Exhaled Ethane, a Marker of Lipid Peroxidation, Is Elevated in Chronic Obstructive Pulmonary Disease

PAOLO PAREDI, SERGEI A. KHARITONOV, DAVID LEAK, SIMON WARD, DAVID CRAMER, and PETER J. BARNES

Department of Thoracic Medicine, Imperial College School of Medicine at the National Heart and Lung Institute; Department of Biochemistry, Imperial College of Science, Technology and Medicine; and Lung Function Unit, Royal Brompton Hospital, London, United Kingdom

Ethane is a product of lipid peroxidation and can be measured in the exhaled air as an index of oxidative stress. Oxidant/antioxidant imbalance is important in the pathogenesis of chronic obstructive pulmonary disease (COPD). Therefore, we measured exhaled ethane in 22 patients with COPD (mean age ± SEM, 59 ± 8 yr; 19 male) and compared it with other noninvasive markers of oxidative stress and inflammation such as carbon monoxide (CO), measured electrochemically, and nitric oxide (NO), measured by chemiluminescence. Exhaled ethane was collected during a flow and pressure-controlled exhalation into a reservoir, discarding dead space air contaminated with ambient air. A sample of the collected expired air was analyzed by chromatography. Compared with normal subjects (n = 14; eight men; age, 33 ± 2.8 yr), patients with COPD not on steroid treatment (n = 12; FEV_1, 58 ± 6%) had elevated levels of exhaled ethane (2.77 ± 0.25 and 0.88 ± 0.09 ppb, respectively, $p < 0.05$), CO (5.96 ± 0.50 and 2.8 ± 0.25 ppm, $p < 0.05$) and NO (11.86 ± 0.53 and 6.77 ± 0.50 ppb, $p < 0.05$) levels. Ethane was correlated to FEV_1 ($r = -0.67$, $p < 0.05$). Patients receiving steroid treatment (n = 10; FEV_1, 56 ± 2%) had lower levels of ethane (0.48 ± 0.05 ppb) than did steroid-treated patients, whereas CO (5.99 ± 0.63 ppm) and NO (9.11 ± 0.53 ppb) levels were similar in the two treatment groups. Exhaled ethane is elevated, correlates with FEV_1, and is significantly lower in patients treated with steroids, so it may be complementary to the use of NO and CO in assessing and monitoring oxidative stress in COPD.

There is evidence for a role of oxidative stress in the pathogenesis and progression of chronic obstructive pulmonary disease (COPD) (1, 2). Reactive oxygen species (ROS) are unstable compounds with unpaired electrons, capable of initiating oxidation. Several of the inflammatory cells that participate in the inflammatory response such as neutrophils and eosinophils and macrophages release ROS in amounts that exceed the capacity of tissue antioxidant defenses (1, 3). Furthermore, cigarette smoke generates ROS such as peroxynitrates and oxygen free radicals. Smokers have increased numbers of macrophages and neutrophils in their bronchoalveolar lavage. In addition, alveolar macrophages and blood neutrophils of smokers release more superoxide anions (O_2^-) than do those from nonsmoking control subjects (4). A correlation has been demonstrated between O_2^- release by neutrophils and bronchial hyperactivity in patients with COPD, and O_2^- release is increased during acute exacerbations (2). The final result of cigarette smoking and chronic neutrophil inflammation is an oxidant antioxidant imbalance.

One mechanism by which oxidants can cause lung injury is through lipid peroxidation. ROS such as O_2^- and hydrogen peroxide (H_2O_2) released by activated immune and inflammatory cells can induce (5) the lipid peroxidation of polyunsaturated membrane fatty acids, impair membrane function and inactivate membrane-bound receptors and enzymes, increase tissue permeability (6), and therefore promote airflow limitation.

The determination of hydrocarbons in the exhaled air has been proposed as a means to assess lipid peroxidation *in vivo* (7–9) and ethane received particular attention because of its easier and faster chromatographic measurement compared with other hydrocarbons (10). The first analyses of the organic compounds present in the exhaled air from human subjects were performed in the 1960s (11). Since then, the research in this area has progressed slowly because of technical and practical problems such as the influence of ambient hydrocarbons on exhaled breath levels of these gases. We modified a previously developed technique for single-breath analysis of exhaled hydrocarbons (10) by allowing airway dead space washout during exhalation eliminating ambient contamination of the exhaled breath. We applied this simplified technique to the measurement of exhaled ethane in patients with COPD.

Noninvasive markers of inflammation and oxidative stress would be of great benefit in disease management and monitoring and in the assessment of drugs efficacy. In this respect other exhaled gases have already been investigated. In COPD high levels of exhaled nitric oxide (NO) (12) and carbon monoxide (CO) (13) may reflect the release of several mediators, including cytokines and ROS that can induce heme oxygenase-1 (HO-1) and inducible NO synthase (iNOS), the enzymes that catalyze the production of CO and NO respectively.

In view of the role of oxidative stress in the pathogenesis and progression of COPD, we measured exhaled ethane as a marker of lipid peroxidation, and compared it with two other noninvasive volatile markers of oxidative stress and inflammation, NO and CO.

METHODS

Patients

All the patients enrolled met the American Thoracic Society criteria for the diagnosis of COPD (14): a history of productive cough for 3 mo in each of two successive years and FEV_1/VC ratio of less than 60%, the TLC being more than 80% of the predicted value. Furthermore, all the patients were ex-smokers with a history of smoking equivalent to at least 20 pack-years. None of the subjects included in the study had a previous medical history of allergic disease or a significant reversibility of airflow obstruction (> 15% or > 200 ml) after inhalation of 400 μg salbutamol via a metered-dose inhaler. The patients were in a stable state, symptoms of COPD, especially the grade of dyspnea, were unchanged, and the gasometric values had not worsened compared with the best previous values.

Twenty-two patients with COPD (mean age ± SEM, 59 ± 8 yr; 19 male) all of whom were confirmed ex-smokers, were studied (10 had received steroid treatment for at least 3 mo) (Table 1). All the steroid-treated patients were receiving inhaled steroids (n = 3 fluticasone propionate, 500 μg/d; n = 7 beclomethasone, 500 μg/d), and seven of them were receiving oral steroids (prednisolone, 25 ± 5 mg/d). None of 14 nonsmoking control subjects (eight men; age, 33 ± 3 yr) had a history of respiratory or cardiovascular disease. There was no history of upper respiratory tract infection for at least 4 wk before the study

(*Received in original form September 7, 1999 and in revised form January 14, 2000*)

Correspondence and requests for reprints should be addressed to Professor P. J. Barnes, Department of Thoracic Medicine, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, UK. E-mail: p.j.barnes@ic.ac.uk

Am J Respir Crit Care Med Vol 162. pp 369–373, 2000
Internet address: www.atsjournals.org

TABLE 1

PATIENT CHARACTERISTICS

	Notsteroid-treated Patients (*n = 12*)	Steroid-treated Patients (*n = 10*)	Normal Subjects (*n = 14*)
Age, yr*	60 ± 18	58 ± 2	33 ± 3
Sex, M/F	10/2	9/1	8/6
FEV_1, % pred*	56 ± 3	56 ± 15	95 ± 9
Smokers	0	0	0
Ex-smokers	12	10	0
Therapy			
Inhaled β-adrenergics	12	10	0
Theophylline	9	7	0
Inhaled steroids	0	10	0
Oral steroids	0	7	0

* Values are means ± SEM.

in all studied subjects. Considering that cigarette smoke can affect the level of exhaled NO, CO and hydrocarbons, current smokers were excluded from this study. The smoking status of all the subjects was confirmed by nicCheck I (DynaGen, Inc., Cambridge, MA), which detects nicotine and its metabolites in the urine. Active and passive smokers (smoke exposure for more than 30 min/d) were excluded from the study. All patients had at least 1 h of rest before gas measurement, in order to eliminate the effect of any possible exposure to high ethane concentrations (7) during their journey to the hospital.

Exhaled Ethane

Exhaled air was collected during a flow and pressure-controlled exhalation into a reservoir discarding dead space air contaminated with ambient air, as previously described (15). A sample (2 ml) of the collected expired air was analyzed for ethane content using gas chromatography (Chromatograph model PU 4500; Philips, Eindhoven, The Netherlands); column Poropak Q 1-3-m × 4 mm; column temperature, 60° C; injector temperature, 140° C; detector temperature, 160° C; signal output to a CR6A integrator (Shimadzu, Kyoto, Japan). The sensitivity of exhaled ethane measurement was 0.4 ppb.

In a preliminary study the reproducibility of this method was proved by the Bland and Altman test (16). The reproducibility of a method reflects the extent to which a result varies when it is repeated. This variability can be assessed plotting the mean of two measurements against their difference, and the method is considered reproducible if the values are within ± 2 standard deviations of the mean. The reproducibility of ethane measurement was also determined as a coefficient of variation (standard deviation/mean value × 100%). The coefficient of variation of exhaled ethane measured during two successive collections at 5-min intervals (single session variability) was 5.4% (n = 22), whereas between-sessions variability (n = 6, 1-d interval) was 6.2%. The levels of exhaled ethane were strictly exhalation flow-dependent. Six normal subjects and five patients with COPD were instructed to perform a complete exhalation from TLC maintaining two different expiratory flows. Exhaled ethane levels were significantly higher at the lower (5 to 6 L/min) than at the higher exhalation rate (10 to 11 L/min) both in normal subjects (60 ± 8%) and in patients with COPD (65 ± 5%). To reduce the effect of the flow dependency on exhaled ethane levels, all the sample were collected at 10 to 11 L/min. Ethane concentration was equally stable in five polyethylene and five Tedlar reservoirs for 48 h after collection (% increase: 3 ± 1% and 5 ± 2% for the polyethylene and Tedlar reservoirs, respectively).

Exhaled CO and NO Measurements

Exhaled CO was measured by a modified electrochemical sensor with sensitivity from 1 part per million (ppm) to 500 ppm of CO, simultaneously with NO measurement by LR2000 chemiluminescence analyzer (Logan Research Ltd, Rochester, Kent, UK) in order to control exhalation parameters (mouth pressure, 3 ± 0.4 mm Hg; exhalation flow 5 to 6 L/min). The subjects exhaled slowly from TLC over 10 to 15 s trying to keep a constant flow. The mean of two reproducible measurements with less than 5% variation was recorded. Ambient CO was recorded before each measurement and subtracted to the mean value obtained during the maneuvers, as stated by other investigators (17). NO was measured as described previously (18). The response time was 500 ms for NO measurements and the lag time was 1.4 s; therefore, the total response time (lag time + response time) was 1.9 s. This is in compliance with the American Thoracic Society guidelines and European guidelines for exhaled NO measurement, which require a total response time of less than 7 s (18). The response time for CO measurement is < 5 s and the lag time is 200 ms. The accuracy was ± 0.3 ppb for NO and ± 0.2 ppm for CO measurements, this was also in accordance with the European guidelines for exhaled NO measurement (18).

Statistics

All the results were expressed as means ± SEM. Comparisons between groups were made by two-way analysis of variance (ANOVA). A p value < 0.05 was considered significant. The reproducibility of ethane measurement was assessed by the Bland and Altman test (16).

RESULTS

Exhaled Ethane

Ethane levels were elevated in steroid-naïve (2.77 ± 0.25 ppb) compared with steroid-treated (0.48 ± 0.05 ppb) patients ($p < 0.01$) and with normal control subjects (0.88 ± 0.09 ppb, $p < 0.05$) (Figure 1, *panel A*, and Figure 2). There was a correlation between exhaled ethane and FEV_1 in patients not receiving steroid treatment ($r = -0.67$, $p < 0.05$) (Figure 1, *panel B*).

Exhaled CO

Exhaled CO levels were similar in untreated (5.99 ± 0.50 ppm) and in steroid-treated (5.96 ± 0.63 ppm) patients ($p > 0.05$) and higher than in normal subjects (2.8 ± 0.2 ppm, $p < 0.05$) (Figure 3).

Exhaled NO

NO levels were similar in untreated patients (11.86 ± 1.27 ppb) compared with steroid-treated patients (9.11 ± 0.53 ppb, $p > 0.05$), and elevated compared with the control group (6.7 ± 0.5 ppb, $p < 0.05$) (Figure 4).

DISCUSSION

We have demonstrated that patients with COPD have elevated levels of exhaled NO, CO, and ethane, and that the latter correlates with disease severity as assessed by FEV_1. In addition, we found lower exhaled ethane concentrations in steroid-treated patients than in untreated patients. We interpret these findings as confirmation that oxidative stress and lipid peroxidation are increased in the airways of patients with COPD and we suggest that the measurement of exhaled ethane may be complementary in the noninvasive evaluation of inflammation and oxidative stress in the airways.

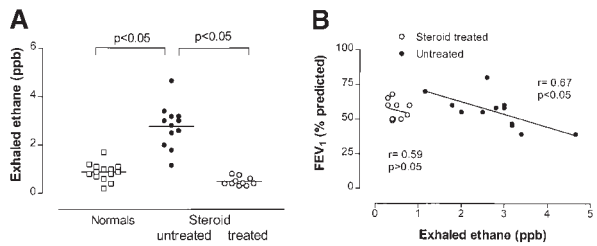

Figure 1. Levels of exhaled ethane (*panel A*) and correlation with FEV_1 levels (*panel B*) in normal subjects (*open squares*), patients with COPD not receiving steroid treatment (*closed circles*) and receiving steroid treatment (*open circles*).

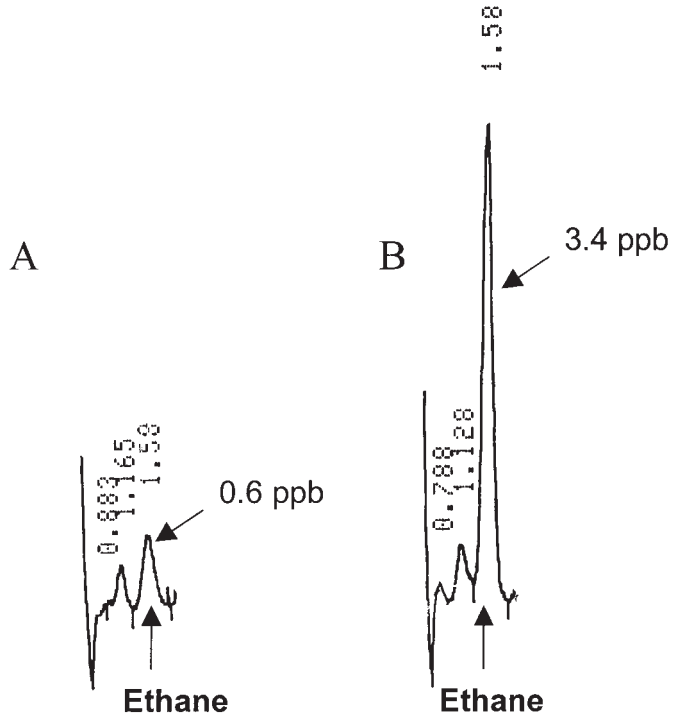

Figure 2. Sample cromatograph of exhaled breath analysis from a normal subject (*panel A*) and from a patient with COPD (*panel B*). The gas chromatogram peak corresponding to ethane (retention time, 1.58 min) is indicated with arrows.

Oxidative stress has been implicated in the pathogenesis of COPD (1, 2). Smoking increases the oxidant-antioxidant imbalance, in part because cigarette smoke itself contains a great number of ROS such as peroxynitrates and in part because it increases the number of inflammatory cells in alveoli, which release ROS such as O_2^- and hydrogen peroxide (H_2O_2) (3, 19), causing oxidation of nucleic acids, proteins, and membrane lipids (19). There is evidence for an increased lipid peroxidation in COPD (20, 21) and breath hydrocarbons have been studied as a measure of its activity (7–9). Polyunsaturated fatty acids are found in the cellular and subcellular membranes and are prone to lipid peroxidation as a result of the extremely weak binding of the hydrogen atoms to the carbon chain. Ethane and pentane are hydrocarbons released during lipid peroxidation in biological tissues. Ethane specifically results from the effects of free radicals on the omega-3 fatty ac-

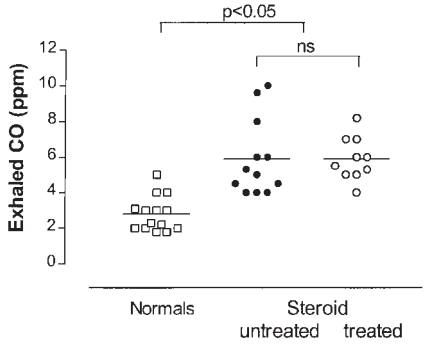

Figure 3. Levels of exhaled carbon monoxide CO in normal subjects (*open squares*), patients with COPD not receiving steroid treatment (*closed circles*) and receiving steroid treatment (*open circles*).

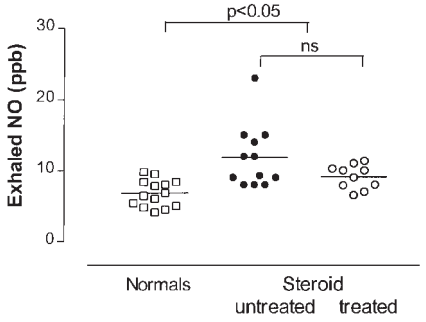

Figure 4. Levels of exhaled nitric oxide NO in normal subjects (*open squares*), patients with COPD not receiving steroid treatment (*closed circles*) and receiving steroid treatment (*open circles*).

ids such as 9,12,15-linolenic acid, whereas pentane derives from the peroxidation of n-6 polyunsaturated acids such as 9,12,15-linoleic and arachidonic acid. Ethane has been used as a noninvasive marker of lipid peroxidation since the 1960s (11) and has been confirmed as a potential marker of lipid peroxidation *in vivo* (9). We favor ethane over pentane as a measure of lipid peroxidation because of the more rapid metabolism of pentane and of its difficult chromatographic separation from isoprene, another product of lipid peroxidation.

We found elevated levels of exhaled ethane in patients with COPD when compared with normal subjects. These results indicate that there is increased lipid peroxidation in COPD, confirming previous studies showing elevated levels of other markers of lipid peroxidation such as urinary and plasma concentrations of 8-isoprostane (20, 21). Furthermore, in keeping with the results of Habib and coworkers (22), who showed that exhaled ethane is correlated with airway obstruction, we found a negative correlation between exhaled ethane and disease severity as assessed by FEV_1. This indicates that in patients with more severe COPD lipid peroxidation is more active and may play a major role in the progression of the disease. Spirometric measurements and arterial blood gas determinations are the best predictor of mortality; however, they provide an incomplete picture of the other elements that are important to define the severity and progression of COPD such as inflammation and oxidative stress (1). Therefore, even if the rate of decline of lung function may return to normal in patients with COPD who are ex-smokers, the measurement of exhaled gases may complement the clinical assessment, being a reflection of the underlying activity of the inflammatory process and oxidative stress. Pathologic studies are necessary to confirm this hypothesis.

Age does not influence the levels of ethane in the exhaled breath (9); therefore, the older age of the patients with COPD compared with the control group does not explain the difference in exhaled ethane levels in these two groups. However, exhaled ethane may not be solely of lung origin but may be transported to the lung for elimination. Ethane is in fact produced in other organs such as the intestine, brain, kidney, liver, heart, diaphragm, and testis (23), therefore, the systemic oxidative stress that characterizes COPD (2) may contribute to the final concentration of ethane in the exhaled breath.

The use of steroid treatment in patients with stable COPD is controversial, even though some patients can benefit from it (24). High bronchoalveolar and sputum (25) eosinophils counts have been suggested as predictors of the efficacy of steroid treatment. In the present study, of the 10 patients receiving steroid treatment, seven were receiving oral as well as inhaled steroids and had lower levels of exhaled ethane than the

untreated patients. This indicates that steroid treatment reduces lipid peroxidation. Steroids, in fact, by reducing inflammation, attenuating the release of oxidants by inflammatory cells (26), and suppressing proinflammatory cytokines production (27) may reduce lipid peroxidation, and therefore the synthesis of ethane. It is noteworthy that the levels of exhaled NO and CO were not different in the two treatment groups. This may be due to the lack of a suppressive effect of inhaled steroids on the neutrophilic inflammation that characterizes COPD (28), reflected in the constant activation of iNOS and HO-1 and therefore in the high production of NO and CO also in steroid-treated patients. Exhaled ethane is a marker of lipid peroxidation and therefore it reflects the damage of cell membranes caused by reactive oxygen species. On the other hand, exhaled CO and NO are an indirect measurement of oxidative stress mediated by HO and iNOS activity. The different effect of steroid treatment on the level of exhaled gases may be the result of a complex interaction with enzymes and release of ROS by inflammatory cells. The measurement of exhaled ethane, CO, and NO may be complementary, providing an estimate of the intensity of the oxidative stress and the actual cell damage. The combined use of exhaled gases as noninvasive markers of oxidative stress is particularly appealing if one considers that decreasing the inflammatory response may prevent structural damage to the airways.

Nitric oxide (NO) is a gas produced by several types of pulmonary cells, including inflammatory, endothelial, and airway epithelial cells. Elevated levels of exhaled NO in asthma (29) are likely to be due to the activation of the inducible form of NO synthase (iNOS) (30) and therefore may reflect airway inflammation. In COPD the measurement of exhaled NO as a noninvasive marker of inflammation has already been investigated (12, 31–33). The finding by some investigators of high levels of exhaled NO (12, 31) in patients with stable COPD was not confirmed by others who showed either normal exhaled NO concentrations (32) or even low levels of this gas (33). These disparities may be due to a diversity of methods used for NO measurements such as excretion rate (32) versus single breath (12), or in patient selection with mild (31) versus severe (33) disease. In the present study NO levels were elevated in COPD and were not influenced by steroid treatment in accordance with our previous data (12). The measurement of exhaled NO may provide a noninvasive method to assess airway inflammation also in COPD. Furthermore, because NO levels are correlated with sputum eosinophil counts (25) and that the latter are a predictor of steroid efficacy, NO measurement may be of assistance in the selection of patients who may benefit from steroid treatment.

CO is a product of heme degradation by HO. The inducible form of HO (HO-1) is activated in the alveolar macrophages of asthmatic patients compared with normal subjects (34) as a result of increased oxidative stress. Elevated levels of CO in exhaled air may reflect the degree of HO-1 induction as confirmed by higher levels of CO during allergen challenge (35).

We found high levels of exhaled CO in patients with COPD who were ex-smokers, as previously shown (13). Heme oxygenase is present in the pulmonary vascular endothelium and alveolar macrophages and can be upregulated by oxidative stress and proinflammatory cytokines (5), thus increasing the production of CO. We presume that the high levels of exhaled CO found in patients with COPD are due to inflammatory cytokines or ROS induced HO-1 expression and therefore that the measurement of exhaled CO may reflect inflammation, oxidative stress, or both.

Measurement of exhaled ethane may be another means of detecting and monitoring cytokine and oxidant-mediated inflammation and of assessing anti-inflammatory treatments. In addition to its potential as a marker of oxidative stress, it is possible that the increase in exhaled ethane may be of pathophysiologic significance. Further studies are necessary to investigate the correlation of exhaled ethane with other markers of lipid peroxidation and the clinical utility of exhaled ethane measurement in the follow-up of patients with COPD.

References

1. Repine, J. E., A. Bast, and I. Lankhorst. 1997. Oxidative stress in chronic obstructive pulmonary disease: Oxidative Stress Study Group. *Am. J. Respir. Crit. Care Med.* 156:341–357.
2. Rahman, I., D. Morrison, K. Donaldson, and W. MacNee. 1996. Systemic oxidative stress in asthma, COPD, and smokers. *Am. J. Respir. Crit. Care Med.* 154:1055–1060.
3. Barnes, P. J. 1990. Reactive oxygen species and airway inflammation. *Free Radic. Biol. Med.* 9:235–243.
4. Ludwig, P. W., and J. R. Hoidal. 1982. Alterations in leukocyte oxidative metabolism in cigarette smokers. *Am. Rev. Respir. Dis.* 126:977–980.
5. Choi, A. M., and J. Alam. 1996. Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am. J. Respir. Cell Mol. Biol.* 15:9–19.
6. Morrison, D., I. Rahman, S. Lannan, and W. MacNee. 1999. Epithelial permeability, inflammation, and oxidant stress in the air spaces of smokers. *Am. J. Respir. Crit. Care Med.* 159:473–479.
7. Habib, M. P., N. C. Clements, and H. S. Garewal. 1995. Cigarette smoking and ethane exhalation in humans. *Am. J. Respir. Crit. Care Med.* 151:1368–1372.
8. Kazui, M., K. A. Andreoni, E. J. Norris, *et al.* 1992. Breath ethane: a specific indicator of free-radical-mediated lipid peroxidation following reperfusion of the ischemic liver. *Free Radic. Biol. Med.* 13:509–515.
9. Kneepkens, C. M., G. Lepage, and C. C. Roy. 1994. The potential of the hydrocarbon breath test as a measure of lipid peroxidation. *Free Radic. Biol. Med.* 17:127–160.
10. Zarling, E. J., and M. Clapper. 1987. Technique for gas-chromatographic measurement of volatile alkanes from single-breath samples. *Clin. Chem.* 33:140–141.
11. Aulik, I. V. 1966. Gas chromatographic analysis of exhaled air and acetylene mixture. *Biull. Eksp. Biol. Med.* 62:115–117.
12. Maziak, W., S. Loukides, S. Culpitt, P. Sullivan, S. A. Kharitonov, and P. J. Barnes. 1998. Exhaled nitric oxide in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 157:998–1002.
13. Culpitt, S., P. Paredi, S. A. Kharitonov, and P. J. Barnes. 1998. Exhaled carbon monoxide is increased in COPD patients regardless of their smoking habit (abstract). *Am. J. Respir. Crit. Care Med.* 157:A787.
14. American Thoracic Society. 1995. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease: ATS statement. *Am. J. Respir. Crit. Care Med.* 152(Suppl.):S77–S120.
15. Paredi, P., S. Loukides, S. Ward, D. Cramer, M. Spicer, S. A. Kharitonov, and P. J. Barnes. 1998. Exhalation flow and pressure-controlled reservoir collection of exhaled nitric oxide for remote and delayed analysis. *Thorax* 53:775–779.
16. Bland, J. M., and D. G. Altman. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1:307–310.
17. Zayasu, K., K. Sekizawa, S. Okinaga, M. Yamaya, T. Ohrui, and H. Sasaki. 1997. Increased carbon monoxide in exhaled air of asthmatic patients. *Am. J. Respir. Crit. Care Med.* 156:1140–1143.
18. Kharitonov, S., K. Alving, and P. J. Barnes. 1997. Exhaled and nasal nitric oxide measurements: recommendations: The European Respiratory Society Task Force. *Eur. Respir. J.* 10:1683–1693.
19. Kinnula, V. L., J. D. Crapo, and K. O. Raivio. 1995. Generation and disposal of reactive oxygen metabolites in the lung. *Lab. Invest.* 73:3–19.
20. Morrow, J. D., B. Frei, A. W. Longmire, J. M. Gaziano, S. M. Lynch, Y. Shyr, W. E. Strauss, J. A. Oates, and L. J. Roberts II. 1995. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers: smoking as a cause of oxidative damage. *N. Engl. J. Med.* 332:1198–1203.
21. Pratico, D., S. Basili, M. Vieri, C. Cordova, F. Violi, and G. A. Fitzgerald. 1998. Chronic obstructive pulmonary disease is associated with an increase in urinary levels of isoprostane $F_{2\alpha}$-III, an index of oxidant stress. *Am. J. Respir. Crit. Care Med.* 158:1709–1714.
22. Habib, M. P., L. J. Tank, L. C. Lane, and H. S. Garewal. 1999. Effect of vitamin E on exhaled ethane in cigarette smokers. *Chest* 115:684–690.

