

Central and peripheral airway/alveolar sites of exhaled nitric oxide in acute asthma

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ABSTRACT

Introduction Central airway nitric oxide flux (J'_{awNO}) and peripheral airway/alveolar nitric oxide concentration (C_{ANO}) during asthma exacerbation has not been investigated after correction for axial NO back-diffusion.

Methods After measuring exhaled NO (fraction of exhaled nitric oxide (F_{ENO}); ppb) at 50, 100, 150 and 200 ml/s, J'_{awNO} (nl/s) and C_{ANO} (ppb) were calculated using the two-compartment model and corrected for axial NO back-diffusion. Fifteen (8 males), non-smoking, patients with moderate-to-severe treated (inhaled corticosteroid (ICS) and inhaled long-acting β_2 -agonist (LABA)) asthma, age 57 ± 13 years (mean \pm SD), were studied at baseline, during exacerbation prior to oral corticosteroid, and during recovery after an 8 day tapering prednisone course. Based on earlier asthma studies without correction, it was hypothesised that with correction for NO axial back-diffusion, the incidence of abnormal J'_{awNO} and C_{ANO} at baseline and after exacerbation would be $\geq 30\%$ in 15 patients with asthma with 80% power.

Results At baseline when clinically stable, after 180 μ g of albuterol, forced expiratory volume in 1 s (FEV_1 ; litres) was $78 \pm 26\%$ predicted ($p=0.009$) with increased F_{ENO} at 50 ml/s ($p=0.01$) and J'_{awNO} ($p=0.02$), but C_{ANO} was normal compared with the controls. During exacerbation FEV_1 (litres) was $57 \pm 20\%$ predicted ($p=0.02$), with increased F_{ENO} at 50 ml/s ($p=0.01$) and J'_{awNO} ($p=0.004$), but C_{ANO} was normal. Recovery results were similar to baseline. Two of 15 patients with asthma always had normal exhaled NO gas exchange.

Conclusions The central airways were the major site of abnormal NO flux in 13 of 15 patients with moderate–severe asthma when stable and during exacerbation and could be easily detected with abnormal F_{ENO} at 50 ml/s. C_{ANO} was normal.

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Measurement of the fraction of exhaled nitric oxide (F_{ENO} ; ppb) is a relatively simple, reproducible and non-invasive test for monitoring airway inflammation.¹ Increased F_{ENO} in asthma is accepted as a surrogate for predominantly eosinophilic-mediated inflammation in central airways and increased response to inhaled corticosteroid (ICS).^{1–5} The currently accepted method of measuring F_{ENO} at a single constant expiratory flow rate, usually 50 ml/s, is incapable of separating out whether the source of increased NO production is the large central airways or the peripheral small airway/alveolar site, or both.⁶ However, increased F_{ENO} predominantly reflects large central airways. Therefore, several investigators have developed newer techniques to discriminate NO gas exchange

between large central airways and peripheral smaller airways/alveolar compartments.^{7–12}

Using a two-compartment model developed by Tsoukias and George,¹¹ central airway NO flux and peripheral airway/alveolar concentration can be estimated by measuring F_{ENO} at multiple expiratory flows and plotting NO output versus expiratory flow. The slope of the linear regression line between NO output versus flow reflects the peripheral airway/alveolar NO concentration (C_{ANO} ; ppb), whereas the y -intercept reflects central airway NO flux J'_{awNO} (nl/s).¹¹ We^{13 14} and others^{8 12 15–21} using this model¹¹ have previously reported NO gas exchange in central as well as peripheral airway/alveolar sites in those with mild^{8 12 15 18–21} and moderate–severe^{8 13 14 16 17 20} clinically stable asthma. However, two groups independently^{12 22} have also demonstrated, both experimentally and theoretically, axial back-diffusion of NO from proximal to peripheral airway/alveoli, against the direction of exhalation. Furthermore, this may contaminate peripheral airway/alveolar levels, leading to an underestimation of central airway NO flux and an overestimation of peripheral airway/alveolar NO levels. Axial NO back-diffusion has not been previously accounted for in those with asthma with abnormal expiratory airflow limitation.

The current prospective outpatient study evaluated F_{ENO} using the two-compartment model before and after correcting for NO axial back-diffusion^{12 22} in clinically stable, non-smoking, treated patients with asthma with moderate–severe expiratory airflow limitation who were not previously studied. Our goal was to evaluate and partition central airway versus peripheral airway/alveolar sites of NO concentration at baseline, during an ensuing spontaneous asthma exacerbation prior to initiation of oral corticosteroid, and following recovery with oral corticosteroid. This has not been investigated previously. Based on our earlier asthma studies^{13 14} without correction for axial NO back-diffusion, we hypothesised the incidence of abnormal J'_{awNO} and C_{ANO} at baseline and after exacerbation to be $>30\%$ in 15 patients with asthma with 80% power after correction for axial NO back-diffusion.

METHODS

Patient selection

We recruited adult patients with moderate–severe persistent asthma who were on maintenance combination inhaled corticosteroid (ICS)/inhaled long-acting β_2 -agonist (LABA), fluticasone propionate 250 μ g/salmeterol 50 μ g twice a day (F250/

S50) (Advair250/50, GlaxoSmithKline, Research Triangle Park, North Carolina, USA) for at least 1 year. Smoking history was <5 pack-years and currently all were non-smokers for at least 5 years. All asthma patients were clinically stable for at least 8 weeks and were monitored in our tertiary referral outpatient clinic for ≥ 3 years. Their asthma symptoms and spirometry were optimal when compared with clinical and spirometric results obtained over the previous ≥ 3 years. When needed for acute relief, medical treatment included a short-acting inhaled β_2 -agonist (albuterol sulphate metered dose inhaler (MDI) or solution) and inhaled antimuscarinic agent ipratropium bromide (Atrovent MDI or solution, Boehringer-Ingelheim Pharmaceuticals, Ridgefield, Connecticut, USA). All those with asthma were off oral corticosteroids, antibiotics and leukotriene-modifying agents for at least 8 weeks before entry into the study. Serum eosinophils and immunoglobulin E (IgE) were measured only at baseline. All subjects with asthma had demonstrated bronchodilator reversibility within the past 2 years defined as an increase in forced expiratory volume in 1 s (FEV_1) 200 ml and $\geq 12\%$, 15 min after 180 μg of albuterol via MDI. Our goal was to study prospectively exhaled NO gas exchange in the initial 15 patients with moderate–severe asthma who developed an acute exacerbation requiring oral corticosteroids.

Normal controls

Normal values for $F_{E}NO$ were obtained from 40 normal subjects (20 males), age 57 ± 9 years (mean \pm SD), who were asymptomatic, healthy lifelong non-smokers with no history of lung disease and not on any medications. No provocative bronchoconstrictor challenge tests were done on normal subjects. All subjects with asthma and normal subjects studied had given informed consent for participation. This study was approved by both Lakewood Regional Medical Center IRB and Western IRB, Olympia, WA, and registered as NCT00576069.

Measurement of exhaled NO gas exchange

All subjects abstained from food and coffee for 2 h and alcohol for 12 h prior to studies. Exhaled NO was measured in triplicate prior to spirometry at four separate constant expiratory flow rates: 50, 100, 150 and 200 ml/s, and the mean of three values obtained within 10% of each other was reported using a Sievers NOA 280 chemiluminescence analyser with varying expiratory airflow resistors (GE Analytical Instruments, Boulder, Colorado, USA) as previously described.^{13 14} Furthermore, to avoid nasal NO contamination, a mouth pressure of >5 cm H_2O was used, as previously recommended.²³ The NO analyser was calibrated daily with a known concentration (45 ppm) and before each patient and control subject with NO-free air. The technique of Tsoukias and George¹¹ was used to calculate central airway NO maximal flux (J'_{awNO} , nl/s) (γ -intercept) and steady state peripheral airway/alveolar NO concentration (C_{ANO} , ppb) (slope) using a linear regression line for each subject with a minimum of three expiratory flow rate data points and r^2 of ≥ 0.9 . Correction was made for potential underestimation of large airway NO flux due to axial back-diffusion of NO using the method of Condorelli *et al*²² by multiplying large airway NO flux J'_{awNO} by a factor of 1.7. Furthermore, to adjust for possible spurious overestimation of values for peripheral lung C_{ANO} , initial uncorrected large airway NO flux J'_{awNO} (nl/s) was divided by a correction factor of 0.53 and subtracted from initial uncorrected small airway/alveolar C_{ANO} .²² This could yield negative or near zero values for peripheral lung C_{ANO} .²² This correction factor was determined in normal subjects and those with mild asthma using flow rates of 50–250 ml/s.²² Investigators responsible for measuring $F_{E}NO$ gas

exchange and lung function (CFT, CF and AK) were blinded to the therapeutic intervention. Spirometry and NO gas exchange were measured at the initiation of the study when subjects with asthma were clinically stable at baseline, and subsequently prior to initiation of oral corticosteroid during exacerbation, and then during recovery immediately after treatment with an 8 day course of tapering oral corticosteroid.

Lung CT studies

Our goal was to include only patients with asthma without clinically occult significant lung CT-scored emphysema or bronchiectasis. Therefore, all subjects with asthma had high-resolution, thin-section scans of the lung obtained using a helical 64 slice multidetector-row CT (Model Sensation 64, Siemens, Malvern, Pennsylvania, USA). Images were obtained at 5 mm collimation at intervals of 6 mm using 120 kVp and varying mA dependent upon patient size. Reconstructed 1 mm slices were obtained every 9 mm using a window width of 850 HU and level of -600 HU with an edge-enhancing algorithm. Images were scored by a radiologist (Mark J Schein) as 0 to 100, none to worst emphysema, using picture templates which we previously validated using inflated whole lung specimens.²⁴

Lung function studies

When clinically stable at baseline, and during recovery following treatment with oral corticosteroid, subjects with asthma were instructed to continue all their medications, except to withhold MDI albuterol sulphate and/or ipratropium bromide for 6 h, and the LABA DPI (dry powder inhaler) for 24 h prior to testing. Obviously, during asthma exacerbation, medications were not withheld. Our techniques for measuring spirometry, thoracic gas volumes and airway resistance using plethysmography, and diffusing capacity have been previously published.^{13 14}

Protocol: therapeutic intervention with oral corticosteroid

Once selected for this prospective, single-blind, open label to patient study, patients with asthma were continued on maintenance combination ICS plus inhaled LABA. Concurrent acute relief medications when needed included aerosolised and/or nebulised albuterol sulphate and ipratropium bromide. Definition of asthma exacerbation requiring oral corticosteroids included increasing complaints of cough \pm sputum, chest tightness, wheezing and shortness of breath for ≥ 1 day. These symptoms were not relieved despite at least three treatments with aerosolised and/or nebulised 2.5 mg albuterol sulphate and 0.5 mg ipratropium bromide within 8 h and there was a concomitant decrease in $FEV_1 \geq 0.3$ litres from baseline. In addition, absence of fever and no acute change on a chest x-ray were required. The decision to initiate oral corticosteroids was determined by the emergency ward or other physician who was initially unaware of the study until the patient with asthma informed the physician, and the Principal Investigator was immediately notified. Asthma exacerbation was treated with an 8 day tapering course of oral prednisone starting with 40 mg. Concurrent use of antibiotics was optional and was used in eight of the subjects with asthma. No laboratory study was initiated to definitively determine the aetiology of superimposed acute bronchitis. Measurement of $F_{E}NO$ was not used to guide clinical treatment.

Statistical methods

In our previous studies^{13 14} we noted that $>60\%$ of patients with clinically stable moderate–severe asthma had significantly increased peripheral lung C_{ANO} without correction for NO axial back-diffusion²² and, after re-examination, $>30\%$ after

correction.¹³ In the current study, we needed to evaluate a minimum of 15 patients with moderate–severe asthma at baseline and during exacerbation to achieve 80% power to detect a $\geq 30\%$ incidence of increased peripheral C_{ANO} after correction for NO axial back-diffusion.²² Forty normal age-matched subjects were compared with patients with asthma at baseline using two-group unpaired t tests. Data for those with asthma were initially analysed to determine normality distribution using Shapiro–Wilks test. Because of non-parametric data, analysis of variance (ANOVA) using ranks with repeated measurement was used, with clinical status including stable baseline, exacerbation and recovery as the within factor. Paired t test was used to compare change in clinical status within the same group. S+version for Windows was used for analysis (Tibco Software, Palo Alto, California, USA). Values for $p < 0.05$ were considered significant.

RESULTS

Baseline studies

We prospectively studied 15 (8 males) patients with moderate–severe asthma, age 57 ± 13 years (mean \pm SD) who had been treated with inhaled combination ICS+LABA for 3.6 ± 0.4 years. Results of lung function studies in normal subjects and those with asthma before and 15 min after 180 μ g of albuterol by MDI are presented in table 1. Those with asthma had baseline vital capacity of 3.0 ± 1.0 litres (87 \pm 29% predicted), total lung capacity 5.8 ± 0.9 litres (108 \pm 19% predicted), residual volume 2.8 ± 1.0 litres (143 \pm 47% predicted), functional residual capacity 3.5 ± 0.9 litres (133 \pm 62% predicted), single breath diffusing capacity 23 ± 6 ml/min/mm Hg (98 \pm 27% predicted) and diffusing capacity/alveolar volume 5.0 ± 0.8 ml/min/mm Hg/l (126 \pm 20% predicted). Compared with 40 age-matched normals, values for forced vital capacity (FVC), FEV₁ and FEV₁/FVC were significantly decreased at baseline. High-resolution, thin-section lung CT emphysema scores²⁵ were 9 ± 5 (mean \pm SD), indicating none or trivial emphysema, and no bronchiectasis was noted in the 15 subjects with asthma. At baseline, serum IgE was 215 ± 154 mg/dl and total blood eosinophil count $0.42 \pm 0.36 \times 10^9$ cells/l in the 15 subjects with asthma.

Baseline NO production

Compared with age-matched normal subjects, when clinically stable, baseline values for F_ENO at 50 ml/s, ppb ($p=0.01$), F_ENO

at 100 ml/s, ppb ($p=0.005$), J' _{awNO}, nl/s ($p=0.02$) and C_{ANO}, ppb ($p=0.04$) in the subjects with asthma were significantly increased prior to Condorelli correction.²² The value for central airway NO flux J' _{awNO} after correction for axial back-diffusion of NO remained significantly increased ($p=0.02$). However, after correcting²² for NO axial back-diffusion, values for C_{ANO} were zero or slightly negative (not statistically different from zero) both in normal subjects and all those with asthma ($p=0.2$), indicating no or trivial peripheral airway/alveolar C_{ANO} concentration. Two of the 15 subjects with asthma at baseline had normal values for NO gas exchange despite abnormal spirometry (see table 1 and figures 1–5).

Spirometry and NO production during asthma exacerbation prior to initiation of oral corticosteroid

During asthma exacerbation which occurred on average 101 ± 10 days (mean \pm SD) after baseline, there was a significant decrease in FVC and FEV₁ both before and after 180 μ g of albuterol. During asthma exacerbation, compared with baseline, there was a significant increase in F_ENO production at 50 ml/s ($p=0.003$), at 100 ml/s ($p<0.001$) and in J' _{awNO} ($p=0.004$) both before and after correction ($p=0.004$) for NO axial back-diffusion. There was increased C_{ANO} before ($p=0.001$), but not after ($p=0.8$) correction for NO axial back-diffusion in all those with asthma. These results allowed us to reject the hypothesis that $\geq 30\%$ of those with asthma after correction for NO axial back-diffusion would have increased C_{ANO}. Two of the subjects with asthma who had normal NO gas exchange at baseline had similar normal values during exacerbation. These two subjects had similar spirometry and serum IgE and total eosinophil count to the other 13 subjects with asthma with abnormal NO gas exchange (see table 1 and figures 1–5).

Spirometry and NO production in recovery after an 8 day oral corticosteroid course

After 10 ± 2 days (mean \pm SD) following initiation of an 8 day tapering course of oral prednisone, there was a significant increase in spirometry ($p \leq 0.005$), compared with values obtained during acute exacerbation. There was also a significant decrease in F_ENO production at 50 ml/s ($p<0.001$), at 100 ml/s ($p=0.001$), J' _{awNO} and C_{ANO} compared with values obtained during exacerbation ($p=0.01$). Results for J' _{awNO} ($p=0.01$) were

Table 1 Lung function and nitric oxide (NO) gas exchange in normal subjects and patients with asthma at baseline, during exacerbation and following recovery

	Normal control 57 \pm 9 years n = 40	Stable baseline asthma F250/S50 57 \pm 13 years n = 15	Asthma exacerbation F250/S50 n = 15	Asthma recovery F250/S50 plus oral corticosteroid n = 15
FVC (litres) pre/post 180 μ g of albuterol	3.6 \pm 1.0	\leftrightarrow 3.2 \pm 0.9/3.3 \pm 0.9, $p=0.2$	\leftrightarrow 2.5 \pm 0.9/2.8 \pm 0.9, $p=0.004/p=0.14$	\leftrightarrow 3.2 \pm 0.8/3.2 \pm 0.7, $p=0.005/p=0.19$
FVC % predicted pre/post	97 \pm 13	88 \pm 20/94 \pm 22, $p=0.2$	66 \pm 17/74 \pm 17, $p=0.001/p=0.001$	80 \pm 17/88 \pm 22, $p<0.001/p=0.06$
FEV ₁ (litres) pre/post	2.8 \pm 0.7	2.1 \pm 0.8/2.2 \pm 0.8, $p=0.009$	1.5 \pm 0.7/1.8 \pm 0.7, $p<0.001/p<0.001$	2.1 \pm 0.7/2.2 \pm 0.6, $p<0.001/p=0.1$
FEV ₁ % predicted pre/post	93 \pm 14	71 \pm 23/78 \pm 26, $p=0.004$	49 \pm 20/57 \pm 20, $p<0.001/p=0.02$	68 \pm 21/77 \pm 24, $p<0.001/p=0.02$
FEV ₁ /FVC % pre/post	79 \pm 5	66 \pm 13/68 \pm 14, $p=0.005$	61 \pm 13/64 \pm 13, $p=0.06/p=0.4$	70 \pm 12/70 \pm 12, $p=0.07/p=0.2$
F _E NO at 50 ml/s (ppb)	22 \pm 10	40 \pm 23 $p=0.01$	90 \pm 47 $p=0.003$	44 \pm 19 $p<0.001$
F _E NO at 100 ml/s (ppb)	14 \pm 9	24 \pm 11 $p=0.005$	53 \pm 27 $p<0.001$	27 \pm 9 $p=0.001$
Large airway NO (nl/s)	1.0 \pm 0.6	1.7 \pm 1.1 $p=0.02$	4.2 \pm 2.8 $p=0.004$	2.0 \pm 1.0 $p=0.01$
Large airway NO (nl/s) corrected	1.7 \pm 1.0	3.0 \pm 1.8 $p=0.02$	7.2 \pm 4.8 $p=0.004$	3.4 \pm 1.7 $p=0.01$
Small airway/alveolar NO (ppb)	3.9 \pm 2.4	5.6 \pm 3.0 $p=0.04$	11 \pm 6 $p=0.001$	5.9 \pm 2.2 $p=0.01$
Small airway/alveolar NO (ppb) corrected	1.9 \pm 2.3	2.2 \pm 2.7 $p=0.2$	2.6 \pm 4.3 $p=0.8$	1.9 \pm 2.4 $p=0.4$
Statistical comparison		Baseline asthma vs age-matched normals	Exacerbation vs stable asthma	Recovery vs exacerbation

Lung function and NO results are mean \pm SD. Measurements of NO gas exchange were obtained prior to spirometry. Spirometry obtained in patients with asthma are before and after 180 μ g of albuterol by metered dose inhaler. Corrected indicates the NO gas exchange values in this table have been corrected for axial NO back-diffusion.²² F250/S50, fluticasone propionate 250 μ g/salmeterol 50 μ g; F_ENO, fraction expired nitric oxide; FEV₁, forced expiratory volume in 1 s; FVC, forced expiratory volume; ppb, parts per billion.

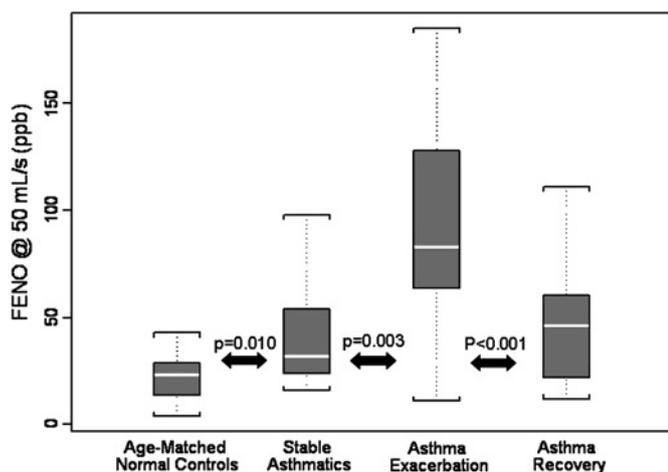


Figure 1 Measurement of total exhaled nitric oxide ($F_{E}NO$) at 50 ml/s in age-matched normal subjects and patients with asthma on fluticasone 250 μ g/salmeterol 50 μ g when stable at baseline, during exacerbation and following recovery. ppb, parts per billion. Each figure notes the median, 1–3 interquartile with 5–95% bars in normal subjects and those with asthma, and asthma outliers are represented by a line.

unchanged after correction for NO axial back-diffusion. However, C_{ANO} was significantly decreased before ($p=0.01$) but not after ($p=0.4$) correction for NO axial back-diffusion. The two subjects with asthma who had normal values for NO gas exchange at baseline and despite exacerbation also had normal values during recovery after oral corticosteroid. Their response to oral corticosteroid was similar to that of those with asthma with elevated NO gas exchange (see table 1 and figures 1–5).

DISCUSSION

Spirometry and exhaled NO at multiple expiratory flow rates were measured to determine the contribution of central and peripheral airway/alveolar NO production sites using the two-compartment model.¹¹ Data were obtained initially at baseline, during asthma exacerbation prior to initiating oral corticosteroid, and subsequently during recovery immediately following an 8 day course of tapering oral corticosteroid. At baseline, despite optimal clinical stability, there was significantly

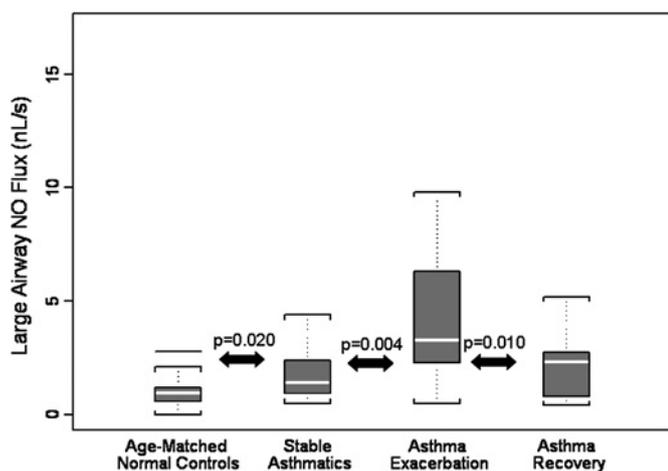


Figure 2 Measurement of large/central airway nitric oxide (NO) flux (nl/s) in age-matched normal subjects and patients with asthma on fluticasone 250 μ g/salmeterol 50 μ g when stable at baseline, during exacerbation and following recovery. NO values are uncorrected for axial back-diffusion.²²

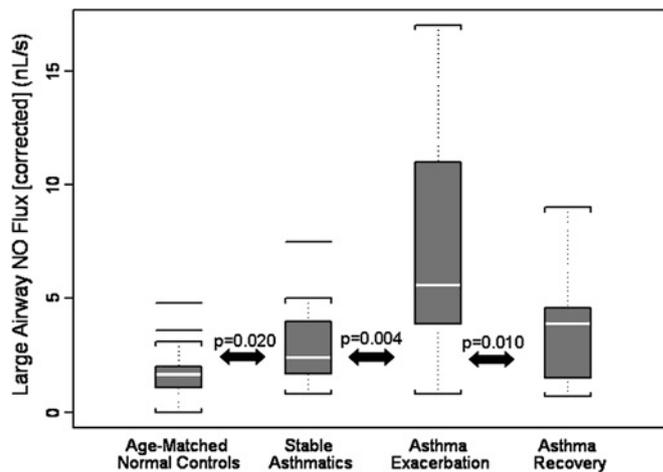


Figure 3 Measurement of large/central airway nitric oxide (NO) flux (nl/s) adjusted for axial NO back-diffusion²² in age-matched normal subjects and patients with asthma on fluticasone 250 μ g/salmeterol 50 μ g when stable at baseline, during exacerbation and following recovery.

abnormal spirometry and both increased central airway NO flux and peripheral airway/alveolar C_{ANO} before correction for NO axial back-diffusion.²² However, after correction, peripheral airway/alveolar C_{ANO} was normal compared with 40 age-matched healthy, non-smoking controls with normal lung function. During asthma exacerbation, with greater expiratory airflow limitation, central airway NO flux, (J'_{awNO}) was increased before and after correction for NO axial back-diffusion.^{12–22} However, peripheral airway/alveolar C_{ANO} was significantly increased before but not after correction for NO axial back-diffusion.^{12–22} The results suggest that central airways are the predominant site of increased NO gas exchange in 13 of 15 patients with asthma with moderate–severe expiratory airflow limitation studied at baseline and following exacerbation. Two of the 15 studied patients with asthma had normal values for NO gas exchange at baseline, during exacerbation and following recovery after oral corticosteroid. These two patients may have a non-eosinophilic-mediated inflammatory pathway. However, their clinical and physiological response to 8 day tapering oral corticosteroid treatment was similar to that of the

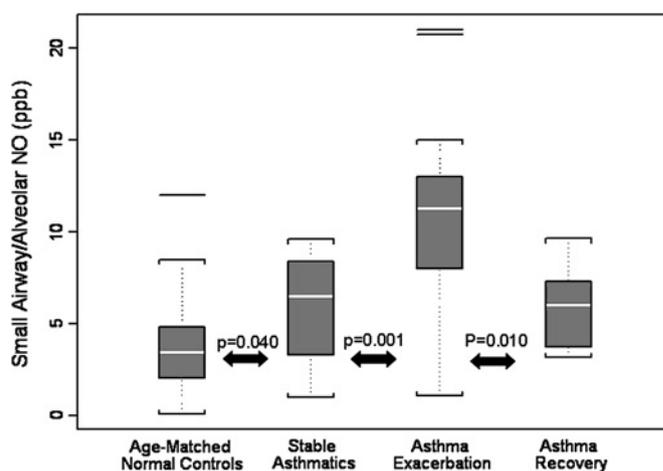


Figure 4 Measurement of small and peripheral airway/alveoli C_{ANO} (ppb) in age-matched normals subjects and patients with asthma on fluticasone 250 μ g/salmeterol 50 μ g when stable at baseline, during exacerbation and following recovery. Nitric oxide (NO) values are uncorrected for axial back-diffusion.²²

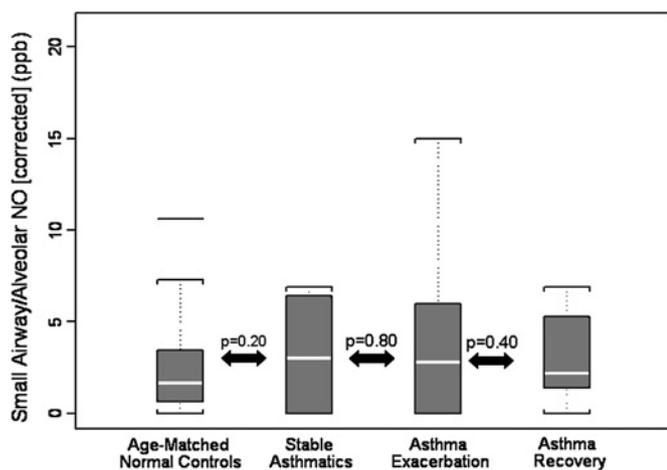


Figure 5 Measurement of small and peripheral airway/alveoli C_{ANO} (ppb) adjusted for nitric oxide (NO) axial back-diffusion²² in age-matched normal subjects and patients with asthma on fluticasone 250 μ g/salmeterol 50 μ g when stable at baseline, during exacerbation and following recovery.

other 13 subjects with asthma, with increased central airway J'_{awNO} flux. Furthermore, measurement of $F_{E}NO$ at 50 ml/s, which reflects predominantly large airway NO flux, may be both more relevant and practical, especially when peripheral airway/alveolar NO is normal.

Peripheral airway/alveolar NO production and inhaled corticosteroid

It has been reported that increased central airway NO flux and peripheral lung C_{ANO} in asthma reflects predominantly eosinophilic-mediated inflammatory pathways, presumably stimulated by upregulated or overexpressed inducible nitric oxide synthase (iNOS), or less probably constitutive nitric oxide synthase (cNOS).^{1–6} The present study contradicts in part our previous observations^{13–14} of increased peripheral lung C_{ANO} in 60% of clinically stable patients with moderate–severe asthma that did not take into account axial NO back-diffusion.²² Upon re-examination of our original data,^{13–14} and after correcting for axial NO back-diffusion,²² peripheral C_{ANO} still remained increased in 30% of those with asthma.¹⁵ The discrepancy with the present results may in part be related to different cohorts of patients with asthma and the therapeutic regimen. Previous studies uncorrected for axial NO back-diffusion^{12–22} noted increased central airway NO flux and increased peripheral airway/alveolar C_{ANO} in those with clinically stable asthma with mild^{15–21} and moderate–severe obstruction^{13–14–16–17} despite ICS. Review of published data from van Veen *et al*¹⁶ suggests normal peripheral C_{ANO} production after we corrected for NO axial back-diffusion.²² Alternatively, previously published results, also without correction for axial NO back-diffusion,^{12–22} by Hogman *et al*,⁸ Brindicci *et al*²⁰ and Shin *et al*,²¹ reported increased central NO flux but normal peripheral C_{ANO} in clinically stable patients with asthma with mild²¹ and moderate–severe^{8–20} expiratory airflow limitation despite ICS. Kerckx and Van Muylem¹² noted normal peripheral airway/alveolar NO concentration in those with mild asthma after correction for NO axial back-diffusion. However, Brindicci *et al*²⁰ reported increased C_{ANO} during exacerbation in those with moderate–severe asthma. However, re-examination of their published data²⁰ suggests normal C_{ANO} after correction for NO back-diffusion. The reduction in C_{ANO} following oral corticosteroid in the present study is similar to our previous experi-

ence¹³ and that of Berry *et al*¹⁷ in those with clinically stable asthma prior to correction for NO axial back-diffusion. We favour the new hypothesis that <30% of those with moderate–severe asthma at baseline and after exacerbation have increased C_{ANO} ($p < 0.001$) with a 95% upper confidence limit for incidence of increased C_{ANO} of 22%. We would have to study 15 additional subjects with asthma to achieve a 95% upper confidence limit for an incidence of increased $C_{ANO} < 10%$ ($p < 0.001$).

Potential mechanism(s) for peripheral NO before and after correction for axial NO diffusion

Previously, Silkoff *et al*⁵ reported that methacholine-induced bronchoconstriction in subjects with asthma, with reduction in epithelial surface area, decreased NO production by ~15%. The partial pressure of NO in the small airway and alveolar area (P_{LNO}) is equal to the production of NO by surrounding tissue (V_{NO})/diffusing capacity of NO from air space into surrounding blood vessels (D_{LNO}).²⁶ Therefore, either an increase in NO tissue production as an inflammatory response (increased iNOS and/or cNOS) or reduced D_{LNO} , or both could account for increased P_{LNO} .²⁶ Lehtimäki *et al*²⁵ have previously shown an inverse relationship between C_{ANO} and pulmonary capillary diffusing capacity in alveolitis. In asthma, we^{13–14} have previously noted that the increase in C_{ANO} was attributed to an increase in NO tissue production, probably related to increased iNOS, and suspect that this is also operant during asthma exacerbation since reduction in D_{LNO} in asthma is unlikely. Furthermore, it would have a relevant effect on NO axial back-diffusion. The increase if any in peripheral lung C_{ANO} in clinically stable patients with asthma is modestly downregulated by inhaled compared with systemic corticosteroids.^{13–14–17}

The original two-compartment model developed by Tsoukias and George *et al*¹¹ to describe central and peripheral airway/alveolar site of NO concentration failed to account for axial back-diffusion of NO from the central airways towards the alveoli. This error may have led to underestimation of central airway NO flux and overestimation of peripheral/alveolar C_{ANO} . Subsequent contributions from Condorelli *et al*²² for NO axial back-diffusion estimated that large airway NO flux is 1.7-fold greater if the flow range is between 50 and 250 ml/s, with trivial NO levels in peripheral lung units in normal subjects (approaching zero). The negligible level of C_{ANO} in normal subjects is probably due to the great affinity between periacinar NO and the haemoglobin molecule in the surrounding blood pool.^{7–11–12–22}

Important experimental observations by Kerckx and Van Muylem²⁷ have also demonstrated the inhomogeneous distribution of NO production in healthy adults, with the majority of exhaled NO coming from very proximal airways and ‘larger’ peripheral airways of <17 generation, with little contribution from the lung periphery after correcting for axial NO back-diffusion. The response following histamine-induced bronchoconstriction in normal subjects has been studied by Verbanck *et al*²⁸ who noted its potential effect to increase convective flow of NO and $F_{E}NO$ at 50 ml/s by a paradoxical decrease in axial back-diffusion of NO. Additionally, Kerckx *et al*¹² reported the inhomogeneous airway contribution of NO production in those with stable asthma without expiratory airflow obstruction and noted a minimal peripheral contribution of NO production, similar to normal subjects, after correcting for axial NO back-diffusion. These observations are similar to those noted by Condorelli *et al*²² and reinforce the present observations in those with asthma with abnormal expiratory airflow limitation. It is possible that during an acute asthma exacerbation, the correction factor for axial NO back-diffusion would be smaller, due to

a smaller cross-sectional area available for back-diffusion of NO through constricted airways. This would result in a potential underestimation of C_{ANO} and overestimation of J'_{awNO} using the current technique and interpretation. There are currently no experimental data available on back-diffusion of NO during acute asthma.

Large airway NO flux and ICS, and potential mechanism(s)

Central airway NO flux is the product of NO production in bronchial wall mucosa (C_w) and bronchial NO diffusing capacity (D_{awNO}) which reflects transfer of NO from the airway wall to the lumen of expired air and is proportional to the lumen surface area and transfer coefficient.^{10–26} While we did not measure C_w and D_{awNO} , this could be estimated as noted by Silkoff *et al*¹⁰ using low exhaled flow rates (5–10 ml/s) and application of a non-linear method. We suspect that in those with both clinically stable and unstable asthma moderate dose ICS (F250/S50) is probably unable significantly to suppress increased bronchoreactive iNOS and/or resident cNOS in the larger airways to reduce bronchial wall (C_w) NO production effectively. Review of the individual data in the present study indicates that 2 of the 15 studied subjects with asthma had normal baseline values for large and small airway/alveolar C_{ANO} and without subsequent increase during exacerbation. Despite lack of induced sputum and/or airway cellular analyses, this implies a non-eosinophilic inflammatory-mediated pathway.^{29–30} In summary, in the present asthma cohort, and after correction for NO axial back-diffusion, the central airways remain the predominant site of increased NO gas exchange in the subjects with asthma studied at baseline, following exacerbation and subsequent recovery.

Clinical implications

Several investigators^{2–5, 31–36} have addressed the clinical role of add-on monitoring of exhaled NO at a single expiratory airflow rate, usually at 50 ml/s, to help guide inhaled and/or systemic corticosteroid dosing in those with clinically stable and unstable asthma, in addition to guideline recommendations which include spirometry.³¹ Results of five double-blind randomised control trials^{32–36} comparing add-on F_{ENO} versus usual guidelines including spirometry³¹ were only equivocal. This suggests that routine serial measurements of F_{ENO} for clinical asthma management does not appear necessary. However, multiple issues related to study design question the validity of the conclusions.³⁷ The present study is an extension of our original investigation^{13–14} to define and partition central and peripheral airway/alveolar sites of NO concentration in those with stable asthma at baseline and during exacerbation, initially without^{13–14} and now with correction for NO axial back-diffusion.^{12–22} This requires measuring NO gas exchange at four separate expiratory flow rates. However, the current results suggest predominantly central airway sites of abnormal NO gas exchange, presumably reflective of eosinophilic-mediated inflammation, in 13 of the 15 studied subjects with asthma. While measuring F_{ENO} at a single expiratory flow rate at 50 ml/s does not provide any information about the contribution of C_{ANO} in peripheral sites, it appears that NO overproduction in asthma can be localised to the central airways. Furthermore, it should be emphasised that increased F_{ENO} at 50 ml/s, a surrogate of central airway increased NO flux, correlates only modestly with induced sputa eosinophilia >3%: $r^2=0.26$, $p<0.001$ with a sensitivity of 71% and specificity of 72%,³⁷ and $r^2=0.29$, $p=0.007$ with tissue eosinophilia in treated subjects with severe refractory asthma.⁵ The current asthma results are in contrast to our observations in chronic obstructive pulmonary disease where both large airway and

peripheral NO are normal, yet large airway NO flux was suppressed with moderate but not low dose ICS.³⁸

SUMMARY

We used the two-compartment NO model¹¹ with correction²² for axial back-diffusion of NO to avoid spurious underestimation of large airway NO flux and overestimation of peripheral C_{ANO} . In 13 of 15 the studied clinically stable adult subjects, there was increased NO flux in predominantly central airway sites in those with moderate–severe asthma at baseline with abnormal spirometry despite inhaled corticosteroid. NO production in central airway sites was further increased in the same 13 patients during asthma exacerbation prior to oral corticosteroid and decreased following an 8 day course of tapering oral corticosteroid. This suggests ongoing eosinophilic-mediated inflammation in central airways in these 13 subjects with asthma. Following correction for axial back-diffusion of NO, peripheral airway/alveolar NO values were similar to those of normal subjects. These results are in contrast to our previous observations in other patients with asthma^{13–14} even after correction for axial NO back-diffusion,²² and suggest that peripheral NO concentration in those with asthma may be normal. Increased measurement of F_{ENO} at 50 ml/s may be a practical surrogate for large airway increased NO flux, especially when peripheral lung C_{ANO} is normal as in the present asthma exacerbation study.

Competing interests PS receives royalties from patents licensed to General Electric Instruments and Aperion, both manufacturers of exhaled NO equipment (range US \$2000–US\$3000/annum). Consultant to GE Instruments, Aperion and Aperion (range US\$10 000–\$15 000/annum).

Ethics approval This study was conducted with the approval of the Lakewood Regional Medical Center, Lakewood, California, USA; Western IRB, Olympia, Washington, USA and Registered National Clinical Trials: 00576069.

Contributors AFG designed, developed and supervised this study and was responsible for all patient care and this manuscript. AK, CMS and TM were responsible for data analysis. CFT, AK and CF were responsible for all laboratory studies. SCG and PES provided invaluable input towards data interpretation and manuscript preparation. Fernando Camacho of Damos, Toronto, Ontario, Canada provided invaluable statistical help.

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REFERENCES

1. Kharitonov SA, Gonio F, Kelly C, *et al*. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. *Eur Respir J* 2003;**21**:433–8.
2. Green RH, Brightling CE, McKenna S, *et al*. Asthma exacerbations and sputum eosinophil counts: a randomized controlled trial. *Lancet* 2002;**360**:1715–21.
3. Taylor DR, Pijnenburg MW, Smith AD, *et al*. Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax* 2006;**61**:817–27.
4. Ricciardolo FL, Sterk PJ, Gaston B, *et al*. Nitric oxide in health and disease of the respiratory system. *Physiol Rev* 2004;**84**:731–65.
5. Silkoff PE, Lent AM, Busacker AA, *et al*. Exhaled nitric oxide identifies persistent eosinophilic phenotype in severe refractory asthma. *J Allergy Clin Immunol* 2005;**116**:1249–55.
6. American Thoracic Society. Recommendations for standardized procedure for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children. *Am J Respir Crit Care Med* 2005;**60**:2104–17.
7. George SC, Hogman M, Permutt S, *et al*. Modeling pulmonary nitric oxide exchange. *J Appl Physiol* 2004;**96**:831–9.
8. Hogman M, Holmkvist T, Wegener T, *et al*. Extended nitric oxide analysis applied to patients with COPD, allergic asthma, and allergic rhinitis. *Respir Med* 2002;**96**:24–30.
9. Pietropaoli AP, Perillo IB, Torres A, *et al*. Simultaneous measurement of nitric oxide production by conducting and alveolar airways of humans. *J Appl Physiol* 1999;**87**:1532–42.
10. Silkoff PE, Sylvester JT, Zamel N, *et al*. Airway nitric oxide diffusion in asthma. Role in pulmonary function and bronchial responsiveness. *Am J Respir Crit Care Med* 2000;**161**:1218–28.
11. Tsoukias NM, George SC. A two-compartment model of pulmonary nitric oxide exchange dynamics. *J Appl Physiol* 1998;**85**:653–66.
12. Kerckx Y, Michilis A, Van Muylem A. Airway contribution to alveolar nitric oxide in healthy subjects and stable asthma patients. *J Appl Physiol* 2008;**104**:918–24.

13. **Gelb AF**, Flynn-Taylor C, Nussbaum E, *et al.* Airway and alveolar nitric oxide inflammation in treated asthma. *Am J Respir Crit Care Med* 2004;**170**:737–41.
14. **Gelb AF**, Flynn Taylor C, Shinar CM, *et al.* Role of spirometry and exhaled nitric oxide to predict exacerbations in treated asthmatics. *Chest* 2006;**129**:1492–9.
15. **Lehtimäki L**, Kankaanranta H, Saarelainen S, *et al.* Inhaled fluticasone decreases bronchial but not alveolar nitric oxide output in asthma. *Eur Respir J* 2001;**18**:635–9.
16. **vanVeen IH**, Sterk PJ, Schot R, *et al.* Alveolar nitric oxide versus measures of peripheral airway dysfunction in severe asthma. *Eur Respir J* 2006;**27**:951–6.
17. **Berry M**, Hargadon B, Morgan A, *et al.* Alveolar nitric oxide in adults with asthma: evidence of distal lung inflammation in refractory asthma. *Eur Respir J* 2005;**25**:986–91.
18. **Mahut B**, Delacourt C, Zerah- Lancner F, *et al.* Increase in alveolar nitric oxide in the presence of symptoms in childhood asthma. *Chest* 2004;**125**:1012–18.
19. **Paraskakis E**, Brindicci C, Fleming L, *et al.* Measurement of bronchial and alveolar nitric oxide production in normal children and children with asthma. *Am J Respir Crit Care Med* 2006;**174**:260–67.
20. **Brindicci C**, Ito K, Barnes PJ, Kharitonov SA. Differential flow analysis of exhaled nitric oxide in patients with asthma of differing severity. *Chest* 2007;**131**:1353–62.
21. **Shin HW**, Rose-Gottron CM, Cooper DM, *et al.* Airway diffusing capacity of nitric oxide and steroid therapy in asthma. *J Appl Physiol* 2004;**96**:65–75.
22. **Condorelli P**, Shin HW, Aledia AS, *et al.* A simple technique to characterize proximal and peripheral nitric oxide exchange using constant flow exhalations and axial diffusion model. *J Appl Physiol* 2007;**102**:417–25.
23. **Silkoff PE**, McClean PA, Slutsky AS, *et al.* Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. *Am J Respir Crit Care Med* 1997;**155**:260–7.
24. **Gelb AF**, Hogg JC, Muller NL, *et al.* Contribution of emphysema and small airways in COPD. *Chest* 1996;**109**:353–9.
25. **Lehtimäki L**, Kankaanranta H, Saarelainen S, *et al.* Extended exhaled NO measurement differentiates between alveolar and bronchial inflammation. *Am J Respir Crit Care Med* 2001;**163**:1557–61.
26. **Hyde RW**, Geigel EJ, Olszowska AJ, *et al.* Determination of production of nitric oxide by lower airways of humans—theory. *J Appl Physiol* 1997;**82**:1290–6.
27. **Kerckx Y**, Van Muylem A. Axial distribution heterogeneity of nitric oxide airway production in healthy adults. *J Appl Physiol* 2009;**106**:1832–9.
28. **Verbanck S**, Kerckx Y, Schuermans D, *et al.* Effect of airways constriction on exhaled nitric oxide. *J Appl Physiol* 2008;**104**:909–11.
29. **Wenzel SE**, Schwartz LB, Langmack EL, *et al.* Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999;**160**:1001–8.
30. **Haldar P**, Pavord ID. Noneosinophilic asthma: a distinct clinical and pathologic phenotype. *J Allergy Clin Immunol* 2007;**119**:1043–52.
31. **Global strategy for asthma management and prevention.** <http://www.ginasthma.org>.
32. **Szeffler SJ**, Mitchell H, Sorkness CA, *et al.* Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults: a randomized controlled trial. *Lancet* 2008;**372**:1065–72.
33. **de Jongste JC**, Carraro S, Hop WC, *et al.* Daily telemonitoring of exhaled nitric oxide and symptoms in the treatment of childhood asthma. *Am J Respir Crit Care Med* 2008;**179**:93–7.
34. **Shaw DE**, Berry MA, Thomas M, *et al.* The use of exhaled nitric oxide to guide asthma management: a randomized controlled trial. *Am J Respir Crit Care Med* 2007;**176**:231–7.
35. **Pijnenburg MW**, Bakker EM, Hop WC, *et al.* Titrating steroids on exhaled nitric oxide in children with asthma: a randomized controlled trial. *Am J Respir Crit Care Med* 2005;**172**:831–6.
36. **Smith AD**, Cowan JO, Brassett KP, *et al.* Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. *N Engl J Med* 2005;**352**:2163–73.
37. **Berry MA**, Shaw DE, Green RH, *et al.* The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. *Clin Exp Allergy* 2005;**35**:1175–9.
38. **Gelb AF**, Taylor CF, Krishnan A, *et al.* Central and peripheral airway sites of nitric oxide gas exchange in COPD. *Chest* 2010;**137**:575–84.

Lung alert

Non-invasive ventilation (NIV) immediately after extubation reduces ventilatory failure and 90-day mortality in patients with hypercapnia

This randomised open controlled trial took place from 2005–7 in three Spanish intensive care units. The aim was to assess whether non-invasive ventilation (NIV) would prevent respiratory failure and hence re-intubation, an independent risk factor for mortality, nosocomial pneumonia and length of hospital stay in patients who developed hypercapnia (arterial carbon dioxide tension >45 mm Hg) during a spontaneous breathing trial prior to extubation. The secondary end point was 90-day mortality.

One hundred and sixty-four consecutive patients were registered and 106 underwent randomisation. Fifty-four were assigned NIV (mean pressure 17/4 cm H₂O) immediately after extubation for a maximum of 24 h while the control group was given oxygen alone. Both groups were monitored for ventilatory failure and, if found, were either managed with re-intubation or rescue NIV according to predefined criteria.

Statistically significant differences in the development of respiratory failure were found in the NIV group compared with the controls (15% vs 48%), the impact occurring within the first 24 h. There was, however, no difference in the rate of re-intubation or in the length of stay in hospital or in the intensive care unit (ICU). This may have been a result of rescue NIV being deployed in both groups; 20/25 controls in respiratory failure met the criteria for rescue NIV, leading to 75% avoiding re-intubation, a better outcome than in previous reports.

Although overall mortality in the ICU and hospital did not differ, 90-day mortality was significantly reduced in the NIV group, suggesting longer term benefits of NIV which cannot be explained by re-intubation avoidance alone and warrants further assessment.

► **Ferrer M**, Sellares J, Valencia M, *et al.* Non-invasive ventilation after extubation in hypercapnic patients with chronic respiratory disorders: randomised controlled trial. *Lancet* 2009;**374**:1082–8.

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