Increased content of thiobarbituric acid-reactive substances and hydrogen peroxide in the expired breath condensate of patients with stable chronic obstructive pulmonary disease: no significant effect of cigarette smoking

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The imbalance between oxidants and antioxidants is known to play an important role in the pathogenesis of chronic obstructive pulmonary disease (COPD). Cigarette smoking is the most frequent factor responsible for development of COPD by leading to oxidant overload in the lower airways, due to presence of its own oxidants and to recruitment and activation of pulmonary phagocytes.

We aimed to determine whether (1) patients with stable COPD have higher thiobarbituric acid-reactive substances (TBARs, an end-product of lipid peroxidation) and H₂O₂ levels in expired breath condensate than healthy subjects who have never smoked; (2) COPD subjects who are current smokers exhale more TBARs and H₂O₂ than COPD ex-smokers and those who have never smoked; and (3) concentration of TBARs correlates with H₂O₂ levels in the breath condensate of COPD patients.

The TBAR and H₂O₂ content in expired breath condensate of 17 healthy nonsmoking subjects and 44 patients (11 current smokers, 20 ex-smokers and 13 who had never smoked) with stable COPD [forced expiratory volume in 1 s (FEV₁) 63·3±16·3% and FEV₁ reversibility 5·2±4·3% predicted value] was measured spectrofluorimetrically by the thiobarbituric acid and homovanillic acid methods, respectively.

The mean concentrations of TBARs and H₂O₂ in the expired breath condensate of COPD subjects were 12·0 (0·48–0·86 μM vs. 0·04±0·14 μM; P<0·05) and 10 times (0·48±0·67 μM vs. 0·05±0·07 μM; P<0·005) higher than in healthy controls. Current smokers with COPD did not exhale more H₂O₂ than COPD ex-smokers and those who had never smoked. TBARs levels shared only a tendency to be higher in the breath condensate of smoking COPD subjects than in that of ex-smokers (0·92±1·49 μM vs. 0·35±0·44 μM) and of COPD subjects who had never smoked (0·92±1·49 μM vs. 0·30±0·53 μM). No correlation was found between TBAR and H₂O₂ levels in the whole COPD group. These variables did not correlate with cigarette smoking status and the time from smoking cessation.

Subjects with stable COPD exhibit increased lipid peroxidation and H₂O₂ generation in the airways. Current cigarette smoking does not distinguish COPD subjects with respect to TBARs and H₂O₂ exhalation.

Introduction

Cigarette smoking is the major factor implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD) (1). Cigarette smokers have an increased number of macrophages and neutrophils in the lower airways (2,3). These cells release large amounts of hydrogen peroxide (H₂O₂) (2,4) which, after conversion into hydroxyl radicals (5), can cause peroxidation of the polyunsaturated fatty acids of cell membranes (6,7). In addition, cigarette smoke itself contains many free radicals which are capable of reacting with biomolecules present in the airways (5,8). Some of the H₂O₂ which is decomposed by antioxidant enzymes may evaporate from the alveolar lining fluid and may be exhaled with the expiratory air. Similarly, some lipid peroxidation products, such as ethane, pentane and thiobarbituric acid-reactive substances (TBARs), are volatile and may be present in expired breath (9,12). Patients with COPD and asymptomatic cigarette smokers have been reported to exhale more H₂O₂ than healthy nonsmokers (13,14). As cigarette smoking enhances oxidant generation in the airways (7,13) it is possible that COPD patients who
still smoke have higher concentration of H$_2$O$_2$ and lipid peroxidation products in expired air than ex-smokers or those COPD subjects who have never smoked. Recent studies, however, have provided conflicting data showing that COPD patients who still smoked could exhale lower amounts of H$_2$O$_2$ than ex-smokers with stable COPD (14). The interindividual variability in antioxidant potential (10) or the induction by cigarette smoke of some compensatory mechanisms providing protection against reactive oxygen species including H$_2$O$_2$ (15) may be possible explanations of this observation. On the other hand, the above-mentioned differences between COPD smokers and ex-smokers in H$_2$O$_2$ exhalation are based on the analysis of a small group of patients and therefore bias cannot be excluded (14). In addition, no studies on TBAR exhalation in COPD subjects have been published so far. Therefore, in this study we aimed to compare the concentration of TBARs and H$_2$O$_2$ in the expired breath condensate of 17 healthy nonsmoking subjects and 44 patients with stable COPD and to investigate whether the exhalation of these compounds was correlated with or dependent upon current smoking status. We report here that patients with stable COPD exhale 12 and 10 times more TBARs and H$_2$O$_2$ than healthy nonsmoking subjects and that these variables do not correlate with cigarette smoking.

