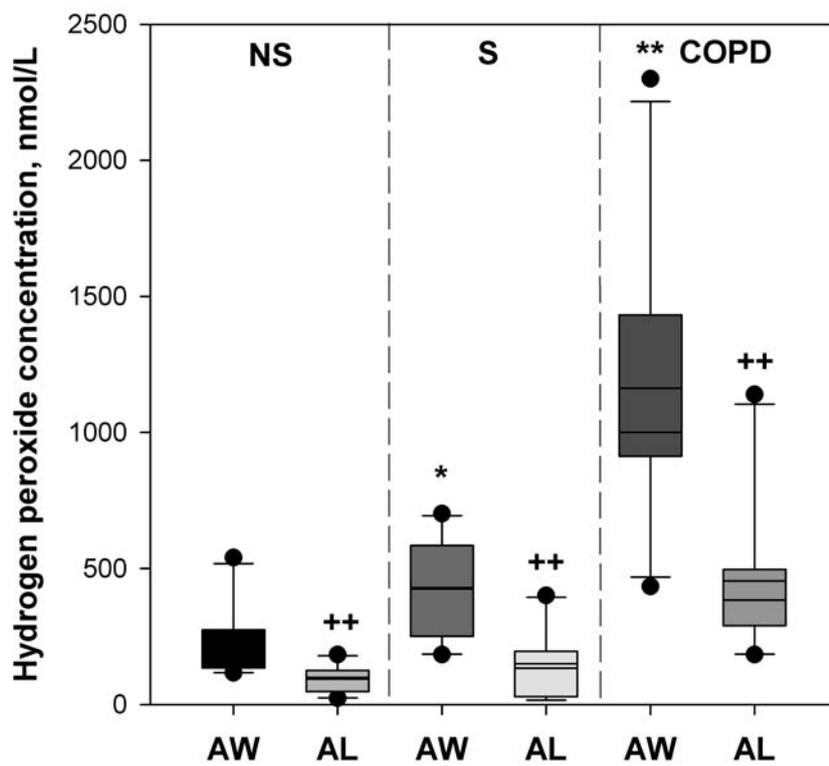


Figure 2

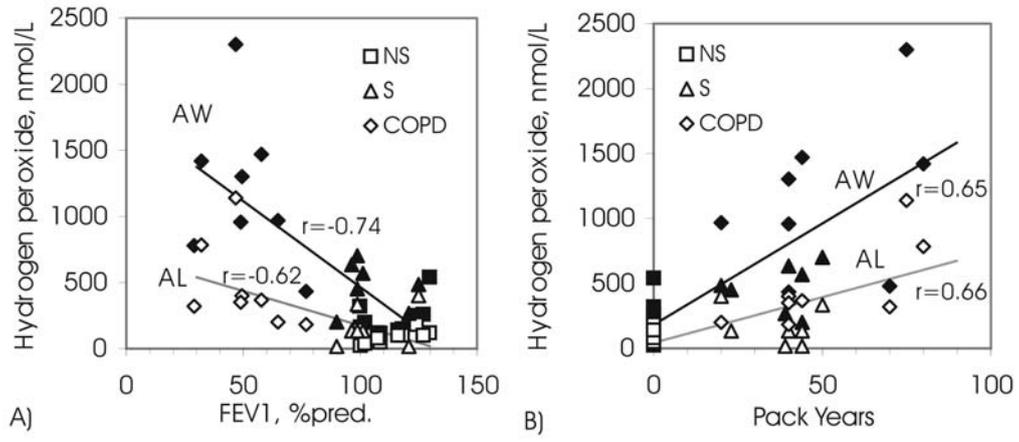


Figure 3: