ERJ Express. Published on July 2, 2009 as doi: 10.1183/09031936.00177508

BRONCHIAL NITRIC OXIDE IS RELATED TO SYMPTOM RELIEF DURING FLUTICASONE TREATMENT IN COPD

Lauri Lehtimäki[1,2], Hannu Kankaanranta[1,3], Seppo Saarelainen[2], Ilkka Annila[2], Tiina Aine[2], Riina Nieminen[1], Eeva Moilanen[1]

[1]The Immunopharmacology Research Group, Medical School, University of Tampere; and The Science Centre, Tampere University Hospital, Tampere, Finland.

[2]Department of Respiratory Medicine, Tampere University Hospital, Tampere, Finland.

[3]Department of Respiratory Medicine, Seinäjoki Central Hospital, Seinäjoki, Finland.

Corresponding author: Lauri Lehtimäki, MD

The Immunopharmacology Research Group, Medical School

FIN-33014 University of Tampere

Finland

lauri.lehtimaki@uta.fi

fax: +358 3 35518082

Short title: Inflammation and treatment response in COPD

ABSTRACT

High levels of exhaled nitric oxide (NO) predict favorable response to inhaled corticosteroids in asthma, but the ability of exhaled NO or inflammatory markers in exhaled breath condensate (EBC) to predict steroid-responsiveness in COPD is not known.

We measured alveolar and bronchial NO output, levels of leukotriene B_4 (LTB_4), cysteinyl leukotrienes (cysLTs) and 8-isoprostane in EBC, spirometry, body plethysmography and symptoms in 40 subjects with COPD before and after 4 weeks of treatment with inhaled fluticasone (500 µg b.i.d.).

Five subjects (12.5 %) with COPD had significant improvement in lung function during fluticasone treatment, while 20 subjects (50 %) had significant decrease in symptoms. High baseline bronchial NO flux was associated with higher increase in FEV_1/FVC ($r=0.334$, $p=0.038$) and more symptom relief ($r= -0.317$, $p=0.049$) during the treatment. Baseline EBC levels of LTB_4, cysLTs or 8-isoprostane were not related to response to fluticasone treatment. Inhaled fluticasone decreased bronchial NO flux but not alveolar NO concentration or markers in EBC.

High levels of bronchial NO flux are related to symptom relief and improvement of airway obstruction during treatment with inhaled fluticasone in COPD. Markers of inflammation or oxidative stress in EBC are not related to steroid-responsiveness in COPD.

Keywords: chronic obstructive pulmonary disease, corticosteroids, 8-isoprostane, leukotrienes, nitric oxide, spirometry

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterised by chronic bronchitis and emphysema causing an airflow obstruction which, by definition, is not fully reversible (1). High numbers of neutrophils and macrophages are typical to the airway inflammation in COPD, but also eosinophils are present in some subjects (2). As treatment with inhaled corticosteroids (ICS) is usually more efficient in eosinophilic than in neutrophilic airway inflammation, the role of ICS in the treatment of stable COPD is controversial. Treatment with ICS is currently recommended for patients with severe COPD and frequent exacerbations (1). ICS are less effective in COPD than in asthma, but also some subjects with COPD gain from ICS by improving their lung function and health status (3). High levels of markers of eosinophilic inflammation, like exhaled NO concentration (4) and sputum eosinophils (5), predict favourable response to ICS in asthma, and these markers may also be useful in titrating the ICS dose in long term asthma management (6,7). There are also some studies showing that high levels of sputum eosinophils in COPD predict favourable response to short course of oral prednisolone (8) or ICS (9,10), but the role of exhaled NO or inflammatory markers in exhaled breath condensate (EBC) in predicting response to ICS in COPD is not known.

Exhaled NO has usually been measured at a single exhalation flow rate of 50 ml/s (11). More information on airway inflammation and its anatomical location can be gained by measuring exhaled NO at multiple flow rates and calculating alveolar NO concentration ($C_{A_{NO}}$) and bronchial NO flux ($J'aw_{NO}$) (12-14). COPD has been associated with decreased (15) or normal (16,17) $J'aw_{NO}$ (NO from central large airways) and increased (15,16) or normal (17) $C_{A_{NO}}$ (NO from alveoli and small peripheral airways). Varying results are likely explained by differences in smoking status and heterogeneity in the inflammatory status and the use of ICS. Furthermore, $C_{A_{NO}}$ has been shown to

correlate negatively with FEV_1 in COPD (15), which is in line with the small airways being the main site of airflow limitation in COPD.

Airway inflammation can be non-invasively assessed also by collecting exhaled breath condensate (EBC) and analysing levels of biomarkers in the condensate. Leukotriene B_4 (LTB_4) is a chemotactic factor for neutrophils (18), and increased levels of LTB_4 have been found in states of neutrophilic airway inflammation like COPD (19,20). Cysteinyl leukotrienes (cysLTs) have been associated with eosinophilic inflammation (21), and increased cysLT concentrations in EBC have been found in asthmatic subjects (19,22). The effect of oxidative stress can be assessed by measuring the EBC level of 8-isoprostane, a marker of lipid peroxidation (23).

In cross-sectional studies ICS have been reported to have no effect on EBC levels of LTB_4 (24), and to slightly decrease (15) or to have no effect (17) on bronchial NO output. However, there are no interventional studies on the effect of ICS on non-invasive markers of pulmonary inflammation, or on the ability of these markers to predict steroid-responsiveness in COPD. The aim of the current study was to assess the effect of ICS on bronchial NO flux and alveolar NO concentration, and on the levels of LTB_4, cysLTs and 8-isoprostane in EBC in patients with COPD, and to study if these markers can predict the individual effect of ICS on lung function and symptoms in COPD.

METHODS

Subjects

Patients with COPD were recruited among subjects referred from primary care for diagnostic assessment to the Department of Respiratory Medicine at Tampere University Hospital. The inclusion criteria were symptoms compatible with COPD (cough, sputum production and chest tightness), post-bronchodilator $FEV_1/FVC < 0.7$, smoking-history ≥ 20 pack-years, and emphysema on high resolution computed tomography (HRCT) of the lungs. Exclusion criteria were history of asthma, significant diurnal variation in home peak expiratory flow monitoring, any other pulmonary disease, arterial oxygen tension less than 8.0 kPa, or diabetes. Only reliever medication with short-acting β_2-agonists was allowed. Possible ICS or theophylline were withdrawn for at least 4 weeks before the first measurements. The study was approved by the Ethics Committee of Tampere University Hospital and all subjects gave their written informed consent.

Study protocol

Spirometry (Vmax 20C, SensorMedics, Yorba Linda, CA, USA) and body plethysmography (Autobox 6200, SensorMedics) were measured before and after inhaled salbutamol (400 μg). Two-week home peak expiratory flow monitoring was conducted to rule out asthma-like diurnal variation in airway obstruction. HRCT of the lungs was scanned (Siemens Somatom Plus 4, Siemens Medical, Erlangen, Germany). In addition, exhaled NO was measured, exhaled breath condensate was collected, and the subjects filled in a symptom questionnaire. The same measurements excluding HRCT were repeated after 4 weeks of treatment with inhaled fluticasone propionate (Flixotide Diskus 500 μg b.i.d., GlaxoSmithKline, Ware, UK).

Exhaled NO

Exhaled NO was measured with a Sievers NOA 280 analyser (Sievers Instruments, Boulder, Colorado, USA) at exhalation flow rates of 50, 100, 200 and 300 ml/s. The desired exhalation flow rates were achieved by letting the patients exhale through a mass flow meter connected to a computer-controlled adjustable flow restrictor that kept the flow rate steady at the desired level (25,26).

$C_{A_{NO}}$ and $J'aw_{NO}$ were calculated with the linear method as previously described by using exhalation flow rates of 100, 200 and 300 ml/s (12,27). Exhaled NO output (= exhaled NO concentration × exhalation flow rate) was plotted against exhalation flow rate and a linear regression was set (Microsoft Excel). Slope and intercept of the regression line are approximates of $C_{A_{NO}}$ and $J'aw_{NO}$, respectively.

Axial backward diffusion of NO from bronchial compartment to alveoli may cause falsely high $C_{A_{NO}}$ and falsely low $J'aw_{NO}$ especially in subjects with high $J'aw_{NO}$. $C_{A_{NO}}$ and $J'aw_{NO}$ adjusted for trumpet shape airways and axial diffusion ($C_{A_{NO}}(TMAD)$ and $J'aw_{NO}(TMAD)$) were calculated according to the equations 1 and 2 as described by Condorelli *et al* (28).

$$C_{A_{NO}}(TMAD)\ [ppb] = C_{A_{NO}}\ [ppb] - J'aw_{NO}\ [nl/s] / 0.86\ [l/s] \quad \text{(Eq 1)}$$

$$J'aw_{NO}(TMAD)\ [nl/s] = J'aw_{NO}\ [nl/s] \times 1.7 \quad \text{(Eq 2)}$$

The difference between $C_{A_{NO}}$ and $C_{A_{NO}}(TMAD)$ is dependent on individual $J'aw_{NO}$ and there may therefore be differences in result profiles between $C_{A_{NO}}$ and $C_{A_{NO}}(TMAD)$. On the contrary, $J'aw_{NO}(TMAD)$ is precisely 1.7 times higher than $J'aw_{NO}$ in every subject, and therefore all the

correlations to other markers or treatment changes in $J'aw_{NO}$ and $J'aw_{NO}$(TMAD) are exactly the same, but the absolute values are 1.7 times higher in $J'aw_{NO}$(TMAD).

Exhaled breath condensate

Exhaled breath condensate was collected during 15 min of tidal breathing with Ecoscreen condenser (Ecoscreen, Jaeger, Hoechberg, Germany) while wearing nose clips. The samples were stored at -70°C until assayed. LTB_4, 8-isoprostane and cysLT concentrations in the condensates were measured by immunoassay with a detection limit of 2 pg/ml (Cayman Chemical Company, Ann Arbor, Michigan, USA).

Symptom scoring

The subjects filled in a Finnish translation of the St George's Respiratory Questionnaire (SGRQ) containing questions and scoring on three aspects of the disease (*symptom* frequency and severity, *activities* that cause or are limited by breathlessness, and the *impact* of the disease on social functioning with psychological disturbances resulting from the disease) and a *total score*. Each score ranges from 0 to 100 with higher score meaning worse disease. A change of at least 4 points is considered as a significant change in the disease state (29).

Statistics

Based on power calculations, forty patients was needed to the final analysis to give the study a power of ≈ 90 % to detect a change of 0.5 SD in each parameter during the fluticasone treatment (effect size 0.5) with an α of 0.05. Distributions of the NO parameters and inflammatory markers in breath condensate were non-normal, while lung function parameters and symptom scores were normally distributed (Shapiro-Wilk test). Ex-smokers and current smokers were compared using t-

test for normal data, Mann-Whitney U-test for non-normal data and Fisher's exact test for binary variables. Changes in parameters during the treatment were analysed using paired t-test for normal data and Wilcoxon signed rank test for non-normal data. Correlations between baseline inflammatory markers and changes in lung function or symptoms during the treatment were analysed with Spearman's rank correlation. SPSS 12.0.1 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Results are given as mean ± SEM for normally distributed data and as median [inter-quartile range] for non-normal data. P-value < 0.05 was considered as statistically significant.

RESULTS

To obtain forty patients to the final analysis, fifty-five patients needed to be recruited. Four subjects were excluded due to technical problems in NO measurements, 5 had an exacerbation during the study period, 2 subjects were not willing to continue in the study, and timing of the study medication and follow-up was wrong in 4 subjects. The forty subjects included in the analysis had NO results of good quality and there was high linearity ($r \geq 0.96$) between NO output and flow rate at range 100 - 300 ml/s.

Majority (n=30) of the patients had moderate COPD (stage II) according to the GOLD-criteria (1), while 3 subjects had stage I, 6 subjects stage had III, and 1 subject had stage IV COPD. None of the subjects had signs of interstitial lung disease on HRCT scans.

Spirometry and body plethysmography, NO parameters and inflammatory markers in exhaled breath condensate according to the current smoking status are presented in Table 1. Current smokers had slightly better lung function, and lower $FE_{NO0.05}$ and bronchial NO flux as compared to ex-smokers. There were no differences in other NO parameters or inflammatory markers in breath condensate between current and ex-smokers. Seventeen subjects had reversibility in $FEV_1 \geq 12$ % and 200 ml after salbutamol inhalation, but there were no differences in baseline pulmonary function or any of the inflammatory measures between responders and non-responders to salbutamol (data not shown).

Five out of 40 subjects used ICS during the enrollment, and ICS were stopped for 4 weeks before entering the study. EBC levels of 8-isoprostane (16.1 [10.8 – 19.1] vs 9.1 [7.3 – 14.1], p=0.024) and Cys-LTs (30.0 [7.4 – 58.7] vs 2.8 [1.0 – 12.7], p=0.040) were higher in those who used ICS at the

enrollment, but there were no differences in NO parameters, lung function or symptoms between those who used ICS at enrollment and those who did not.

Levels of inflammatory markers, spirometry and body plethysmography and symptom scores before and after fluticasone treatment are presented in Table 2. Fluticasone treatment decreased bronchial NO flux and $FE_{NO_{0.05}}$ but had no effect on alveolar NO concentrations. There were no changes in breath condensate levels of LTB_4, cysLTs or 8-isoprostane.

Fluticasone treatment decreased residual volume but had no other effects on mean pulmonary function (Table 2). However, 5 out of the 40 patients (12.5 %) had improvement in $FEV_1 \geq 12$ % and 200 ml during the fluticasone treatment. The relative number of responders tended to be higher in ex-smokers (3 out of 11 [27.3 %]) than in current smokers (2 out of 29 [6.9 %], p=0.117). Baseline bronchial no flux correlated positively with the change in post-bronchodilator FEV_1/FVC (r = 0.334, p = 0.038, Figure 1), and this correlation was even stronger in ex-smokers (r = 0.621, p = 0.042) but non-significant in current smokers (r = 0.152, p = 0.432). Baseline $C_{A_{NO}}$ correlated negatively with the relative change in FEF_{75} (r = -0.340, p = 0.037, Figure 1), while baseline $C_{A_{NO}}$(TMAD) correlated negatively with the relative change in FEF_{75} (r = -0.395, p = 0.014) and positively with the relative change in functional residual capacity, FRC (r = 0.341, p=0.036). Thus, low baseline $C_{A_{NO}}$ and $C_{A_{NO}}$(TMAD) were associated with better functional outcome (increase in FEF_{75} and decrease in FRC). There were no other significant correlations between baseline inflammatory markers and change in lung function.

Fluticasone treatment decreased symptoms measured by SGRQ (Table 2), and 20 patients (50 %) had significant decrease in symptoms defined as decrease in SGRQ total score at least 4 points. The relative number of responders was similar in ex-smokers (6 out of 11 [54.5 %]) and current smokers

(14 out of 29 [48.3 %], p=1.000). Baseline $FE_{NO0.05}$ (r = -0.373. p=0.013) and bronchial NO flux (r = -0.317, p=0.049) correlated negatively with the change in SGRQ total score during fluticasone treatment, i.e. subjects with high bronchial NO output had more pronounced decrease in symptoms during the treatment. These correlations were similar in subgroups of ex-smokers and current smokers. Baseline $FE_{NO0.05}$ and bronchial NO flux were higher in those patients who gained a decrease of at least 4 points in SGRQ total score during fluticasone treatment (Figure 2). Other baseline inflammatory markers were not related to symptom change during the treatment.

DISCUSSION

In the present study we found that inhaled fluticasone decreased bronchial NO flux but had no effect on alveolar NO concentration in patients with COPD. Fluticasone improved lung function significantly (increase in $FEV_1 \geq 12$ % and 200 ml) only in 5 out of 40 subjects, but 20 subjects had significant symptom relief. Baseline bronchial NO flux correlated with increase in post-bronchodilator FEV_1 / FVC and decrease in symptoms during fluticasone treatment, while baseline alveolar NO concentration correlated negatively with the change in FEF_{75} during the treatment. Levels of 8-isoprostane, LTB_4 and cysLTs in EBC were not affected by fluticasone treatment, and these markers were not related to fluticasone induced changes in lung function or symptoms.

The multiple flow rate method is a promising extension of exhaled NO measurement, as it allows separate assessment of NO output in large central airways (bronchial NO flux) and in the peripheral small airways / pulmonary parenchyma (alveolar NO concentration) (12-14). This method has been shown to be suitable for measuring central and peripheral inflammation in airway diseases and parenchymal diseases (15-17,25,27). A further extension of the model to theoretically simulate for the effects of spatial heterogeneity in pulmonary inflammation has recently been introduced (30).

In COPD and asthma, eosinophilic rather than neutrophilic inflammatory activity predicts favourable response to ICS (5,8-10). Nitric oxide output is associated mainly with eosinophilic airway inflammation (31-33) while LTB_4 (chemotactic factor for neutrophils) and 8-isoprostane (marker of lipid peroxidation) are associated with neutrophilic inflammation (18,23). It is therefore understandable that NO parameters but not EBC LTB_4 and 8-isoprostane decreased during fluticasone treatment and were associated with the response of symptoms and lung function to ICS. It might be that indices of neutrophilic inflammatory activity, like LTB_4 and 8-isoprostane in EBC,

could better predict response to drug treatment specifically aimed to tackle neutrophilic inflammation.

We found that inhaled fluticasone decreased bronchial NO flux but had no effect on alveolar NO concentration. The decrease in bronchial NO output is in line with the effect of fluticasone in asthma (34) and suggests that there is at least some steroid-responsive component in the airway inflammation in these subjects. The decrease in bronchial NO flux following fluticasone treatment may be explained by decrease in inflammatory mediators needed to increase the expression of inducible NO synthase (iNOS), or by direct inhibitory effects of glucocorticoids on iNOS expression (35,36). The lack of effect on alveolar NO concentration during fluticasone treatment suggests either that there is no steroid-sensitive iNOS expression in small airways / lung parenchyma in COPD or that the deposition of fluticasone from a dry-powder inhaler is not sufficient in the lung periphery. A trial with systemic glucocorticoids could clarify this issue, as oral prednisone has been shown to decrease alveolar NO concentration in asthmatics on regular ICS treatment (37).

Only a minority of patients had significant improvement in lung function (increase in $FEV_1 \geq 12$ % and ≥ 200 ml) during the fluticasone treatment, but half of the subjects had significant decrease in symptoms. This may be related to differences in the underlying causes of impaired lung function and symptoms in COPD. Airway obstruction in COPD is caused mainly by irreversible structural changes that are not related to current inflammatory activity (small airway fibrosis and loss of alveolar attachments due to emphysema) (38), and to lesser extent by inflammation related factors like mucus secretion and mucosal oedema. However, cough and sputum production are affected by the current degree of inflammatory activity. This difference in causative factors between symptoms and impaired lung function may explain why the subjects were more responsive to fluticasone in

terms of symptoms rather than of lung function, and why baseline inflammatory markers were able to predict better the effect of fluticasone on symptoms than on lung function.

The higher the baseline bronchial NO flux, the higher was the improvement in post-bronchodilator FEV_1/FVC in ex-smokers. This suggests that high bronchial NO flux is associated with steroid-sensitive large airway inflammation, the treatment of which improves lung function. However, the negative correlation between baseline alveolar NO concentration and relative change in FEF_{75} during fluticasone treatment (i.e. the higher the baseline alveolar NO concentration the lower the improvement in FEF_{75}) is more difficult to interpret. One explanation might be that high alveolar NO concentration in COPD is not caused by a steroid-sensitive peripheral inflammation but by irreversible structural changes. Alveolar NO concentration can be increased either by increased peripheral NO production, or by decreased diffusing capacity of NO from alveolar air to pulmonary circulation (pathological changes in lung parenchyma or high ventilation to perfusion ratio) (12,39). Emphysema destroys pulmonary tissue available for gas transfer and thereby decreases pulmonary diffusing capacity of NO and could, in fact, also increase alveolar NO concentration. Thus, subjects with more pronounced emphysema might have higher alveolar NO concentration because of decreased diffusing capacity of NO, but due to loss of alveolar attachments lower ability to improve small airway function during ICS treatment.