Material and Methods

REAGENTS

Horseradish type II peroxidase (HRP, 200 U mg$^{-1}$ solid), 4-hydroxy-3-methoxy-phenylacetic acid (homovanillic acid), thiobarbituric acid (TBA) and tetramethoxypropane were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other compounds were purchased from POCH (Gliwice, Poland) and were of analytical grade. Sterile deionized water (conductivity 0·05 S cm$^{-1}$, Milli Q Plus Water Purification System, New York, U.S.A.) was used throughout the study. Thirty per cent H$_2$O$_2$ solution was diluted 100-fold with phosphate-buffered saline (PBS, pH 7·4) and stored at 4°C in the dark. An aqueous solution of HRP (1 U ml$^{-1}$) with the addition of 100 μM homovanillic acid was freshly prepared before the assay.

STUDY POPULATIONS

The study subjects included 17 healthy volunteers and 44 patients with COPD (Table 1) who had not suffered from any infectious disease for at least 3 months prior to the study. Healthy subjects who had never smoked and with no history of respiratory or atopic disease were members of our medical staff and were free of any medication. Routine physical examination showed nothing abnormal. Fifty-two patients with COPD were recruited from the Medical University Outpatient Department COPD register. They had not taken inhaled or oral corticosteroids for the last 3 months. Subjects were asked to stop any medication except short-acting β$_2$-agonists (salbutamol or fenoterol on demand), anticholinergics (ipratropium bromide) and theophylline and to come to the clinic after a 4-week wash-out period for collection of expired breath condensate and to perform the lung function test. The intake of mucolytics (bromhexine, ambroxol, N-acetylcysteine) was not allowed during the study due to their possible antioxidant activity (16–18). Spirometry was performed with a Flowscreen (Erich Jaeger GmbH Co., Germany) equipped with software compatible to American Thoracic Society standards (19) between 0900 and 1100 hours and subjects refrained from the use of inhaled drugs (β$_2$-agonists and anticholinergics) and oral theophylline for 6 and 12 h before lung function measurement, respectively. The inclusion criteria were the ability to stop other than the above recommended therapy and a forced expiratory volume in 1 s (FEV$_1$) increase below 10% of the predicted value 15 min after 200 μg of salbutamol (two puffs from the metered dose inhaler). Forty-four of the COPD patients fulfilled these criteria. Eleven of them were current smokers, 20 were ex-smokers and 13 had never smoked (Table 2). All patients were asked about their smoking and disease history and their medical documentation was carefully analysed. Patients had not suffered from any COPD exacerbation during the 3 months before the study. The number of exacerbations per year during the 2 years prior to the visit date in the whole COPD group ranged from 1 to 8 (mean 3 ± 2). In current smokers, ex-smokers and those COPD patients who had never smoked, the mean number of exacerbations per year reached 3 ± 1, 3 ± 2 and 3 ± 2, respectively. The duration of COPD varied from 24 to 436 months (mean 115 ± 91) for the whole group and 71 ± 51, 150 ± 107 and 98 ± 71 months for current smokers, ex-smokers and COPD patients who had never smoked, respectively. This study was approved by the Medical University Ethics Committee and all subjects gave informed consent before participation.

COLLECTION OF AIR CONDENSATE

The expired breath condensate was collected just before the lung function tests as described previously (12,13). Briefly, patients were asked to breathe out spontaneously through a
cally using a Perkin Elmer Luminescence Spectrometer.

Therefore, our smoke itself causes formation of reactive oxygen species in the airways (13).

