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DOTTORATO DI RICERCA IN FISIOPATOLOGIA RESPIRATORIA SPERIMENTALE

Ciclo XXII

Role of fractional exhaled nitric oxide in asthma

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ABSTRACT

Chronic inflammation in asthma is a key feature of the disease and monitoring is an essential component of asthma management. Inflammation is present in both central and peripheral bronchi and can be measured by non invasive markers, such as fractional exhaled nitric oxide (FeNO). FeNO is increased in asthmatics, as compared to normal subjects and is lowered by inhaled corticosteroids (ICS) with a dose-response relationship that is more evident for asthmatic patients with high levels of FeNO. FeNO measuring is easy to be performed in almost all patients including children and can be helpful as a tool contributing to asthma diagnosis and evaluation of the response to antinflammatory therapy with ICS. Smoking affects FeNO measurements and this has to be considered when evaluating patients with asthma who smoke. The different measurement of bronchial and alveolar FeNO can give information on the distribution of inflammation in the bronchial tree and is of particular interest for clinical pharmacology. Nonetheless, the clinical application of FeNO still needs to be clarified but it is clearly nowadays one of the most used non-invasive markers giving information on the inflammatory component of the disease.

EXHALED NITRIC OXIDE AND AIRWAY INFLAMMATION

Asthma is a respiratory disease associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment. Airway narrowing is the final common pathway leading to symptoms and physiological changes in asthma. Several factors contribute to the development of airway narrowing in asthma but the most important is chronic inflammation of the lower airways in which many cells and cellular elements play a role.

There is now good evidence (1) that the clinical manifestations of asthma-symptoms, sleep disturbances, limitations of daily activity, impairment of lung function, and use of rescue medications-can be controlled with appropriate treatment, mainly antinflammatory drugs, such as inhaled corticosteroids (ICS). When asthma is controlled, there should be no more than occasional recurrence of symptoms and severe exacerbations should be rare. Monitoring of asthmatic symptoms is a key feature of the management of asthma to evaluate the response to therapy and to titrate ICS dose to the lower effective dose (1).

Whereas symptoms and lung function can be easily monitored, assessment of airway inflammation is more difficult and non-invasive markers of airway inflammation are preferred to more invasive tests like bronchoscopy. The evaluation of airway inflammation associated with asthma may be undertaken by examining spontaneously produced or hypertonic saline-induced sputum for eosinophilic or neutrophilic inflammation or by analysis of exhaled breath condensate in which some inflammatory

compounds are detectable. In addition, levels of fractional exhaled nitric oxide (FeNO) have been suggested as non-invasive markers of airway inflammation in asthma (2).

Nitrogen oxides (NOx), regarded in the past primarily as toxic air pollutants, have recently been shown to be bioactive species formed endogenously in the human lung. The relationship between the toxicities and the bioactivities of NOx must be understood in the context of their chemical interactions in the pulmonary microenvironment. Nitric oxide synthase (NOS) is a newly identified enzyme system active in airway epithelial cells, macrophages, neutrophils, mast cells, autonomic neurons, smooth muscle cells, fibroblasts, and endothelial cells (figure 1).

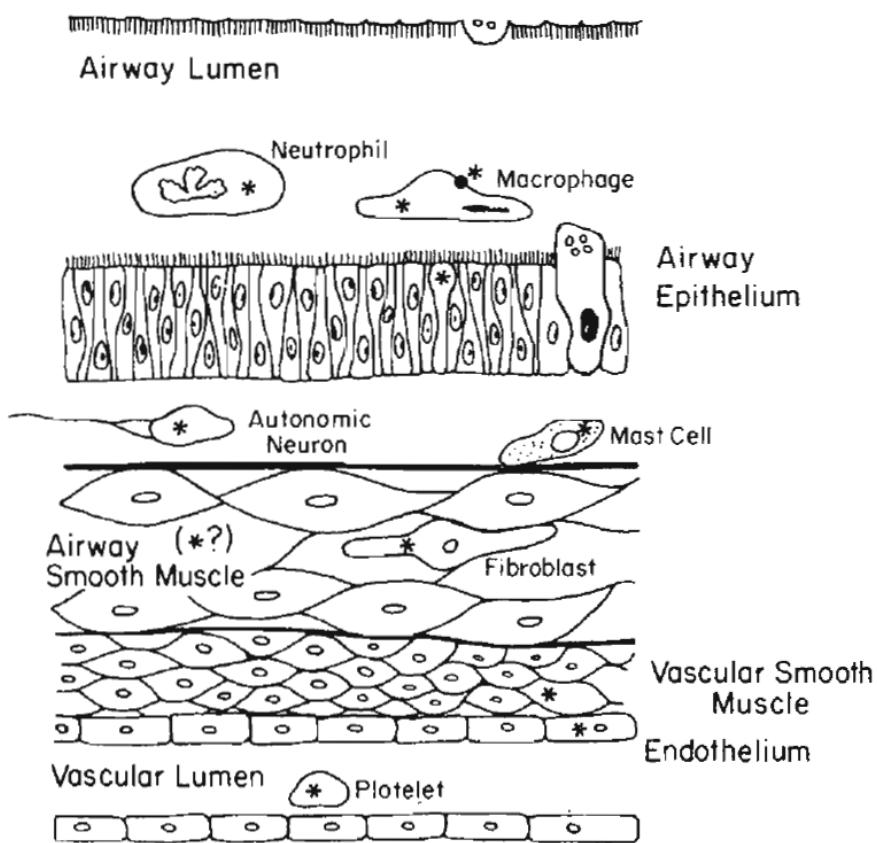


Figure 1: sites of NOS activity in the lung; the asterisks (*) mark cell types in which NOS activity has been demonstrated (from ref 3).

The chemical products of NOS in the lung vary with disease states, and are involved in pulmonary neurotransmission, host defence, and airway and vascular smooth muscle relaxation. Both endogenous and exogenous NOx react readily with oxygen, superoxide, water, nucleotides, metalloproteins, thiols, amines, and lipids to form products with biochemical actions ranging from bronchodilation and bacteriostasis (S-nitrosothiols) to cytotoxicity and pulmonary capillary leak (peroxynitrite), as well as those with frank mutagenic potential (nitrosamines). Nitric oxide (NO) is a mediator of vasodilatation and bronchodilatation synthesised from L-arginine by the enzyme NO synthase, which is either constitutive or induced by lipopolysaccharides and/or cytokines (3).

The role of nitric oxide in airway inflammation was first reported in international medical journals in the beginning of the 80'. To investigate the presence of NO synthase in asthma, Hamid and collaborators immunostained bronchial biopsies from non-steroid-treated people with asthma and non-asthmatic controls with specific polyclonal antisera to purified inducible NOS and to a selected peptide sequence of the same enzyme (4). Immunoreactivity was seen in the epithelium and some inflammatory cells in 22 of 23 biopsies from people with asthma, but in only 2 of 20 controls. To assess the relation of cytokines to NOS induction, bronchial epithelial cells in culture were stimulated with tumour necrosis factor (TNF alpha). Inducible enzyme immunoreactivity was found only in the treated cells. The existence of inducible NOS in human lungs suggests that increased production of NO, probably induced by cytokines, may be relevant to the pathology of asthma.

Kharitonov and colleagues measured FeNO in 67 control subjects and 61 non-steroid-treated asthmatics demonstrating that asthmatics had a significantly higher peak of FeNO as compared to healthy subjects (figure 2). These high concentrations may

reflect induction of NOS and measurement of FeNO concentrations may be clinically useful in detection and management of cytokine-mediated inflammatory lung disorders (2).

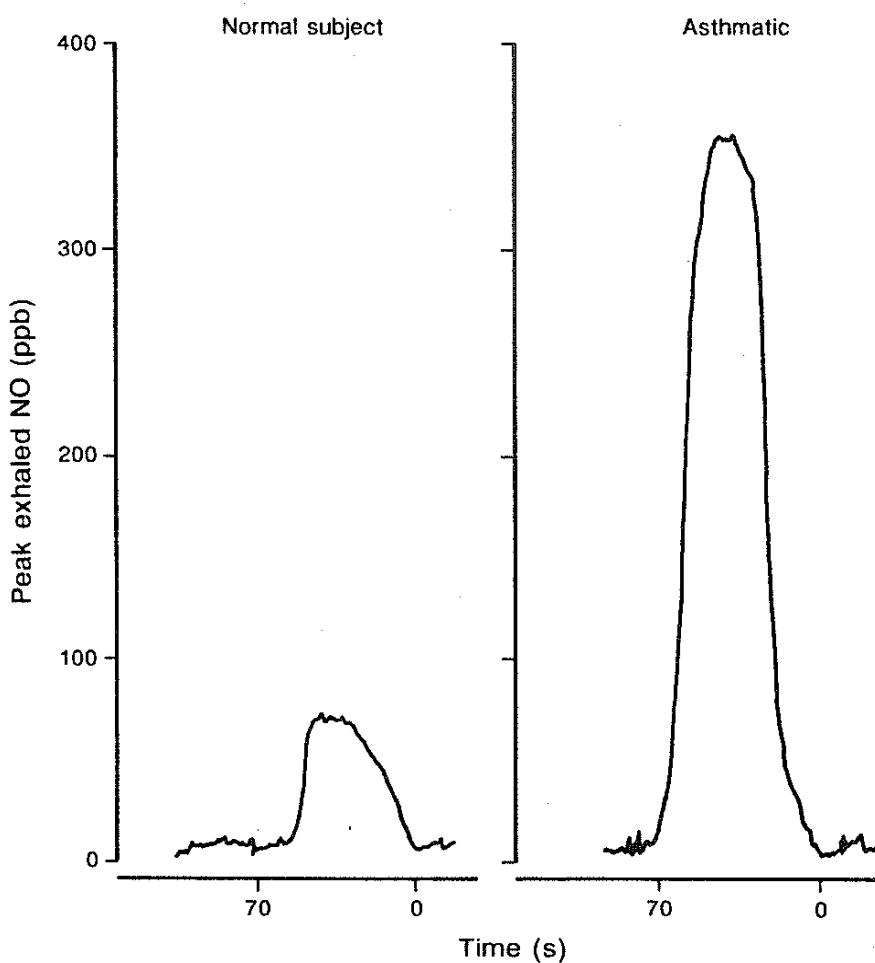


Figure 2: representative original traces of exhaled NO in normal subject and asthmatic patient (from ref 2).

INHALED STEROIDS AND FeNO MEASUREMENT

A factor influencing FeNO measurements is antinflammatory therapy as it has been observed that FeNO is elevated in untreated patients with asthma but not in patients treated with ICS (figure 3). This may reflect an inhibitory effect of glucocorticoids on the induction of the enzyme NO synthase in the respiratory tract (2).

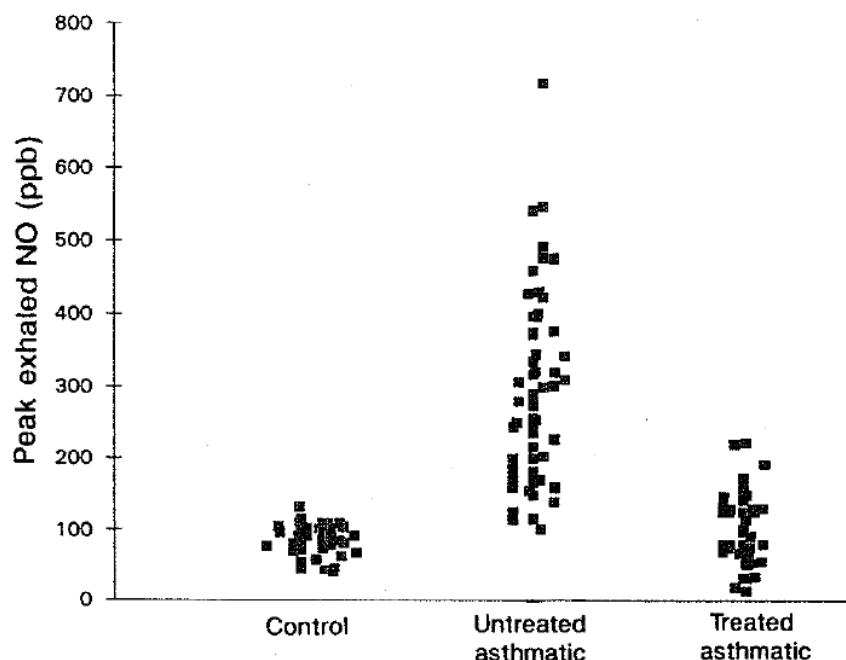


Figure 3: peak FeNO concentrations in control subjects (n=67), untreated asthmatic (n=61) and treated asthmatic (n=52) groups (from ref 2).

Kharitonov and colleagues studied the effect of an ICS (budesonide 800 micrograms twice daily via a dry powder delivery system for 3 weeks) on exhaled NO in 11 patients with mild asthma in a double-blind crossover randomized-order placebo-controlled study. FeNO was significantly reduced from a baseline value of 203 ± 29 parts per billion (ppb) to 120 ± 26 ppb after 3 weeks of treatment, whereas there was no change after a matched placebo (169 ± 20 ppb at baseline compared with 184 ± 16 ppb after 3 weeks). A significant and progressive fall in FeNO was found from week 1 to 3.

In the same trial, no significant change in forced expiratory volume in one second (FEV₁) after inhaled steroids was observed (although mean FEV₁ was 92% predicted normal at baseline), although there was a reduction in airway responsiveness to methacholine (approximately 2.5 doubling dilutions). These results add further support to the view that the elevated levels of FeNO in asthma may derive from induction of an inducible isoform of NOS and indicate that FeNO may be a useful way of monitoring the anti-inflammatory effects of glucocorticoids and other anti-inflammatory treatments in asthma (5).

Silkoff et al. (6) determined the dose response and the reproducibility of the FeNO fall following inhaled beclomethasone dipropionate (BDP) therapy in non-steroid-treated asthmatic patients. For four 1-week periods (period 1 to period 4), the following regimens were administered to 15 non-steroid-treated asthmatic patients received in sequential order placebo and 3 increasing BDP doses (100, 400 and 800 µg/day). After 1 week at each dose level, the subjects came to the laboratory for measurement of FeNO.

FeNO levels fell progressively from visit 1 to visit 5 and all doses of BDP resulted in a significant change in FeNO from placebo treatment, but with significant separation of only the 100µg and 800µg doses (figure 4). A post hoc inspection separated subjects into those with baseline FeNO of 60 to 100 ppb (n=6) and >100 ppb (n=9), on the assumption that airway inflammation would be mild to moderate and moderate to severe in these two groups, respectively. The low-FeNO group showed a modest fall in FeNO with 100 µg/day, but no further decline in FeNO as the dose of BDP was increased. The high-FeNO group showed a progressive fall in FeNO at each dose level, eventually reaching a similar level of FeNO as the low-FeNO group (figure 4).

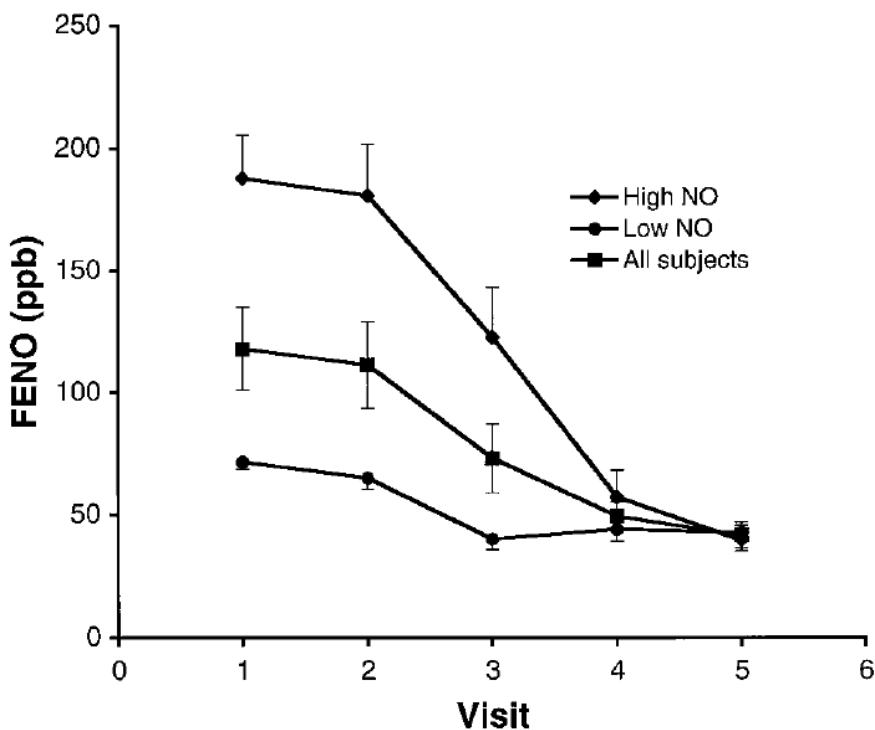


Figure 4: change in FeNO at each visit corresponding to baseline, placebo treatment, and then increasing doses of BDP. The FeNO trend for all the subjects ($n = 15$) is shown together with separate trends for high-baseline ($n = 6$) and low-baseline ($n = 9$) FeNO groups (from ref 6).

Reproducibility was assessed in a four periods study in which 12 non-steroid-treated asthmatic patients received placebo treatment for 7 days (period 1), 200 µg/day of BDP for 14 days (period 2), washout on placebo treatment until the FeNO was within 15% of baseline (period 3), and 200 µg/day of BDP for 14 days (period 4). There were no significant differences between FeNO in the two placebo periods or between the two BDP periods, confirming that the fall in FeNO after two identical administrations of BDP separated by placebo washout was highly reproducible (figure 5).

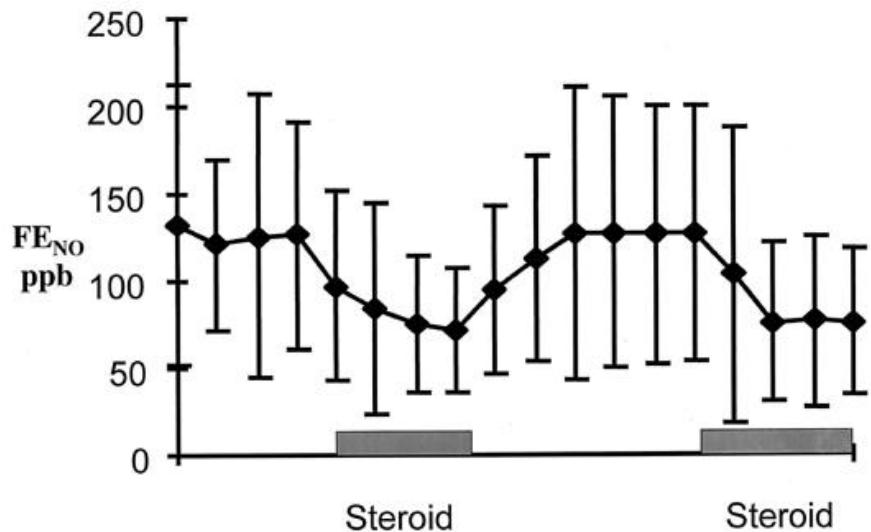


Figure 5: changes in FeNO (mean \pm SD) in two placebo periods and two periods of treatment with 200 μ g/day of BDP (steroid; from ref 6).

SMOKING AND FeNO MEASUREMENT

Another factor influencing FeNO levels in exhaled air is smoking. The first study reporting measurements of FeNO concentrations in asthmatic outpatients and in non-smoking and smoking healthy controls was published in *The Lancet* in 1994. Persson and collaborators (7) demonstrated that in single exhalations, FeNO showed a peak suggestive of airway origin in both controls and asthmatic patients. The peak FeNO concentration was higher in asthmatic patients and lower in smokers than in non-smoking controls (figure 6). The findings support a role for NO in the host defence response in asthma and suggest that FeNO measurements can discriminate between different types of lung disorders. These findings have implications for the use of FeNO measurements in asthmatic smokers that can be up to one third of the total asthmatic population.

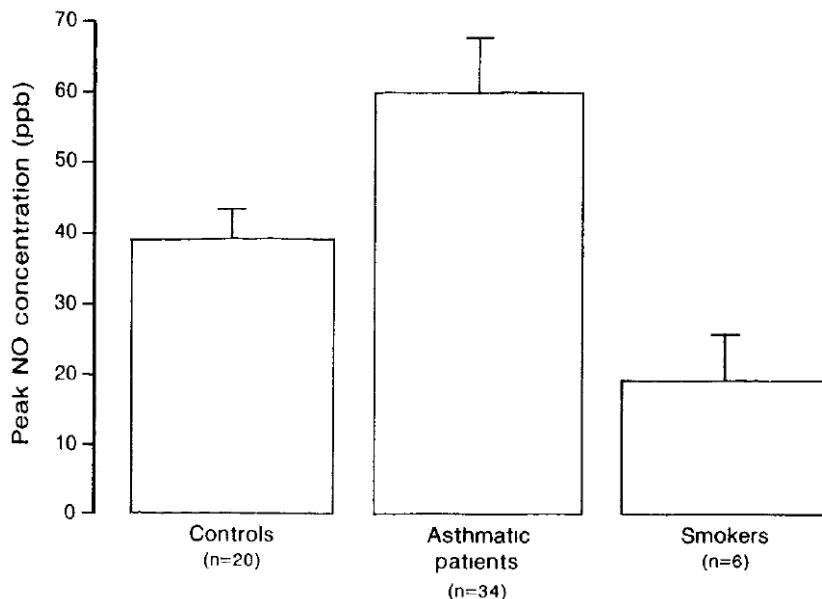


Figure 6: mean (SEM) peak FeNO concentrations in single exhalations after 15-s breath holding in healthy controls, asthmatic patients and smokers (from ref 7).

The mechanism by which smoking causes FeNO reduction is not fully understood, but may include reduction in NO synthesis due to feedback inhibition induced by high concentrations of NO contained in cigarette smoke. NO oxidation or interaction with other molecules present in tobacco smoke might also occur. However, regardless of the mechanism of FeNO reduction reported in smokers, it is generally assumed that FeNO should not be assessed in asthmatic patients who smoke. Perhaps, consequently, this population has been excluded from clinical trials that have explored the potential of FeNO as a biomarker in asthma management.

Interestingly, Michilis et al. strongly suggested that it is the change in FeNO values, rather than absolute cut-off points (i.e. individualised FeNO profiles), that may be meaningful for the longitudinal assessment of asthma control in daily practice (8). Therefore, in a study recently published in the European Respiratory Journal (9), the authors investigated whether, despite the FeNO reduction reported in smoking asthma patients, changes in FeNO might also be significantly related to changes in asthma control in this population. To do this, FeNO was monitored on several occasions in smoking and non smoking patients attending a tertiary asthma clinic. Its ability to reflect improvement or worsening of asthma control over time was compared in both groups, using the Asthma Control Questionnaire (ACQ) as a gold standard for the assessment of asthma control. FeNO and the Asthma Control Questionnaire (ACQ) were recorded at least once in 411 non smoking (345 with at least two visits) and 59 smoking (51 with at least two visits) asthma patients. The study confirmed that, compared with non smokers, FeNO is reduced in smoking asthma patients (18.1 ppb versus 33.7 ppb) despite similar mean ACQ scores (1.5 versus 1.7). However, this reduction does not appear to suppress its ability to reflect asthma control in smoking patients, provided changes in FeNO values detected by repeated measurements are

considered. In fact, a decrease in FeNO of less than 20% precludes asthma control improvement in non smoking (negative predictive value, NPV=78%) and in smoking patients (NPV=72%).

An increase in FeNO of less than 30% is unlikely to be associated with deterioration in asthma control in both groups of patients (NPV=86% and 84% in non smoking and smoking patients, respectively). Overall, the importance of sequential FeNO measurements in both smokers and non smokers is to distinguish whether or not ongoing changes or a sudden change in respiratory symptoms are/is due to changes in airway inflammation, possibly requiring a change in anti-inflammatory therapy. It is concluded that, even in smokers, sequential changes in FeNO have a relationship with asthma control, indicating that cigarette smoking does not obviate the clinical value of measuring FeNO in asthma among smokers. Moreover, it has been suggested that the effect of smoking on FeNO lasts no longer than 15-30 min and can be ignored by simply asking the patient on the time of the last cigarette and not making FeNO measurement earlier than 30 min after that.

When considering both smoking and ICS therapy as two known factors affecting FeNO measurements, interestingly, in the study by Michilis et al. (9) when patients were treated with high-to-medium ICS doses, FeNO no longer had the ability to reflect an improvement in asthma control for smoking patients, whereas for non smoking patients, its ability was only slightly reduced. A similar trend was observed with respect to asthma control deteriorations. These results confirm the overall reduction of the ability of FeNO to reflect asthma control in patients treated with high-to-medium ICS doses. In addition, it appears that confounding factors, such as high ICS doses and tobacco smoking, which are known to reduce FeNO, would have a cumulative interfering effect that may eventually suppress the ability of FeNO to reflect asthma

control. This suggests that the effect of these confounding factors might have to be taken into account when using FeNO to assess asthma control.

MULTIPLE FLOWS FeNO MEASUREMENT

As long as it is strongly agreed that peripheral airways are involved in the pathogenesis of asthma and inflammation is widely present in the whole bronchial tree including peripheral bronchioles and alveoli (1, 10), several tests have been proposed to measure the level of peripheral airway inflammation (11). FeNO measured at 50 ml/s mainly assesses the bronchial origin of exhaled NO, whereas alveolar NO concentration, measured in the compressible compartment, implicates deep lung inflammation. Two compartment models of pulmonary NO production have been described (12) which can be used to calculate the alveolar contribution to exhaled NO concentration by using a model of NO diffusion in airways and measurements of exhaled NO concentrations at multiple flow rates (13).

In a study by Berry et al. (14) in 13 normal subjects, 25 mild to moderate asthmatics and 27 patients with refractory asthma, a positive correlation was found between alveolar NO concentration and bronchoalveolar lavage (BAL) eosinophil count (figure 7) but not with bronchial wash or sputum eosinophil count. Alveolar NO concentration was increased in patients with refractory asthma compared with mild-to-moderate asthma and normal controls and reduced by treatment with prednisolone (figure 8). These findings support the hypothesis that alveolar nitric oxide is a measure of distal airway inflammation and suggest that distal lung inflammation is present in refractory asthma.

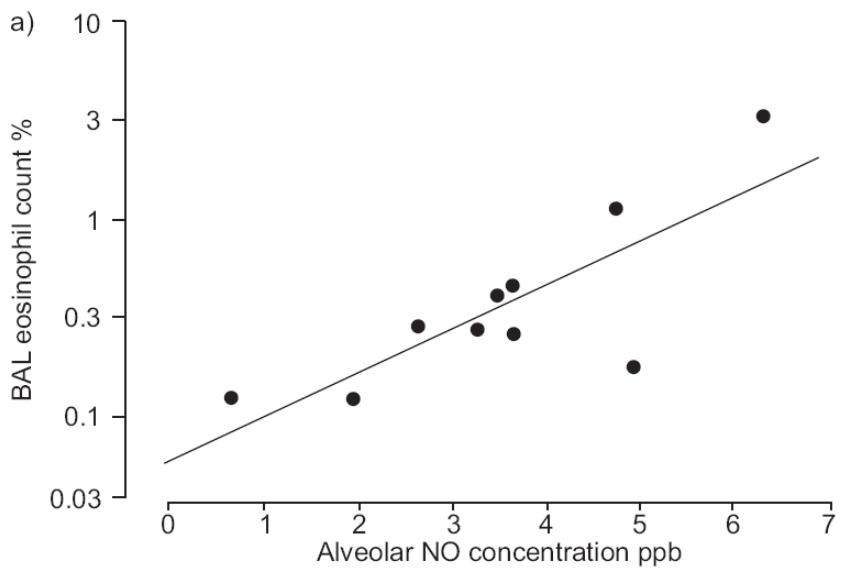


Figure 7: Association between bronchoalveolar lavage (BAL) eosinophils and alveolar NO concentration ($r=0.79$, $p=0.006$; from ref 14).

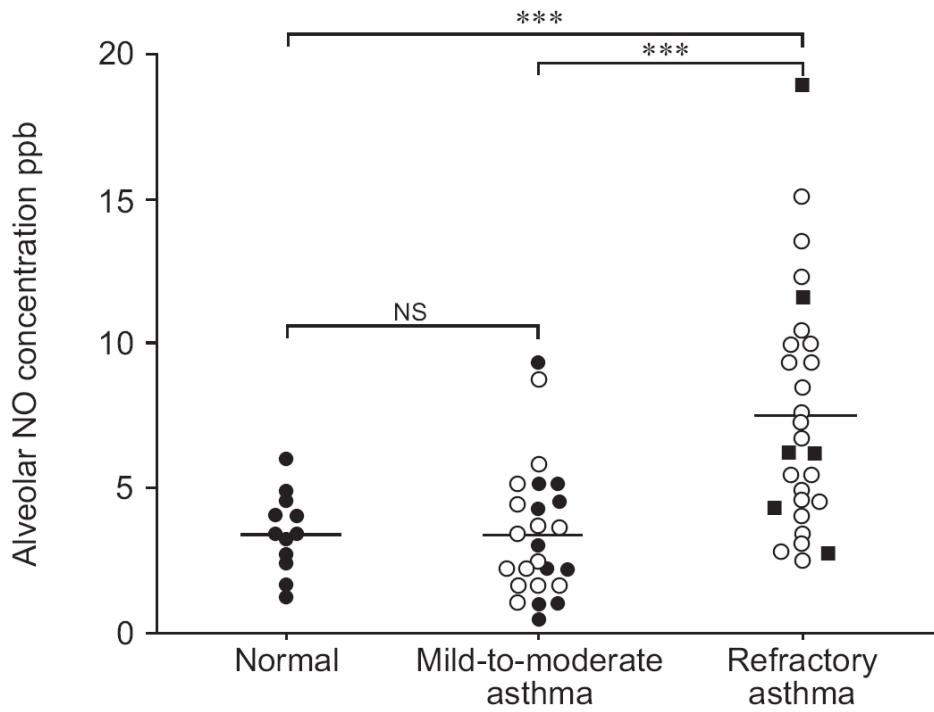


Figure 8: Alveolar NO concentration in normal controls compared to mild-to-moderate asthma and refractory asthma. ●: steroid naive; ○: inhaled steroid treated only; ■: oral and inhaled steroid treated. ***: $p<0.001$; NS: non significant (from ref 14).

Despite the evidence of a reduction in alveolar NO with oral corticosteroids (14), unlike bronchial NO, alveolar NO production is not reduced by ICS in patients with asthma, suggesting that it may be derived from a site not accessed by the inhaled drug (figure 9).

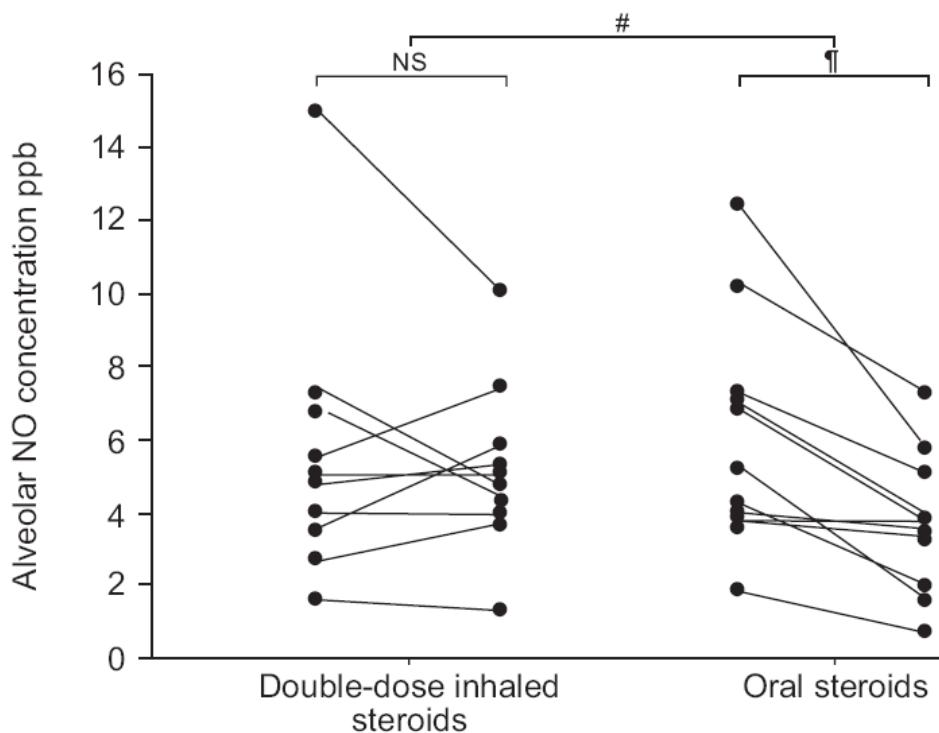


Figure 9: Changes in alveolar NO concentration in patients who received a doubling of their inhaled steroid dose ($n=10$) and those who received oral corticosteroids ($n=11$). #: $p=0.034$; ¶: $p=0.002$; NS: non significant (from ref 14).

However, partitioning exhaled NO in its bronchial and alveolar sources deserves further scrutiny. Although it has been recently suggested that the small airway epithelium is the major source of NO production (15), multiple sources have been identified in the lungs and several analytical techniques have been developed to capture this rich feature (16, 17).

RECENT EVIDENCE ON FeNO MEASUREMENT

The use of FeNO to monitor the effectiveness of asthma treatment is increasing because of its reported association with the presence of inflammation in asthma. For this reason, in the last decade 257 clinical trials in humans were published on the topic of nitric oxide and asthma (18). To standardize FeNO measurement methods, a guideline was released by the European Respiratory Society and the American Thoracic Society in the year 2005 (19).

NO is commonly measured in respiratory clinics with a stationary machine that is similar in appearance and dimensions to a desktop computer, connected to a nitric oxide tank used for calibration. In the last 5 years, a small, portable NO analyzer was introduced to allow repeated measurements by patients outside the clinics. One of the trials evaluating the usefulness of this portable NO analyzer was conducted in UK general practice in collaboration with the University of Parma, Department of Clinical Sciences (20). The study involved 96 asthmatic patients and demonstrated that FeNO measurements performed with a new hand-held monitoring device are reproducible, simple and feasible in General Practice in the majority of patients of different age and asthma severity. Indeed, Success rate of FeNO measurements was 78% and the intra-subject coefficient of variation was 8.7%. Moreover, an overall reducing effect of ICS on FeNO regardless of the smoking habit of the patients was demonstrated. Inhaled corticosteroid treatment had an overall reducing effect on the FeNO value vs. patients not on the ICS. Finally, a high percentage of patients with different severities of asthma and regardless of their treatment with ICS and current smoking habit (current and/or ex-smokers) had highly elevated FeNO levels still significantly above the normal values,

suggesting that their current therapy was possibly insufficient to control the underlying degree of airway inflammation and asthma symptoms.

The availability of FeNO measurements raised the question whether this could be a valid and convenient alternative to standard methods for diagnosis and monitoring of asthma. Price et al. (21) constructed two decision trees to compare FeNO measurement with standard diagnostic testing and guideline recommendations for management. The use and cost of each strategy, as well as associated outcomes, such as diagnostic accuracy, are derived from the medical literature. For asthma diagnosis, FeNO measurement was compared with lung function and reversibility testing, bronchial provocation and sputum eosinophil count. For asthma management, the impact on asthma control, including inhaled corticosteroid use, exacerbations and hospitalizations, of monitoring with FeNO measurement vs symptoms and lung function was evaluated as in standard care. Resource use (direct costs calculated from a UK health-care payer perspective) and health outcomes were evaluated over a 1-year time frame. The conclusion of this study is that asthma diagnosis based on FeNO measurement alone is less costly and more accurate than standard diagnostic methods and asthma management based on FeNO measurement is less costly than asthma management based on standard guidelines and provides similar health benefits. The application of FeNO measurement in clinical practice can therefore play an important role in diagnosing and assessing airway disease.

As long as FeNO measurements are used to interpret the aetiology of nonspecific respiratory symptoms, identify potential responders to ICS therapy, and monitor underlying disease activity in asthma, there are significant areas of uncertainty regarding how results ought to be interpreted, especially in the treatment of patients with chronic asthma over time. This is because, unlike induced sputum eosinophils,

which are absent unless airway inflammation is present, a number of non pathological factors may influence FeNO, occasionally giving rise to increased levels even in healthy individuals.

Clinically significant cut-points for FeNO have been based on the relationship between FeNO and induced sputum eosinophil counts, as well as levels which are prognostically important in relation to ICS therapy. In general, low FeNO values (<25 parts per billion [ppb]) are associated with minimal airway eosinophilia (22). In patients with diagnosed asthma, this generally implies good asthma control or, if the patient is symptomatic, the need to consider explanations for their symptoms other than active eosinophilic airway inflammation. Conversely, high FeNO values (>50 ppb) indicate active eosinophilic airway inflammation and the likelihood of deterioration in asthma control if the dose of ICS is reduced, even if the patient is asymptomatic (22). However, this broad approach leaves open some unresolved questions as some patients with asthma may have FeNO levels that are higher than normal despite good asthma control and it is possible that individualized personal best values might be more clinically useful than population-derived reference values. Indeed, values deemed to be clinically normal and abnormal should perhaps be based on FeNO levels obtained when an individual patient's asthma is respectively well controlled and poorly controlled.

To address this issue, Smith and colleagues (23) analyzed data obtained before and after a trial of oral prednisone (30mg/d for 14 days), and also from a previously published study in which patients had their dose of inhaled corticosteroid adjusted using either FeNO or symptoms/lung function to optimize treatment. The aim of the study was to identify the relationship between reference values for FeNO and personal best levels obtained after a course of oral prednisone in patients with mild to moderate persistent asthma. Secondly, a comparison of the personal best FeNO levels after

prednisone with those obtained at loss of control and during optimized treatment with inhaled steroid was performed. These comparisons were designed to clarify whether anti-inflammatory treatment ought to be guided by reference values or individual clinically-based cut-points for FeNO.

Overall, data from Smith et al. indicate that when measuring FeNO in relation to asthma control and its treatment, target FeNO levels based on group mean data or reference equations have limited value. The absolute values and/or the magnitude of changes in FeNO in relation to personal best obtained in individual patients when asthma is well controlled are more likely to be informative. However, personal best FeNO levels in patients with asthma who have high levels when they are symptomatic do indeed coincide with a particular set of predicted values (24).

The interrelationships between the fraction of FeNO, eosinophilic airway inflammation and steroid responsiveness, together with the ease with which FeNO may be measured, have prompted a series of randomized trials designed to confirm that using FeNO to optimize ICS therapy will improve asthma outcomes. Overall, we must accept that, notwithstanding any weaknesses of the various FeNO-based treatment algorithms, the routine use of FeNO in this setting does not fulfil earlier expectations (25). A recent Cochrane systematic review (26) including six studies (2 adults and 4 children/adolescent) evaluated the efficacy of tailoring asthma interventions based on exhaled nitric oxide in comparison to clinical symptoms (with or without spirometry/peak flow) for asthma related outcomes in children and adults. Of 1053 participants randomised, 1010 completed the trials. In the meta-analysis, there was no significant difference between groups for the primary outcome of asthma exacerbations or for other outcomes (clinical symptoms, FeNO level and spirometry). In post-hoc analysis, a significant reduction in mean final daily dose inhaled corticosteroid per adult

was found in the group where treatment was based on FeNO in comparison to clinical symptoms, (mean difference -450 mcg; 95% CI -677 to - 223 mcg budesonide equivalent/day). However, the total amount of inhaled corticosteroid used in one of the adult studies was 11% greater in the FeNO arm. In contrast, in the paediatric studies, there was a significant increase in inhaled corticosteroid dose in the FeNO strategy arm (mean difference of 140 mcg; 95% CI 29 to 251, mcg budesonide equivalent/day).

It can be concluded that tailoring the dose of inhaled corticosteroids based on exhaled nitric oxide in comparison to clinical symptoms found only modest benefit at best and potentially higher doses of inhaled corticosteroids in children. The role of utilising exhaled nitric oxide to tailor the dose of inhaled corticosteroids cannot be routinely recommended for clinical practice at this stage and remains uncertain. It is understandable, but perhaps unfortunate, that the most rigorous studies to date have focused narrowly on how FeNO might be used to improve asthma outcomes in relation to ICS treatment. However, the pathophysiology of airway disease is heterogeneous, with many overlap syndromes giving rise to nonspecific symptoms which are only weakly correlated with abnormal lung function. FeNO measurements shed complementary light on the underlying inflammatory phenotype and, more importantly, on the potential response to antiinflammatory treatment. This was first assessed either by empiric “trials of steroid” or, with reference to before/after changes in spirometry, whereas serial or repeated FeNO measurements in individual patients may provide additional diagnostic as well as prognostic insights (25).

That “asthma is a chronic inflammatory disorder” has been shouted from the rooftops for over 20 years, and the case for assessing airway inflammation in clinical practice has been strongly made. Practical issues have impeded the wider use of other non-invasive markers of inflammation such as induced-sputum and exhaled-breath

condensate techniques. The standard of proof to support the adoption of FeNO, which is more accessible, ought to be rigorous but not narrowly focused. A working party of the American Thoracic Society is currently drawing up guidelines for the clinical use of FeNO measurements, and we await their statement with interest (25).

CONCLUSIONS

FeNO is a non invasive marker of airway inflammation easy to be performed and can be helpful as a tool contributing to asthma diagnosis and evaluation of response to antinflammatory therapy with ICS. FeNO peak is increased in asthmatics, as compared to normal subjects and is lowered by ICS with a dose-response relationship that is more evident for asthmatic patients with high levels of FeNO. Smoking affects FeNO measurements and this has to be considered when evaluating patients with asthma who smoke. However, the change in repeated measures in the same patient, even if smoker, can be meaningful for the longitudinal assessment of asthma control in daily practice. The clinical application of FeNO still needs to be clarified but it is clearly one of the most used non-invasive markers giving information on the inflammatory component of the disease. The possibility of measuring FeNO derived from either bronchial or alveolar regions by models dividing the lungs in two or more compartments can give information on the distribution of inflammation in the bronchial tree and is of particular interest for clinical pharmacology. The recent development and clinical application of new formulations of ICS which deliver small drug particles, able to reach the more peripheral airways, is of interest because inhaled therapy was limited to the proximal airways in the past. Several studies evaluated the usefulness of measuring FeNO in adults and children but few data are available regarding the different measurement of bronchial and alveolar FeNO which can be particularly useful to optimize therapy in patients with evidence of high values of alveolar FeNO. Future studies and the upcoming American Thoracic Society guidelines for the clinical use of FeNO measurements will clarify the clinical value of this important tool.

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BOTH BRONCHIAL AND ALVEOLAR EXHALED NITRIC OXIDE ARE
REDUCED WITH EXTRAFINE BECLOMETHASONE DIPROPIONATE IN
ASTHMA

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Short title: effects of beclomethasone on exhaled nitric oxide

ABSTRACT

Background: Exhaled nitric oxide (NO) is a non-invasive marker of airway inflammation. Beclomethasone dipropionate (BDP) is the only inhaled corticosteroid available as both extrafine and non-extrafine HFA pMDI formulation. The present study was designed to evaluate whether the different patterns of lung deposition of two HFA BDP formulations, are associated with a different effect on bronchial and alveolar NO.

Methods: This was a prospective double blind, randomized, controlled, cross-over study. After a 2-week placebo run-in period without inhaled corticosteroids, asthmatic patients were randomized to extrafine BDP 100 µg bid or non-extrafine BDP 250 µg bid for two 2-week periods separated by a 2-week washout period.

Results: 14 patients (5 males) mean age 37 years, mean baseline FEV₁ 83 % of predicted were analyzed. Exhaled bronchial NO was significantly ($p<0,001$) reduced in both treatment groups as compared to the last week of run-in period, whereas alveolar NO was significantly ($p<0,001$) reduced only with extrafine BDP. Moreover, extrafine BDP was superior to non-extrafine BDP in both parameters ($p<0,05$).

Conclusions: extrafine but not non-extrafine BDP HFA formulation lowers both bronchial and alveolar exhaled NO in asthmatic patients. ICS distribution throughout the whole bronchial tree could be important in patients who do not gain optimal control of inflammation with conventional non-extrafine ICS.

INTRODUCTION

Asthma is a chronic inflammatory disease characterised by symptoms of variable severity associated with functional alterations and pathological abnormalities such as airway inflammation and remodelling. Anti-inflammatory treatment with inhaled corticosteroids (ICS) constitutes the cornerstone of asthma management and is the most effective long-term therapy [1].

Airway inflammation is present in all forms of asthma including mild and asymptomatic cases [2] and involves both large and small airways [3, 4], the latter gaining attention over the past 15 years thanks to more specific and new non-invasive assessing techniques [5].

The fractional concentration of exhaled nitric oxide (FeNO) is one of the most widely used and convenient non-invasive markers in exhaled breath to monitor airway inflammation in adults and children [6,7]. NO is a gaseous signalling molecule participating in airway physiology, which levels are increased even in mild asthmatics and both in allergic and nonallergic asthma [8-10]. FeNO derives from endogenous NO production by synthases present in airway epithelium and inflammatory cells, with genetic factors accounting for a large proportion of the variation in FeNO and for the correlation between FeNO and serum total IgE [11, 12]. FE_{NO} originates from the intrapulmonary airways in asthma [13] and two compartment models of pulmonary NO production have been described [14], which can be used to calculate the bronchial (J_{NO}) and alveolar contribution (C_{alv}) to exhaled NO concentration [15].

C_{alv} is elevated in conditions associated with distal lung inflammation, such as alveolitis [16] and chronic obstructive pulmonary disease [17] and has been related to BAL eosinophil cationic protein levels in asthmatic children [18] and to BAL eosinophil counts in asthmatic adults [19].

The need for treating asthma inflammation uniformly throughout the lower airways and has led to the introduction of extrafine ICS with small-particle formulation as a particle size of 3–5 μm is optimal for delivery to the conducting airways but particle sizes of 1 μm (extrafine) are needed to target the smaller airways too [20].

The switch from CFC to HFA-propelled MDIs leads to the reformulation of beclomethasone dipropionate (BDP) both as extrafine and non-extrafine formulation [21]. Small-particle aerosols, such as extrafine BDP, which have particle sizes around 1 μm have been shown to allow a greater lung deposition of the drug as a proportion of ex-actuator amount, resulting in an equivalent dose ratio of 1:2,5 with non extrafine formulations [21] and a greater proportion that deposits peripherally [22].

There is little doubt from the large body of published evidence that extrafine formulations are effective in improving clinical indices of asthma control, lung function and inflammation as well as traditional formulations and some evidence is available supporting an added benefit of extrafine formulations [23-24]. Whether small particles are better than larger particles in terms of their effect on small airway function, however, is an area of conflicting evidence and studies designed to address this question are often biased by using different drugs and/or devices [25-26].

The present study was designed to evaluate whether the different patterns of lung deposition of two BDP HFA formulations (extrafine 100 μg vs. non-extrafine 250 μg), are associated with a different effect on bronchial and alveolar exhaled nitric oxide in adults with mild to moderate asthma.

METHODS

Subject characteristics

Sixteen mild to moderate asthmatic patients ($\text{FEV}_1 > 60\%$) according to GINA guidelines (1) with $\text{FeNO} > 40 \text{ ppb}$ (standard flow 50 ml/s) were screened and 14 participated in the study. Patients were recruited by a pneumologist from outpatient clinic at the Parma University Hospital between April and July 2008.

All patients had a diagnosis of asthma and objective measures of airway responsiveness and/or documented airway obstruction and a positive skin-prick test result to at least two common allergens (cat dander, house dust mite, grass pollen, *Aspergillus fumigatus*). Current smokers and patients with a smoking history of > 5 pack-years were excluded. Patients with near fatal asthma, evidence of symptomatic respiratory lower tract infection in the 8 weeks preceding the screening visit, hospitalization for asthma or patients on 3 or more courses of oral corticosteroids in the previous 6 months were not included. Additionally, patients were excluded if treated with anticholinergic agents or antihistamines in the previous 2 weeks and/or inhaled or intranasal corticosteroids or leucotriene receptor antagonists in the previous 4 weeks.

None of the patients were receiving food supplements containing L-arginine, and none were on a nitrate-rich or nitrate-restricted diet that might influence exhaled NO levels. During the study, diaries were supplied to assess patients' compliance to treatments calculated in terms of % of the prescribed daily dose. Compliance was assessed with diary cards and accepted as sufficient when 75% of scheduled treatment was assumed.

The study was performed in accordance with the Good Clinical Practice guidelines recommended by the International Conference on Harmonization of Technical Requirements, was approved by the Ethics Committee of University Hospital of Parma, and all participants gave written informed consent before inclusion.

Study Design

This was a randomized, double-blind, double-dummy, cross-over design study. The whole study period was 8 weeks, during which patients were evaluated in 9 visits (Fig. 1).

Patients satisfying inclusion and exclusion criteria entered the 2-week run-in period and all anti asthmatic drugs other than as needed salbutamol were withdrawn. Nasal decongestants were allowed for the treatment of hay fever.

After a 2-week placebo run-in period, patients were randomized to receive extrafine BDP 100 µg bid (daily dose 200 µg) or non-extrafine BDP 250 µg bid (daily dose 500 µg). Both treatments were administered by means of HFA pressurized metered dose inhalers (pMDI) for two 2-week periods, with a 2-week wash-out period in- between (Figure 1). The wash-out period was considered long enough to re-establish the original values of exhaled NO. To allow recovery of NO values, only the use of short-acting b2 adrenergic agents was allowed, whenever needed, during the run-in and washout periods.

Spirometry

Spirometry was performed at each visit according to international recommendations [27] at least 6 h after administration of any short-acting beta-2 agonist. A flow-sensing spirometer connected to a computer for data analysis (Vmax 22, Sensor Medics, Yorba Linda, U.S.A.) was used for the measurements. The best value of three manoeuvres was expressed as absolute value (in litres) and as a percentage of the predicted value [28].

Methacholine challenge

Methacholine inhalation challenge was performed according to the European Respiratory Society guidelines [29]. Doubling increasing concentrations of methacholine from 0.03 to 32 mg/mL were delivered by a dosimeter (output, 9 µL per puff; MB3; Mefar; Brescia, Italy) and inhaled. Inhalations were interrupted when FEV₁ decreased by 20% from its post-saline solution value. The provocative dose of methacholine causing a 20% decrease in FEV₁ was determined by linear interpolation of the last two experimental points.

Exhaled NO measurements

Exhaled NO concentration was measured by a chemiluminescence analyzer (NIOX; Aerocrine AB; Stockholm, Sweden) at expiratory rates of 10, 50, 100, 200, and 260 mL/s by applying resistors of 10, 50, 100, 200, and 300 cm H₂O mL/s to maintain the target flow rates. According to ATS/ERS guidelines [30], the patients were comfortably seated, inhaled NO-free air from a reservoir, and then exhaled against different linear resistors. The collection started when dead space time was subtracted from start of exhalation. The analyzer was calibrated with a known NO concentration (200 ppm). The exhalation time was 20 s for 10 mL/s, 10 s for 50 mL/s and 100 mL/s, and 6 s for 200 mL/s and 260 mL/s. The minimum waiting time between measurements was 20 s to allow the patient to rest.

J_{NO} and C_{alv} were calculated by nonlinear regression according to the equation of George et al [15]. The slope and the intercept of a regression line between NO output and exhalation flow rate are C_{alv} and J_{NO}, respectively.

STATISTICS

Data are expressed as mean (SD) or \pm SEM. The distribution of variables was assessed by means of Kolmogorov-Smirnov Goodness-of-Fit test. A non parametric Wilcoxon t test was used to compare NO values at the end of the second week of each treatment period versus baseline values (end of run-in). Statistical significance was set at $p<0.05$. Repeatability of J_{NO} and C_{alv} measurements was assessed with Bland–Altman analysis [31]. The mean values at all visits were plotted on the horizontal axis and the differences of the means at all visits plotted on the vertical axis which shows the amount of disagreement between the measures (via the differences) in order to see how this disagreement relates to the magnitude of the measurements.

RESULTS

All randomized patients completed the 8 week study period with satisfactory treatment compliance. Subject characteristics at baseline (visit 3) are shown in Table 1. Both extrafine and non-extrafine BDP lead to a significant reduction in J_{NO} after 2 weeks treatment compared to baseline (1541 ± 138 pL/s and 1832 ± 142 pL/s vs. 2798 ± 222.9 pL/s respectively; $p<0.001$; Figure 2). However, the J_{NO} values were lower after treatment with BDP extrafine compared to non-extrafine ($p<0.05$; figure 2).

By contrast, only extrafine BDP lead to a significant reduction from baseline (2.1 ± 0.1 ppb vs. 3.7 ± 0.1 ppb; $p<0.001$) in C_{alv} , whereas non-extrafine BDP did not modify C_{alv} levels (3.3 ± 0.2 ppb vs. 3.7 ± 0.1 ppb; Figure 3).

The repeatability of J_{NO} measurements as measured by the Bland–Altman analysis yielded an upper limit of agreement of 446 ppb and a lower limit of –535 ppb. The repeatability of C_{alv} measurements as measured by the Bland–Altman analysis

yielded an upper limit of agreement of 0.80 ppb and a lower limit of -0.96 ppb (Figure 4).

No significant differences were observed in J_{NO} and C_{alv} when comparing the first with the second week of the run in, wash out, or treatment periods.

No correlation was found between both J_{NO} and C_{alv} concentrations and lung function values.

DISCUSSION

The present study shows that in mild to moderate asthmatic patients only extrafine BDP reduced both J_{NO} and C_{alv} concentrations while non-extrafine BDP lead to a significant reduction in J_{NO} values only. This finding suggests that treatment with extrafine BDP can specifically exert anti-inflammatory effects on both central and small airways in asthma.

Previous studies investigating the effect of inhaled steroids on bronchial and alveolar derived NO are available but providing discordant results. The reduction of J_{NO} but not C_{alv} with non extrafine BDP is consistent with previous results obtained with a non-extrafine fluticasone dry powder formulation in patients with asthma confirming that C_{alv} may be derived from a site not accessed by non-extrafine inhaled corticosteroid treatment [16]. By contrast, Robroeks and colleagues failed to demonstrate a different effect on J_{NO} and C_{alv} when comparing extrafine BDP with non-extrafine fluticasone dry powder inhaler (DPI) in children. These results were probably affected by overtreatment due to the relatively high doses of steroids compared and by the lack of sensitivity associated with the study design in which patients were treated with ICS during run-in period [26]. Furthermore, the significant reduction in C_{alv} we found with extrafine BDP treatment is in agreement with the recently published data showing a reduction in C_{alv}

after treatment with a recently developed extrafine formulation of an ICS, ciclesonide [32]. The present study comparing two HFA pMDI formulations of the same drug, BDP, provides the first evidence of a different distribution of the antinflammatory effect in the central and peripheral airways. This is reasonably due to the higher peripheral deposition of the extrafine formulation as any other variable such as different devices or different active drugs is lacking.

In this study, Bland Altman analysis of J_{NO} and C_{alv} measurements demonstrated a high repeatability and clinically acceptable agreement. A single operator made all measurements and the feasibility was further enhanced by the accuracy NO analyser used, which controlled the exhalation parameters and ensured that the measurements were not accepted unless they were performed according to the guidelines. NO concentrations were increased during run-in and wash out periods demonstrating the presence of an ongoing active inflammatory process, and confirming the validity of the study design. Moreover, the lack of difference between the first and the second week of each study period suggests that both J_{NO} and C_{alv} levels are rapidly modified by both ICS treatment initiation and suspension without evidence of carry-over effect, at least when ICS are given at low doses for short periods as in the present study.

Proper anti-inflammatory treatment requires accurate assessment and monitoring of the underlying inflammatory state of the airways and the lack of reduction in C_{alv} levels after treatment with non-extrafine ICS, suggests that the inflammatory process in the peripheral airways is extensive and still relatively undertreated despite ICS administration. The current study shows that patients with normal J_{NO} after non-extrafine ICS treatment can still have room for improvement in C_{alv} , thus suggesting C_{alv} could be successfully used to adjust anti-inflammatory treatment, for example, by the addition of extrafine ICS or to test novel anti-inflammatory therapies. Indeed,

current asthma guidelines [1] recommend treatment based on the assessment of asthma control using symptoms and lung function but studies in many countries have identified that asthma control remains suboptimal despite the existence of effective asthma treatments [33]. The use of exhaled NO levels to guide therapy, especially as regards increase and reduction in ICS dose, although promising [7], is still a matter of discussion as different studies gave discrepant results probably due to study design characteristics [34]. Indeed, exhaled NO is a sensitive marker to assess the local anti-inflammatory effects of ICS as it has been shown that levels of exhaled NO correlate with eosinophils in sputum, is predictive of a response to ICS and an elevated level of FeNO is predictive of asthma relapse following corticosteroid withdrawal [35,36]. Even if the correlation of C_{alv} values with standard spirometry could be of particular interest to the potential clinical application, we did not find any correlation between C_{alv} and lung function parameters, in agreement with previous findings [37], confirming that NO levels are not markers of airflow limitation.

A formal power calculation was not performed when the study was designed, as no reference values were available as effect sizes of ICS on small airway parameters are not yet fully known. Notably, it is reassuring that other intervention studies evaluating effects of small-particle ICS used similar sample sizes [32,38-41].

Although it has been recently suggested that the small airway epithelium is the major source of NO production [42], multiple sources have been identified in the lungs and several analytical techniques have been developed to capture this rich feature. The strength of the two-compartment model we used [14, 15] is its relative simplicity in characterizing NO exchange dynamics. Moreover, this model is the only one described in ATS guidelines for NO measurements to distinguish between bronchial and alveolar NO production [30].

The reports so far still suggest the flow-independent NO parameters are uniquely altered in several disease states such as asthma, cystic fibrosis, scleroderma, alveolitis, COPD, and allergic rhinitis and thus we believe provide pathophysiological insight or assist in the clinical management of inflammatory lung diseases. The balance between model complexity and ease of clinical translation is not yet optimal and provides exciting opportunities for the future.

In summary, the present study has demonstrated that treatment with extrafine inhaled BDP, at a daily dose of 100 μ g bid, improves both bronchial and alveolar exhaled NO in patients with asthma, suggesting a uniform and complete antinflammatory effect. Drug distribution throughout the whole bronchial tree with extrafine formulations could be important in patients who do not gain optimal control of inflammation with conventional non-extrafine ICS.

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Table 1. Subjects characteristics at baseline

Variables	Asthma (n=14)
Age, years	37 (8)
Sex, M/F	5/9
FEV ₁ , L	2.96 (1.0)
FEV ₁ , % predicted	83.0 (14.2)
FVC, L	4.07 (1.0)
FVC, % predicted	95.0 (11.2)
FEV ₁ /FVC (%)	71.6 (8.1)
FE _{NO}	55.7 (18.3)
PD ₂₀ mch FEV ₁ , µg	198.55 (30-1438) ^a

Definition of abbreviations: M=male; F=female; FEV₁=forced expiratory volume in one second; FVC=forced expiratory vital capacity; PD₂₀ mch FEV₁=provocative methacholine dose causing a 20% fall in FEV₁; Values are means (SD); ^ageometric mean (range)

Figure Legends:

Figure 1. Study design

Figure 2. Effects of extrafine and non-extrafine BDP on bronchial nitric oxide (J_{NO}).

Results are expressed as mean \pm SEM. * $p<0.001$ vs baseline; # $p<0.05$ between treatments.

Figure 3. Effects of extrafine and non-extrafine BDP on alveolar nitric oxide (C_{alv}).

Results are expressed as mean \pm SEM. * $p<0.001$ vs baseline; # $p<0.05$ between treatments.

Figure 4. Bland-Altman analysis for the repeatability of J_{NO} values (panel A) and C_{alv} values (panel B).