There are no previous intervention studies on the effect of ICS on these markers of inflammation in COPD, but our results are in line with the tendency towards lower bronchial NO output in steroid-treated COPD patients reported in a cross-sectional study by Brindicci and colleagues (15). Another cross-sectional study reported no difference in EBC-levels of LTB_4 in COPD patients treated or not with ICS (24), which is supported by our current finding of negative effect of fluticasone on EBC-LTB_4.

Smoking has been associated with decreased sensitivity to treatment with ICS in asthma and COPD (40). In the current study smokers tended to be less sensitive than ex-smokers for ICS in terms of lung function improvement, but there was no difference in symptom relief between current and ex-smokers. Further, high bronchial NO flux predicted symptom decrease in response to ICS in both current and ex-smokers, but its association with lung function improvement tended to be stronger in ex-smokers than in current smokers. Smoking might thus differently interfere with the various effects of glucocorticoids in COPD.

When assessing the role of exhaled NO measurement in COPD it is important to exclude asthma, as concomitant asthma with more eosinophilic and steroid-sensitive inflammation might bias the results. According to the GOLD-guidelines, COPD is characterised by "airflow limitation that is not fully reversible" and COPD is diagnosed in subjects with relevant medical history based on a post-bronchodilator FEV_1 / FVC < 0.7 (1). Significant improvement (≥ 12 % and 200 ml) in FEV_1 after administration of bronchodilators is no longer recommended for the differential diagnosis between asthma and COPD (1), as this test is not sensitive nor specific enough (41). Further, in large clinical trials more than half of the subjects with COPD show significant improvement in FEV_1 following maximal bronchodilatation even when asthma has been excluded (42). We believe that all the subjects in the present study really had COPD, as none of them had a previous diagnosis or clinical history of asthma or significant diurnal variation in home PEF-monitoring, and they all had a smoking history ≥ 20 pack-years and emphysema on HRCT. However, 17 of the 40 subjects had significant reversibility in FEV_1 after administration of inhaled salbutamol, but there was no difference in baseline pulmonary function or any of the inflammatory measures between responders and non-responders to salbutamol.

In conclusion, high pre-treatment levels of bronchial NO flux are related to symptom relief and improvement of airway obstruction during treatment with inhaled fluticasone in COPD. Levels of 8-isoprostane, cysLTs and LTB_4 in EBC are not related to steroid responsiveness in COPD.

ACKNOWLEDGEMENTS

The authors thank Mrs Marja-Leena Lampén and Mrs Marika Soukkanen for technical help, and Mrs Heli Määttä for secretarial assistance. The study was supported by grants from Tampere Tuberculosis Foundation, The Research Fund of Tampere University Hospital, Väinö and Laina Kivi Foundation, Finnish Anti-Tuberculosis Association Foundation and the Academy of Finland. The study medication was provided by GSK.

References:

1. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, Zielinski J, Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007; 176: 532-555.

2. Fabbri LM, Romagnoli M, Corbetta L, Casoni G, Busljetic K, Turato G, Ligabue G, Ciaccia A, Saetta M, Papi A. Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2003; 167: 418-424.

3. Burge PS, Calverley PM, Jones PW, Spencer S, Anderson JA, Maslen TK. Randomised, double blind, placebo controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease: the ISOLDE trial. *BMJ* 2000; 320: 1297-1303.

4. Smith AD, Cowan JO, Brassett KP, Filsell S, McLachlan C, Monti-Sheehan G, Peter Herbison G, Robin Taylor D. Exhaled nitric oxide: a predictor of steroid response. *Am J Respir Crit Care Med* 2005; 172: 453-459.

5. Meijer RJ, Postma DS, Kauffman HF, Arends LR, Koeter GH, Kerstjens HA. Accuracy of eosinophils and eosinophil cationic protein to predict steroid improvement in asthma. *Clin Exp Allergy* 2002; 32: 1096-1103.

6. Smith AD, Cowan JO, Brassett KP, Herbison GP, Taylor DR. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. *N Engl J Med* 2005; 352: 2163-2173.

7. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002; 360: 1715-1721.

8. Brightling CE, Monteiro W, Ward R, Parker D, Morgan MD, Wardlaw AJ, Pavord ID. Sputum eosinophilia and short-term response to prednisolone in chronic obstructive pulmonary disease: a randomised controlled trial. *Lancet* 2000; 356: 1480-1485.

9. Brightling CE, McKenna S, Hargadon B, Birring S, Green R, Siva R, Berry M, Parker D, Monteiro W, Pavord ID, Bradding P. Sputum eosinophilia and the short term response to inhaled mometasone in chronic obstructive pulmonary disease. *Thorax* 2005; 60: 193-198.

10. Leigh R, Pizzichini MM, Morris MM, Maltais F, Hargreave FE, Pizzichini E. Stable COPD: predicting benefit from high-dose inhaled corticosteroid treatment. *Eur Respir J* 2006; 27: 964-971.

11. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005; 171: 912-930.

12. Tsoukias NM, George SC. A two-compartment model of pulmonary nitric oxide exchange dynamics. *J Appl Physiol* 1998; 85: 653-666.

13. Silkoff PE, Sylvester JT, Zamel N, Permutt S. Airway nitric oxide diffusion in asthma: Role in pulmonary function and bronchial responsiveness. *Am J Respir Crit Care Med* 2000; 161: 1218-1228.

14. George SC, Högman M, Permutt S, Silkoff PE. Modeling pulmonary nitric oxide exchange. *J Appl Physiol* 2004; 96: 831-839.

15. Brindicci C, Ito K, Resta O, Pride NB, Barnes PJ, Kharitonov SA. Exhaled nitric oxide from lung periphery is increased in COPD. *Eur Respir J* 2005; 26: 52-59.

16. Högman M, Holmkvist T, Wegener T, Emtner M, Andersson M, Hedenström H, Meriläinen P. Extended NO analysis applied to patients with COPD, allergic asthma and allergic rhinitis. *Respir Med* 2002; 96: 24-30.

17. Roy K, Borrill ZL, Starkey C, Hazel AL, Morris J, Vestbo J, Singh D. Use of different exhaled nitric oxide multiple flow rate models in COPD. *Eur Respir J* 2007; 29: 651-659.

18. Martin TR, Pistorese BP, Chi EY, Goodman RB, Matthay MA. Effects of leukotriene B4 in the human lung. Recruitment of neutrophils into the alveolar spaces without a change in protein permeability. *J Clin Invest* 1989; 84: 1609-1619.

19. Montuschi P, Barnes PJ. Exhaled leukotrienes and prostaglandins in asthma. *J Allergy Clin Immunol* 2002; 109: 615-620.

20. Kostikas K, Gaga M, Papatheodorou G, Karamanis T, Orphanidou D, Loukides S. Leukotriene B4 in exhaled breath condensate and sputum supernatant in patients with COPD and asthma. *Chest* 2005; 127: 1553-1559.

21. Kostikas K, Koutsokera A, Papiris S, Gourgoulianis KI, Loukides S. Exhaled breath condensate in patients with asthma: implications for application in clinical practice. *Clin Exp Allergy* 2008; 38: 557-565.

22. Zanconato S, Carraro S, Corradi M, Alinovi R, Pasquale MF, Piacentini G, Zacchello F, Baraldi E. Leukotrienes and 8-isoprostane in exhaled breath condensate of children with stable and unstable asthma. *J Allergy Clin Immunol* 2004; 113: 257-263.

23. Janssen LJ. Isoprostanes: an overview and putative roles in pulmonary pathophysiology. *Am J Physiol Lung Cell Mol Physiol* 2001; 280: L1067-82.

24. Montuschi P, Kharitonov SA, Ciabattoni G, Barnes PJ. Exhaled leukotrienes and prostaglandins in COPD. *Thorax* 2003; 58: 585-588.

25. Lehtonen H, Oksa P, Lehtimäki L, Sepponen A, Nieminen R, Kankaanranta H, Saarelainen S, Järvenpää R, Uitti J, Moilanen E. Increased alveolar nitric oxide concentration and high levels of leukotriene B(4) and 8-isoprostane in exhaled breath condensate in patients with asbestosis. *Thorax* 2007; 62: 602-607.

26. Moilanen E, Lehtimäki L. Method and measuring equipement for measuring nitric oxide concentration in exhaled air. US patent 6,733,463.

27. Lehtimäki L, Kankaanranta H, Saarelainen S, Hahtola P, Järvenpää R, Koivula T, Turjanmaa V, Moilanen E. Extended exhaled NO measurement differentiates between alveolar and bronchial inflammation. *Am J Respir Crit Care Med* 2001; 163: 1557-1561.

28. Condorelli P, Shin HW, Aledia AS, Silkoff PE, George SC. A simple technique to characterize proximal and peripheral nitric oxide exchange using constant flow exhalations and an axial diffusion model. *J Appl Physiol* 2007; 102: 417-425.

29. Jones PW, Quirk FH, Baveystock CM. The St George's Respiratory Questionnaire. *Respir Med* 1991; 85 Suppl B: 25-31; discussion 33-7.

30. Suresh V, Shelley DA, Shin HW, George SC. Effect of heterogeneous ventilation and nitric oxide production on exhaled nitric oxide profiles. *J Appl Physiol* 2008; 104: 1743-1752.

31. Brightling CE, Symon FA, Birring SS, Bradding P, Wardlaw AJ, Pavord ID. Comparison of airway immunopathology of eosinophilic bronchitis and asthma. *Thorax* 2003; 58: 528-532.

32. Payne DN, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *Am J Respir Crit Care Med* 2001; 164: 1376-1381.

33. van den Toorn LM, Overbeek SE, de Jongste JC, Leman K, Hoogsteden HC, Prins JB. Airway inflammation is present during clinical remission of atopic asthma. *Am J Respir Crit Care Med* 2001; 164: 2107-2113.

34. Lehtimaki L, Kankaanranta H, Saarelainen S, Turjanmaa V, Moilanen E. Inhaled fluticasone decreases bronchial but not alveolar nitric oxide output in asthma. *Eur Respir J* 2001; 18: 635-639.

35. Kleinert H, Euchenhofer C, Ihrig-Biedert I, Forstermann U. Glucocorticoids inhibit the induction of nitric oxide synthase II by down-regulating cytokine-induced activity of transcription factor nuclear factor-kappa B. *Mol Pharmacol* 1996; 49: 15-21.

36. Korhonen R, Lahti A, Hämäläinen M, Kankaanranta H, Moilanen E. Dexamethasone inhibits inducible nitric-oxide synthase expression and nitric oxide production by destabilizing mRNA in lipopolysaccharide-treated macrophages. *Mol Pharmacol* 2002; 62: 698-704.

37. Gelb AF, Taylor CF, Nussbaum E, Gutierrez C, Schein A, Shinar CM, Schein MJ, Epstein JD, Zamel N. Alveolar and airway sites of nitric oxide inflammation in treated asthma. *Am J Respir Crit Care Med* 2004; 170: 737-741.

38. Barnes PJ. Small airways in COPD. *N Engl J Med* 2004; 350: 2635-2637.

39. Hyde RW, Geigel EJ, Olszowka AJ, Krasney JA, Forster RE,2nd, Utell MJ, Frampton MW. Determination of production of nitric oxide by lower airways of humans--theory. *J Appl Physiol* 1997; 82: 1290-1296.

40. Adcock IM, Barnes PJ. Molecular mechanisms of corticosteroid resistance. *Chest* 2008; 134: 394-401.

41. Kesten S, Rebuck AS. Is the short-term response to inhaled beta-adrenergic agonist sensitive or specific for distinguishing between asthma and COPD? *Chest* 1994; 105: 1042-1045.

42. Tashkin DP, Celli B, Decramer M, Liu D, Burkhart D, Cassino C, Kesten S. Bronchodilator responsiveness in patients with COPD. *Eur Respir J* 2008; 31: 742-750.

TABLE 1. Lung function, NO parameters and inflammatory markers in exhaled breath condensate in 40 patients with COPD according to the current smoking status.

	Smokers	Ex-smokers	p-value
n	29	11	--
Males / females	18 / 11	9 / 2	0.286[#]
FEV_1 (% pred, post)	64.6 ± 2.7	53.3 ± 4.8	**0.037**[§]
FEV_1 / FVC (post)	0.54 ± 0.02	0.43 ± 0.03	**0.002**[§]
FEF_{75} (% pred)	24.7 ± 1.7	18.7 ± 3.2	0.084[§]
RV (% pred)	150.7 ± 6.7	164.5 ± 17.7	0.481[§]
Raw (% pred)	190.5 ± 10.4	273.6 ± 44.5	0.096[§]
TL_{CO} (% pred)	54.8 ± 2.1	50.3 ± 4.1	0.309[§]
$FENO_{0.05}$ (ppb)	8.8 [5.1 – 13.5]	16.2 [11.5 – 19.1]	**0.025***
$FENO_{0.1}$ (ppb)	5.2 [3.8 8.2]	8.1 [5.8 – 10.3]	0.052*
$FENO_{0.2}$ (ppb)	4.2 [3.1 – 6.2]	5.0 [3.8 – 7.3]	0.131*
$FENO_{0.3}$ (ppb)	3.6 [2.8 – 5.6]	4.7 [3.9 – 6.4]	0.148*
$J'aw_{NO}$ (nl/s)	0.26 [0.07 - 0.41]	0.39 [0.38 – 0.73]	**0.025***
$J'aw_{NO}$ (TMAD) (nl/s)	0.44 [0.12 - 0.70]	0.66 [0.65 – 1.24]	**0.025***
C_{ANO} (ppb)	3.0 [2.3 – 3.8]	3.3 [2.4 – 3.6]	0.680*
C_{ANO}(TMAD) (ppb)	2.8 [1.9 – 3.4]	2.5 [1.5 – 3.6]	0.899*
EBC-LTB_4 (pg/ml)	6.7 [5.0 – 9.0]	6.4 [4.0 – 12.3]	1.000*
EBC-cysLT	8.4 [1.0 – 13.9]	6.6 [1.0 – 12.8]	0.824*
EBC-8-iso (pg/ml)	9.3 [7.3 – 14.2]	10.3 [8.8 – 17.3]	0.280*

post, post-bronchodilator
FEF_{75}, Forced expiratory flow when 75 % of vital capacity is exhaled
RV, residual volume
Raw, airway resistance
TL_{CO}, pulmonary transfer factor for carbon monoxide
$FENO_{0.05}$ fractional exhaled NO concentration at exhalation flow rate of 0.05 l/s
$J'aw_{NO}$, Bronchial NO flux
$J'aw_{NO}$(TMAD), Bronchial NO flux adjusted for trumpet shape airways and axial diffusion
C_{ANO}, Alveolar NO concentration
C_{ANO}(TMAD), Alveolar NO concentration adjusted for trumpet shape airways and axial diffusion
[#] Fisher's exact test for sex-distribution between smokers and ex-smokers
[§] t-test between smokers and ex-smokers
* Mann-Whitney U-test between smokers and ex-smokers

TABLE 2. Lung function, NO parameters, inflammatory markers in exhaled breath condensate and symptom scores in 40 patients with COPD before and after 4 weeks of treatment with inhaled fluticasone.