The concentration of H$_2$O$_2$ in expired breath condensate levels in breath condensate reflected the intensity of H$_2$O$_2$ production in the airways (13). The temperature in the tube vicinity ranged from 0°C to 8°C and allowed condensation of all H$_2$O$_2$ present in the expiratory air (20). Each subject wore a noseclip and rinsed their mouth with distilled water just before and at 7 and 14 min of collection in order to reduce the H$_2$O$_2$ evaporation from saliva (20) and the nasal spaces (13). At the end of collection, the tube was removed from the container and 2–5 ml aliquots of condensate were transferred to Eppendorf tubes and stored at −80°C for not more than 7 days until H$_2$O$_2$ and TBARs measurement. All collections were performed between 0800 and 0900 hours. Cigarette smoke itself causes formation of reactive oxygen species in aqueous solution and expired breath (5,21). Therefore, our smokers refrained from cigarette smoking for 12 h before the visit. If the patient failed to refrain from smoking or to stop medication (as described above), the breath condensate was not collected and the visit was rescheduled within 1–7 days. This procedure allowed us to conclude that H$_2$O$_2$ levels in breath condensate reflected the intensity of H$_2$O$_2$ production in the airways (13).

MEASUREMENT OF HYDROGEN PEROXIDE

The concentration of H$_2$O$_2$ in expired breath condensate was measured according to the method of Ruch et al. (22). Briefly, 600 µl of expired breath condensate was mixed with 600 µl of HRP solution (1 U ml$^{-1}$) containing 100 µM homovanillic acid and was incubated for 60 min at 37°C. Then, the sample was mixed with 150 µl 0·1 M glycine-NaOH buffer (pH 12·0) with the addition of 25 mM EDTA. The homovanillic acid oxidation product, as a measure of the amount of H$_2$O$_2$, was determined spectrophotometrically using a Perkin Elmer Luminescence Spectrometer LS-50B (Norwalk, CT, U.S.A.). Excitation was at 312 nm and emission was measured at 420 nm. As opposed to our previous studies (in which the results were expressed in nanomoles of H$_2$O$_2$ per sample) (12,13) readings were converted into µM using the regression equation $y=(x-x_0)/0·06764$ (where $y$=micromoles of H$_2$O$_2$ per litre of expired breath condensate; $x$=intensity of emission at 420 nm expressed in arbitrary units and; $x_0$=intensity of emission given by reference sample receiving distilled water instead of breath condensate). The lower limit of H$_2$O$_2$ detection was 83 nM and the calibration curve was linear up to an H$_2$O$_2$ concentration of 16·7 µM.

MEASUREMENT OF TBA-REACTIVE PRODUCTS

TBARs are low molecular weight compounds formed via the decomposition of certain primary and secondary lipid peroxidation products which at low pH and elevated temperature participate in a nucleophilic addition reaction with thiobarbituric acid, generating a red fluorescent complex. Malondialdehyde (MDA) is one of the most important TBARs (9). The content of TBARs in breath condensate was determined as previously described (12), except that results are here expressed in µM instead of nanomoles per sample. Briefly, 100 µl of the condensate were mixed with 2 ml of TBA solution (0·67 g dissolved in 100 ml of deionized water, then diluted 1:1 with glacial acetic acid), boiled for 30 min and allowed to cool at ambient temperature, next, chromogen was extracted into 2·5 ml of butanol by vigorous shaking for 1 min. Following centrifugation (10 min, 1500 g, 25°C), TBARs were measured spectrofluorimetrically (excitation 515 nm, emission 546 nm) (23). Readings were converted into µM using the regression equation $y=0·12x−0·07$ (where $y$=µM of TBARs and $x$=intensity of emission at 546 nm expressed in arbitrary units) obtained from three series of experiments with 20

<table>
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<tr>
<th>Table 2. Characteristics of COPD patient subgroups</th>
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<td>Current smokers</td>
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<tr>
<td>n</td>
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<tr>
<td>Age (years)</td>
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<td>Sex (M:F)</td>
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<tr>
<td>Present cigarette consumption</td>
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<tr>
<td>Cumulative cigarette consumption</td>
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<tr>
<td>FEV$_1$ (%)</td>
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<td>FEV$_1$/FVC</td>
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<td>FEV$_1$ reversibility (%)</td>
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FEV$_1$ and FEV$_1$ reversibility are expressed as % predicted. All data are expressed as mean ± sd.

*Time from smoking cessation varied from 6 to 369 months (mean 116 ± 104 months).

†The COPD patients who had never smoked were professionally exposed for more than 20 years to factors that are known to predispose to COPD. Of the men, one was a welder, the second a grinder and two were workers in a coal merchant’s shop. The women were textile industry workers (in dye-works, warehouse with chemicals or the spinning room).

FVC, forced vital capacity.

mouthpiece with a saliva trap connected to the tube and to breathe in with the mouthpiece removed, for 20 min. The collecting part of the tube was covered with ice and salt. The temperature in the tube vicinity ranged from −6 to 0°C and allowed condensation of all H$_2$O$_2$ present in the expiratory air (20). Each subject wore a noseclip and rinsed their mouth with distilled water just before and at 7 and 14 min of collection in order to reduce the H$_2$O$_2$ evaporation from saliva (20) and the nasal spaces (13). At the end of collection, the tube was removed from the container and 2–5 ml aliquots of condensate were transferred to Eppendorf tubes and stored at −80°C for not more than 7 days until H$_2$O$_2$ and TBARs measurement. All collections were performed between 0800 and 0900 hours. Cigarette smoke itself causes formation of reactive oxygen species in aqueous solution and expired breath (5,21). Therefore, our smokers refrained from cigarette smoking for 12 h before the visit. If the patient failed to refrain from smoking or to stop medication (as described above), the breath condensate was not collected and the visit was rescheduled within 1–7 days. This procedure allowed us to conclude that H$_2$O$_2$ levels in breath condensate reflected the intensity of H$_2$O$_2$ production in the airways (13).
H$_2$O$_2$ AND TBAR LEVELS IN COPD SUBJECTS

 Patients with stable COPD exhibited a 10-times higher concentration of H$_2$O$_2$ ($P<0.005$) than healthy nonsmoking subjects. The mean H$_2$O$_2$ level in the expired breath condensate reached 0.48 ± 0.67 µM ($n=44$). However, 16 (36%) COPD patients (nine men, seven women) revealed no detectable H$_2$O$_2$ levels (Fig. 1). Four of them were current smokers, nine ex-smokers and three had never smoked. Mean H$_2$O$_2$ levels in the expired breath condensate in COPD patients who were current smokers did not differ significantly from that found in COPD ex-smokers (0.41 ± 0.54 µM vs. 0.55 ± 0.76 µM). Patients who had never smoked had 0.44 ± 0.65 µM H$_2$O$_2$ in the expired breath condensate. Male COPD patients showed similar H$_2$O$_2$ values compared with women, both in the whole COPD group (0.46 ± 0.62 µM, n=23 vs. 0.51 ± 0.69 µM, n=21) and in the current smokers COPD subgroup (0.27 ± 0.34 µM, n=6 vs. 0.57 ± 0.72 µM, n=5). The mean TBARs level in breath condensate of COPD subjects reached 0.48 ± 0.86 µM and was 12 times higher ($P<0.05$) than that found in healthy controls (Fig. 2). The rate of positive TBARs readings was also higher in COPD subjects than in healthy nonsmoking controls (0.48 vs. 0.12). Although COPD subjects who were permanent smokers revealed 3-1 and 2-6 times higher mean TBARs levels in expired breath condensate than COPD patients who had never smoked (0.92 ± 1.49 µM vs. 0.30 ± 0.53 µM) and COPD ex-smokers (0.92 ± 1.49 µM vs. 0.35 ± 0.44 µM), these differences were not significant, probably due to the high variability of individual results. The rate of positive TBARs readings was similar for all three COPD subgroups; 0.46 for subjects who

Fig. 1. H$_2$O$_2$ concentration in the expired breath condensate of healthy (○, never smoked) and COPD (□, never smoked; ▲, ex-smokers; △, current smokers) subjects. Individual results below the sensitivity of H$_2$O$_2$ method determination (0.083 µM) were assumed to be 0 µM. The mean H$_2$O$_2$ concentration in the whole COPD group was 0.48 ± 0.67 µM. *Significantly different from value found for healthy controls who had never smoked; $P<0.05$.

H$_2$O$_2$ AND TBAR LEVELS IN HEALTHY SUBJECTS

In healthy nonsmoking subjects the mean H$_2$O$_2$ concentration in expired breath condensate was 0.05 ± 0.07 µM ($n=17$) (Fig. 1). In 11 (65%) healthy controls (seven men, four women), no detectable H$_2$O$_2$ concentration was noted. TBARs results were positive only in two of 17 healthy volunteers. The means TBAR concentration in breath condensate for the control group was 0.04–0.14 µM (Fig. 2).

Results

The mean H$_2$O$_2$ concentration in expired breath condensate was 0.05 ± 0.07 µM ($n=17$) (Fig. 1). In 11 (65%) healthy controls (seven men, four women), no detectable H$_2$O$_2$ concentration was noted. TBARs results were positive only in two of 17 healthy volunteers. The means TBAR concentration in breath condensate for the control group was 0.04–0.14 µM (Fig. 2).

100-µl samples containing increasing (0.01–50 µM) tetramethoxypropane concentrations. The lower limit of TBARs detection was 0.05 µM. Control experiments showed that short-acting β$_2$-agonists and anticholinergics which may be exhaled and present in the breath condensate did not form fluorescent complexes with TBA (12).

**STATISTICAL ANALYSIS**

Data from subjects are expressed as mean ± SD. For readings which gave results before the method sensitivity, the H$_2$O$_2$ and TBAR concentration in expired breath condensate was assumed to be 0 nm. The differences between results found in healthy subjects and COPD patients were determined by analysis of variance (ANOVA). A $P$-value of less than 0.05 was considered to be significant. Pearson’s correlation was used to determine the relationships between measured variables. All calculations were performed using Microsoft Excel version 5.0 software.

Fig. 2. Concentration of TBARs in the expired breath condensate of healthy (○, never smoked) and COPD (□, never smoked; ▲, ex-smokers; △, current smokers). Individual results below the sensitivity of TBARs method determination (0.05 µM) were assumed to be 0 µM. The mean TBAR concentration in the whole COPD group was 0.48 ± 0.86 µM. Significantly different from value found for healthy controls who had never smoked; *$P<0.05$; **$P<0.01$. 

**Results**

**H$_2$O$_2$ AND TBAR LEVELS IN HEALTHY SUBJECTS**

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had never smoked; 0.50 for ex-smokers; and 0.45 for current smokers. No significant differences were noted between TBARs concentrations in COPD men and women (0.58 ± 0.98 μM vs. 0.36–0.68 μM) and also in COPD men and women who were current smokers (1.16 ± 1.19 μM, n=6 vs. 0.36 ± 0.68 μM, n=5).

COPD subjects with detectable H₂O₂ in breath condensate exhaled similar amounts of TBARs to those with H₂O₂ levels below the method sensitivity (0.43 ± 0.67 μM, n=8 vs. 0.57 ± 1.14 μM, n=16) and the rate of positive TBARs readings in these subgroups was 0.50 and 0.56, respectively. Only nine of 44 COPD subjects (20%) had negative readings of both TBARs and H₂O₂, while in healthy control group most of the analysed subjects (11 of 17; 65%) revealed levels below the detection sensitivity.

**Discussion**

We found that patients with stable COPD had much higher mean TBARs and H₂O₂ concentrations in their expired breath condensate than healthy nonsmoking subjects. Our findings provide evidence of oxidant overload and increased lipid peroxidation in the airways of COPD subjects and support the hypothesis that inflammatory processes within the airways involve free radical-mediated reactions. Patients with lung disorders accompanied by an increased number of activated inflammatory cells in the respiratory tract exhale more H₂O₂ and volatile lipid peroxidation products such as ethane, pentane and TBARs. It was

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**Table 3. Correlations between H₂O₂ and TBAR concentrations in expired breath condensate and selected clinical parameters in the whole COPD group and particulate subgroups (current smokers, ex-smokers, never smoked)**

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>Whole group</th>
<th>Current smokers</th>
<th>Ex-smokers</th>
<th>Never smoked</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H₂O₂ (μM)</td>
<td>TBARs (μM)</td>
<td>H₂O₂ (μM)</td>
<td>TBARs (μM)</td>
</tr>
<tr>
<td>FEV₁ (%)</td>
<td>0.40**</td>
<td>0.02</td>
<td>0.30</td>
<td>0.22</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
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<td>0.01</td>
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<td>0.23</td>
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<td>Disease duration</td>
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<td>–0.44</td>
<td>0.49</td>
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<tr>
<td>Present cigarette consumption</td>
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<td>ND</td>
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<td>–0.19</td>
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<tr>
<td>Cumulative cigarette consumption</td>
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<td>–0.30</td>
</tr>
<tr>
<td>Number of exacerbations per year</td>
<td>–0.08</td>
<td>0.06</td>
<td>0.51</td>
<td>0.03</td>
</tr>
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*P<0.05; **P<0.01.

FVC, forced vital capacity; ND, not determined.

**CORRELATIONS OF H₂O₂ AND TBARS WITH CLINICAL STATUS, SMOKING HABITS AND LUNG FUNCTION**

Analysis of correlations of exhaled H₂O₂ and TBARs with selected clinical variables showed only a few statistically significant results (Table 3). The concentration of H₂O₂ in expired breath condensate moderately correlated with FEV₁% predicted in the whole COPD group as well in the ex-smokers and never-smoked COPD subgroups. There was also a positive correlation between TBARs levels and FEV₁% predicted and TBARs and disease duration in never smoked COPD subjects and former smokers with COPD, respectively. No associations between cigarette smoking status (present cigarette consumption, cumulative cigarette consumption), H₂O₂ and TBARs were noted. Similarly, H₂O₂ concentration did not correlate with TBARs levels in breath condensate in the whole COPD group (r = -0.02) (Fig. 3) and in the current smokers and ex-smokers COPD subgroups (r=-0.20; r= -0.34, respectively). However, there was a strong positive correlation between H₂O₂ and TBARs in the expired breath condensate of COPD subjects who had never smoked (r=0.85, P<0.0003).

**Fig. 3.** The H₂O₂- and TBAR-dependent distribution of breath condensate samples from 44 COPD subjects (●) and 17 healthy controls (○). All samples from healthy subjects are localized around the point of intersection of the dashed lines showing the sensitivity of H₂O₂ and TBARs methods determination. Twenty-six samples (59%) from COPD subjects are distant from this point. There was no correlation between H₂O₂ and TBAR concentrations in the whole COPD group. Due to overlapping, the number of circles is lower than number of analysed subjects.
proved for subjects with adult respiratory distress syndrome (20,24–26), pneumonia (25), bronchial asthma (12,27,28) and also for asymptomatic cigarette smokers (10,13,29). Alveolar macrophages and polymorphonuclear leukocytes seem to be the main source of exhaled \( \text{H}_2\text{O}_2 \) in persons exposed to cigarette smoke. Both asymptomatic cigarette smokers and subjects with COPD have increased number of these cells in bronchoalveolar lavage fluid (2,3,30). They are activated and produce more reactive oxygen species including \( \text{H}_2\text{O}_2 \) than cells obtained from healthy nonsmoking subjects (2,3). \( \text{H}_2\text{O}_2 \) may also originate from type II pneumocytes, lung microsomes and mitochondria (21,31). As COPD patients use drugs which inhibit cellular response to inflammatory mediators (32,33) the exhalation of \( \text{H}_2\text{O}_2 \) and TBARs could be even higher after cessation of this medication.

Cigarette smoke contains many reactive oxygen species and an aqueous solution of cigarette smoke generates \( \text{H}_2\text{O}_2 \) (34,35). COPD smokers refrained from smoking for 12 h before condensate collection. Therefore, it seems that the \( \text{H}_2\text{O}_2 \) measured in breath condensate did not originate directly from cigarette smoke. There is catalase activity in the alveolar lining fluid (36) which could decompose cigarette smoke-derived \( \text{H}_2\text{O}_2 \) during the 12 h preceding condensate collection. In addition exhaled ethane (an alkene by-product of lipid peroxidation) decreased to baseline at 3 h after the last cigarette in cigarette smokers, but this value was still higher than that of subjects who had never smoked (29).

The effect of chronic cigarette smoking on the metabolic activity of pulmonary phagocytes and free-radical mediated processes may persist for a long time in ex-smokers (37,38). Former smokers had a higher annual decline in FEV\(_1\) than healthy subjects who had never smoked (39) and post-mortem histopathological examination of the bronchial walls of COPD ex-smokers showed distinct signs of inflammation and eosinophilic infiltration (40).

Thus, ex-smokers, especially those suffering from COPD [they represent populations susceptible to effects of smoking (41)] may reveal increased oxidant generation in the airways as a consequence of previous cigarette smoking. The COPD subjects who had never smoked had been working for many years in a dusty environment. In response to dust particles pulmonary phagocytes release inflammatory mediators and reactive oxygen species (42) that may result in increased exhalation of \( \text{H}_2\text{O}_2 \) and TBARs.