23. Habib, M. P., and M. A. Katz. 1989. Source of ethane in expirate of rats ventilated with 100% oxygen. *J. Appl. Physiol.* 66:1268–1272.
24. Boushey, H. A. 1999. Glucocorticoid therapy for chronic obstructive pulmonary disease. *N. Engl. J. Med.* 340:1990–1991.
25. Pizzichini, E., M. M. Pizzichini, P. Gibson, K. Parameswaran, G. J. Gleich, L. Berman, J. Dolovich, and F. E. Hargreave. 1998. Sputum eosinophilia predicts benefit from prednisone in smokers with chronic obstructive bronchitis. *Am. J. Respir. Crit. Care Med.* 158:1511–1517.
26. Marumo, T., V. B. Schini-Kerth, R. P. Brandes, and R. Busse. 1998. Glucocorticoids inhibit superoxide anion production and p22 phox mRNA expression in human aortic smooth muscle cells. *Hypertension* 32:1083–1088.
27. Inoue, H., H. Aizawa, S. Fukuyama, S. Takata, K. Matsumoto, M. Shigyo, H. Koto, and N. Hara. 1999. Effect of inhaled glucocorticoid on the cellular profile and cytokine levels in induced sputum from asthmatic patients. *Lung* 177:53–62.
28. Keatings, V. M., A. Jatakanon, Y. M. Worsdell, and P. J. Barnes. 1997. Effects of inhaled and oral glucocorticoids on inflammatory indices in asthma and COPD. *Am. J. Respir. Crit. Care Med.* 155:542–548.
29. Alving, K., E. Weitzberg, and J. M. Lundberg. 1993. Increased amount of nitric oxide in exhaled air of asthmatics. *Eur. Respir. J.* 6:1368–1370.
30. Nathan, C., and Q. W. Xie. 1994. Regulation of biosynthesis of nitric oxide. *J. Biol. Chem.* 269:13725–13728.
31. Kanazawa, H., S. Shoji, T. Yoshikawa, K. Hirata, and J. Yoshikawa. 1998. Increased production of endogenous nitric oxide in patients with bronchial asthma and chronic obstructive pulmonary disease. *Clin. Exp. Allergy* 28:1244–1250.
32. Rutgers, S. R., T. W. van der Mark, W. Coers, *et al.* 1999. Markers of nitric oxide metabolism in sputum and exhaled air are not increased in chronic obstructive pulmonary disease. *Thorax* 54:576–580.
33. Clini, E., L. Bianchi, M. Pagani, and N. Ambrosino. 1998. Endogenous nitric oxide in patients with stable COPD: correlates with severity of disease. *Thorax* 53:881–883.
34. Horvath, I., L. E. Donnelly, A. Kiss, P. Paredi, S. A. Kharitonov, and P. J. Barnes. 1998. Raised levels of exhaled carbon monoxide are associated with an increased expression of heme oxygenase-1 in airway macrophages in asthma: a new marker of oxidative stress. *Thorax* 53: 668–672.
35. Paredi, P., M. J. Leckie, I. Horvath, L. Allegra, S. A. Kharitonov, and P. J. Barnes. 1999. Changes in exhaled carbon monoxide and nitric oxide levels following allergen challenge. *Eur. Respir. J.* 13:48–52.