	Before	After	p-value
FEV_1 (% pred, post)	61.5 ± 2.5	62.8 ± 2.4	0.300§
FEV_1 / FVC (post)	0.51 ± 0.02	0.51 ± 0.02	0.284§
FEF_{75} (% pred, post)	26.4 ± 2.0	26.5 ± 1.7	0.924§
RV (% pred, post)	137.7 ± 5.7	129.3 ± 4.9	**0.005**§
Raw (% pred, post)	213.4 ± 15.2	212.2 ± 12.7	0.892§
$FENO_{0.05}$ (ppb)	10.3 [6.0 – 17.2]	7.2 [5.1 – 12.2]	**0.001***
$J'aw_{NO}$ (nl/s)	0.35 [0.11 - 0.57]	0.19 [0.10 – 0.38]	**0.001***
$J'aw_{NO}$ (TMAD) (nl/s)	0.60 [0.19 - 0.97]	0.32 [0.17 – 0.65]	**0.001***
$C_{A_{NO}}$ (ppb)	3.1 [2.3 – 3.7]	2.9 [2.4 – 3.8]	0.328*
$C_{A_{NO}}$(TMAD) (ppb)	2.6 [1.9 – 3.5]	2.5 [2.2 – 3.4]	0.811*
EBC-LTB_4 (pg/ml)	6.7 [5.0 – 9.0]	6.4 [4.6 – 8.9]	0.501*
EBC-cysLT	7.5 [1.0 – 13.8]	7.7 [1.0 – 14.4]	0.985*
EBC-8-iso (pg/ml)	9.5 [7.8 – 14.6]	9.7 [7.9 – 12.7]	0.541*
SGRQ symptom score	54.1 ± 3.7	38.7 ± 3.8	**0.001**§
SGRQ activity score	47.9 ± 3.0	43.6 ± 3.4	**0.050**§
SGRQ impact score	23.0 ± 2.2	22.0 ± 2.4	0.568§
SGRQ total score	35.7 ± 2.3	31.3 ±2.6	**0.010**§

post, post-bronchodilator
FEF_{75}, Forced expiratory flow when 75 % of vital capacity is exhaled
RV, residual volume
Raw, airway resistance
$FENO_{0.05}$ fractional exhaled NO concentration at exhalation flow rate of 0.05 l/s
$J'aw_{NO}$, Bronchial NO flux
$J'aw_{NO}$(TMAD), Bronchial NO flux adjusted for trumpet shape airways and axial diffusion
$C_{A_{NO}}$, Alveolar NO concentration
$C_{A_{NO}}$(TMAD), Alveolar NO concentration adjusted for trumpet shape airways and axial diffusion
SGRQ, St George´s Respiratory Questionnaire
§ paired t-test
* Wilcoxon´s test

Figure legends

FIGURE 1. Baseline bronchial NO flux ($J'aw_{NO}$) correlated positively with the change in post-bronchodilatator FEV_1 / FVC (A), while baseline alveolar NO concentration (CA_{NO}) correlated negatively with the relative change in FEF_{75} (B) in 40 current smokers (○) and ex-smokers (×) with COPD during 4 weeks of treatment with inhaled fluticasone.

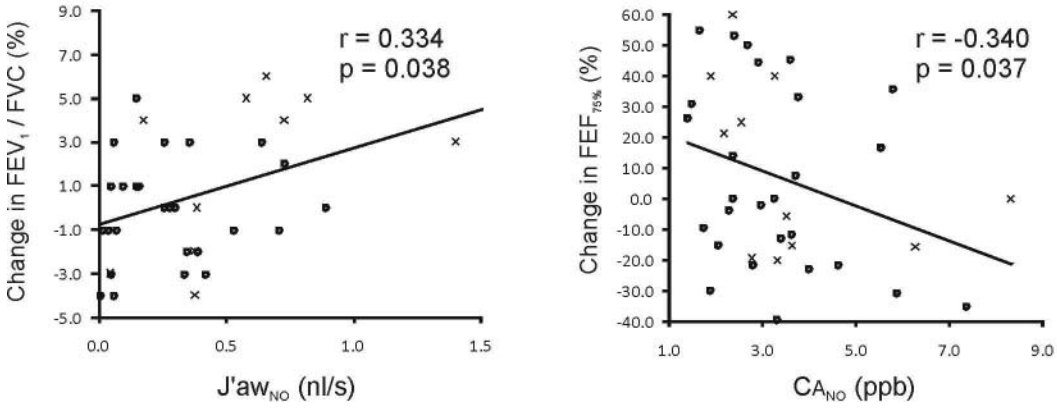

FIGURE 2. Baseline bronchial NO flux in subjects with and without significant decrease in symptoms (decrease of at least 4 points in the St George's Respiratory Questionnaire) during 4 weeks of treatment with inhaled fluticasone. Results are plotted as medians (thick horizontal line) and quartiles (whiskers for the 1st and 4th quartiles, box for the 2nd and 3rd quartiles).