MDA is one of the most important volatile TBARs. It is recognized as an end-product of polyunsaturated fatty acid peroxidation; however, it could be formed during oxidative injury of DNA, proteins or carbohydrates (9). Hence, elevated TBARs levels in expired breath condensate of COPD subjects may reflect the increased intensity of peroxidative damage to lung tissue. The presence of \( \text{H}_2\text{O}_2 \) in expired breath condensate may be a result of several processes, including production of \( \text{H}_2\text{O}_2 \), its diffusion through cell membranes, reactions with various biomolecules, decomposition by catalase or glutathione peroxidase and evaporation from the alveolar lining fluid. Higher exhalation of \( \text{H}_2\text{O}_2 \) may reflect its higher production and risk of peroxidative damage to lung tissue with formation of volatile TBARs. On the other hand, the decrease in exhaled \( \text{H}_2\text{O}_2 \) may result from its higher conversion into hydroxyl radicals with subsequent generation of TBARs in pulmonary parenchyma. In addition, pulmonary glutathione and activity of antioxidant enzymes may rise in response to chronic inflammatory processes and exposition of airways to cigarette smoke (15,30). These may explain the lack of association between exhaled \( \text{H}_2\text{O}_2 \) and TBARs in the whole COPD group and also no significant effect of current cigarette smoking on these two variables. It is consistent with previous studies showing no correlation between exhaled ethane and \( \text{H}_2\text{O}_2 \) and smoking measures in cigarette smokers (10,13). However, analysis of breath condensate obtained from COPD subjects who had never smoked showed a strong positive correlation between \( \text{H}_2\text{O}_2 \) and TBARs, which is consistent with our previous observations in nonsmoking asthmatics (12).

The rate of negative \( \text{H}_2\text{O}_2 \) readings in COPD group was only 1.8-fold lower than that in healthy controls and 36% of COPD subjects had no detectable \( \text{H}_2\text{O}_2 \) in their breath condensate. Surprisingly, COPD subjects with detectable \( \text{H}_2\text{O}_2 \) did not exhale more TBARs than those with negative \( \text{H}_2\text{O}_2 \) readings. This may suggest that some TBARs originate from enzymatic (i.e. independent of free radical) lipid peroxidation which is usually enhanced in sites of inflammation (9), and/or that the exhaled \( \text{H}_2\text{O}_2 \) mostly represents a pool of reactive oxygen species not involved in free radical-mediated reactions leading to lipid peroxidative damage. This \( \text{H}_2\text{O}_2 \) pool may be released into the epithelial lining fluid and evaporate before the induction of lung lipid peroxidative damage. On the other hand, the rate of COPD subjects with both \( \text{H}_2\text{O}_2 \) and TBARs negative readings was 3.25 times lower than that in healthy control group. This indicates that simultaneous measurement of \( \text{H}_2\text{O}_2 \) and TBARs more effectively distinguishes the samples of expired breath condensate obtained from COPD subjects and healthy controls (Fig. 3).

Neither \( \text{H}_2\text{O}_2 \) nor TBARs correlated with spirometric parameters in the whole COPD group. However, in ex-smokers and COPD subjects who had never smoked, TBARs and \( \text{H}_2\text{O}_2 \) correlated positively with FEV\(_1\). Subjects with higher FEV\(_1\) levels may represent those with lower inflammatory damage to lung tissue and perhaps a lower secondary rise in antioxidant enzymes activities. It has been proved that cytokines and endotoxin can upregulate the pulmonary antioxidant defence (43–45). On the other hand, our previous studies have showed a negative correlation between TBARs, \( \text{H}_2\text{O}_2 \) and FEV\(_1\) in asthmatics (12) and this discrepancy may result from the different natures of these diseases. In conclusion, we found that patients with stable COPD had elevated TBAR and \( \text{H}_2\text{O}_2 \) concentrations in their expired breath condensate. This may reflect oxidant overload in the airways and peroxidative damage to lung tissue in COPD subjects. Neither parameter correlated with cigarette smoking status and the majority of clinical parameters. Although measurement of TBARs and \( \text{H}_2\text{O}_2 \) was able to distinguish COPD subjects from healthy controls, this approach failed to find any differences between the COPD subgroups of current smokers, former smokers...
and subjects with occupational COPD who had never smoked.

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References