Figure 1

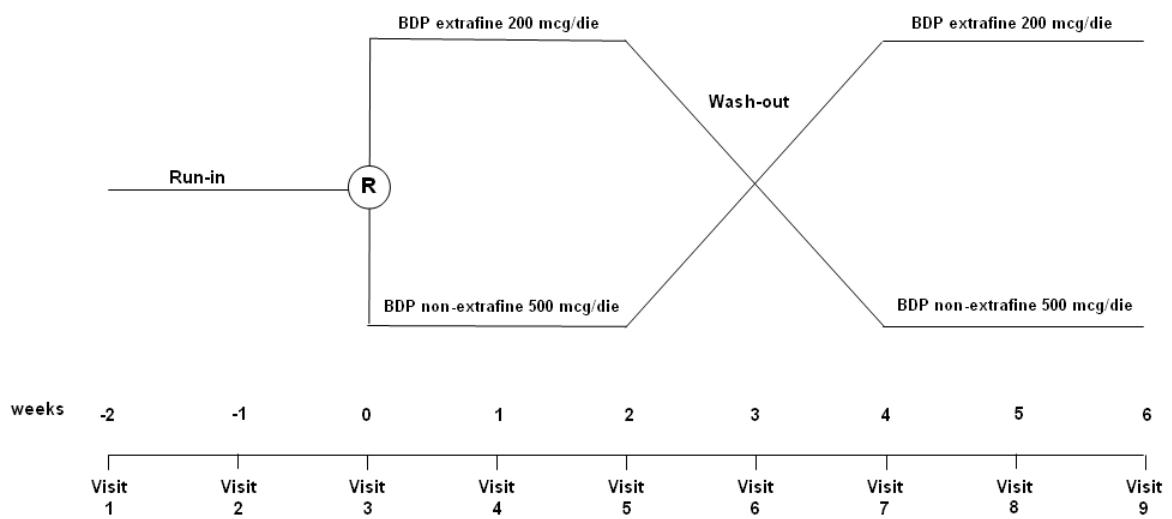


Figure 2

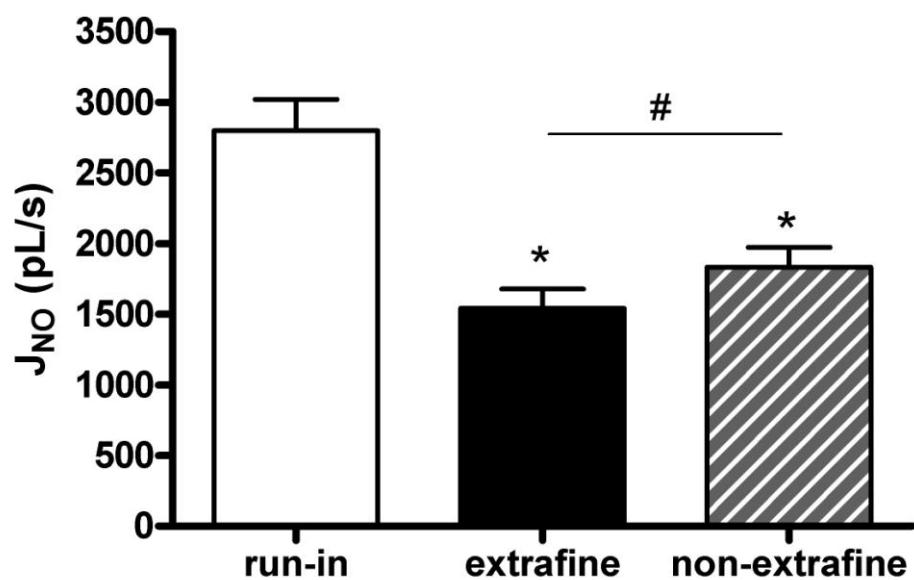


Figure 3

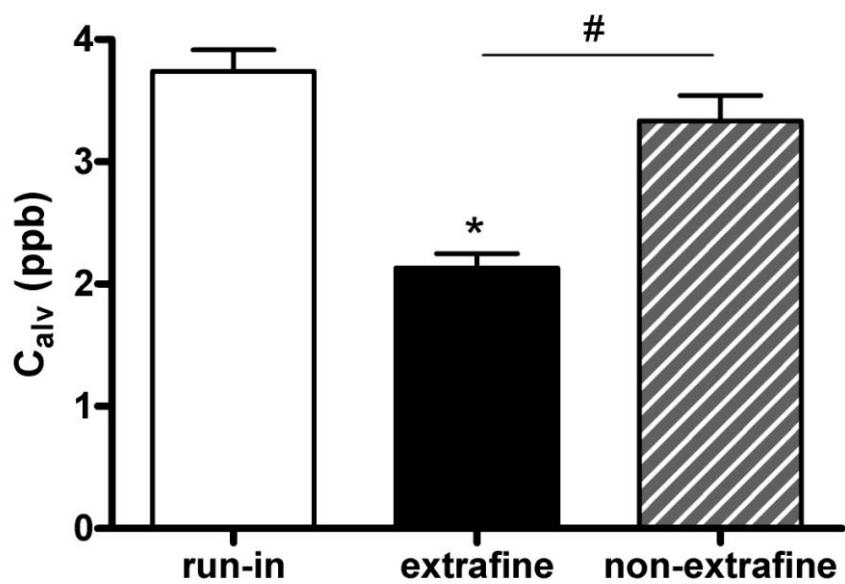
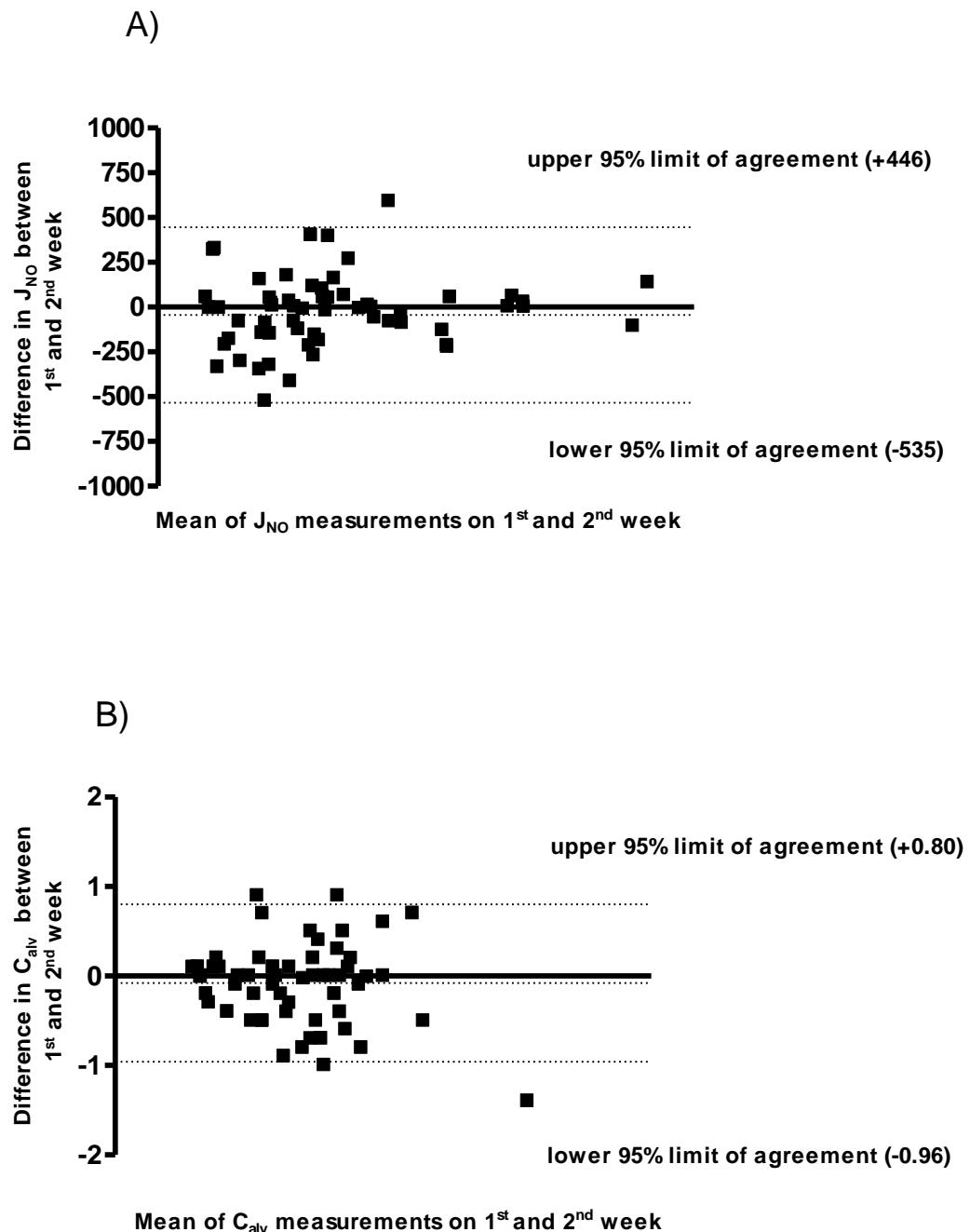


Figure 4



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