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# AIRWAY DIFFUSING CAPACITY OF NITRIC OXIDE AND STEROID THERAPY IN ASTHMA

Hye-Won Shin[1,2], Christine M. Rose-Gottron[4], Dan M. Cooper[3,4], Robert L. Newcomb[4,5], and Steven C. George[1,2]

*Department of Biomedical Engineering [1], Department of Chemical Engineering and Materials Science [2], Department of Pediatrics[3], General Clinical Research Center[4], Center for Statistical Consulting[5], University of California, Irvine, Irvine, CA 92697-2575*

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*Corresponding Author and Requests for Reprints:

Steven C. George, M.D., Ph.D.
Department of Chemical Engineering and Materials Science,
916 Engineering Tower
University of California, Irvine
Irvine, California 92697-2575

Telephone: (949) 824-3941
Fax: (949) 824-2541
e-mail: scgeorge@uci.edu

**ABSTRACT**

Exhaled nitric oxide (NO) concentration is a non-invasive index for monitoring lung inflammation in diseases such as asthma. The plateau concentration at constant flow is highly dependent on the exhalation flow rate and the use of corticosteroids, and cannot distinguish airway and alveolar sources. In subjects with steroid-naïve asthma (n=8) , steroid-treated asthma (n=12), and healthy controls (n=24), we measured flow-independent NO exchange parameters, that partition exhaled NO into airway and alveolar regions, and correlated these with symptoms and lung function. The mean (SD) maximum airway flux ($pl \cdot s^{-1}$) and airway tissue concentration (ppb) of NO were lower in steroid-treated asthmatics compared with steroid-naïve asthmatics (1195 (836) and 143 (66) compared to 2693 (1687) and 438 (312), respectively). In contrast, the airway diffusing capacity for NO ($pl \cdot s^{-1} \cdot ppb^{-1}$) was elevated in both asthmatic groups compared to healthy controls independent of steroid therapy (11.8 (11.7), 8.71 (5.74) and 3.13 (1.57) for steroid-treated and steroid-naïve, and healthy controls, respectively). In addition, the airway diffusing capacity was inversely correlated with both $FEV_1$ and FVC (% predicted), while the airway tissue concentration was positively correlated with FVC. Consistent with previously reported results from Silkoff et. al. (*Am. J. Resp. Crit. Med.*, 161:1218, 2000) using an alternate technique, we conclude that the airway diffusing capacity for NO is elevated in asthma independent of steroid therapy, and may reflect clinically relevant changes in airways.

**Keywords:** NO, model, airways, alveoli, inflammation

## INTRODUCTION

Nitric oxide (NO) was first detected in the exhaled breath of humans more than a decade ago (19) and remains a promising non-invasive index of lung pathophysiology. Substantial evidence suggests that both the airway and alveolar regions are significant sources of exhaled NO ($F_{E_{NO}}$) (8, 20, 37, 42, 44-46, 48, 52, 53). Thus, in contrast to a respiratory gas like $CO_2$ that is evolved predominantly in the alveolar compartment and whose presence in the exhaled breath primarily reflects alveolar gas exchange, $F_{E_{NO}}$ measurements might lead to specific insights about pathophysiology throughout the respiratory tract. Guidelines for characterizing $F_{E_{NO}}$ by the American Thoracic Society (ATS) and the European Respiratory Society (ERS) include only the plateau concentration in phase III, $C_{NOplat}$, at a constant exhalation flow rate (3, 29). However, a single measurement of $C_{NOplat}$ cannot distinguish airway and alveolar contributions, and thus may not be the optimal parameter to describe pulmonary NO exchange.

The potential for greater clinical insight is accompanied by the need for new and robust analytical approaches to characterize NO in the exhaled breath. Because NO is produced throughout the respiratory tract, factors like expiratory flow rate substantially influence the NO concentration in the exhaled breath (21, 47, 54). To account for this and other determinants of NO concentration, we and others have described NO exchange using a biologically-relevant two-compartment model (airway and alveolar compartments) and a series of flow-independent NO exchange parameters (20, 42, 48, 52). The flow-independent parameters potentially provide clinically relevant

information about NO exchange. For example, the alveolar NO concentration is elevated in allergic alveolitis (alveolar inflammation), while airway wall NO flux is elevated in asthma (bronchial inflammation) (37).

Inflammation is characteristic of asthma, and induces the expression of several steroid-sensitive enzymes such as nitric oxide synthase and glutaminase which impact nitric oxide metabolism (2, 23, 43). Consequently, corticosteroids, which attenuate the inflammatory process, also reduce the concentration of nitric oxide in the exhaled breath (31, 41). This feature of corticosteroid therapy may be useful in monitoring the inflammatory status of the airways, but, by reducing the concentration of NO in the exhaled breath to near normal, may mask steroid-independent alterations in airway NO physiology that are of potential clinical significance.

The airway diffusing capacity of NO ($D_{awNO}$) is the conductance for the transfer of NO between the airway wall and the gas stream (48, 52, 53). It depends on both the physical features of the airway wall (e.g., airway surface area or tissue thickness) and the rate of chemical consumption (4, 53), both of which may be altered in asthma. Recently, Silkoff et. al. (48) demonstrated that $D_{awNO}$ was elevated in asthma independent of steroid therapy by measuring multiple $C_{NOplat}$ at small flow rates (< 50 ml/s). However, values for the flow-independent NO exchange parameters may depend on the breathing maneuver and analytical technique utilized. Thus, the goal of the present study was to apply our alternate breathing and analytical technique (20 second pre-expiratory breathhold followed by a decreasing flow-rate maneuver) in asthma to confirm the results of Silkoff et. al. (48) and potentially provide additional insight into the pathophysiology that marks chronic asthma.

## METHODS

*Subjects.* 24 healthy adults and 20 subjects with a clinical history of asthma (8 steroid-naïve and 12 steroid-treated) participated in this study. Inclusion criteria for the healthy subjects was an $FEV_1/FVC > 0.80$; exclusion criteria was a history of smoking at any time, heart disease or lung disease. Inclusion criteria for the asthma group were a clinical history of reversible bronchoconstriction, and a current $FEV_1/FVC < 0.75$ regardless of the use of corticosteroids; exclusion criteria were a history of smoking at any time, heart disease, and lung disease other than asthma. We then subdivided the adults with a clinical history of asthma into 2 groups: 1) steroid-naïve and 2) steroid-treated. In addition, each of the adult subjects with asthma also completed a previously validated asthma control questionnaire (see Appendix A) to assess clinical symptoms of asthma over the past seven days (27, 28). Subject characteristics are presented in Table 1 including details of their clinical history. The Institutional Review Board at the University of California, Irvine approved the protocol, and written informed consent was obtained from all subjects.

*Experimental Protocol*. Each subject performed two types of exhalation maneuvers -- one necessary to estimate the flow-independent NO exchange parameters and the other according to the ATS guidelines (3). The first maneuver was five repetitions of a 20-second pre-expiratory breathhold followed by a decreasing flow rate (from ~6% to ~1% of vital capacity per second) maneuver (53) to estimate several flow-independent NO exchange parameters. A positive pressure of > 5 cm $H_2O$ was maintained to

prevent nasal contamination during the breathhold (3), and a Starling resistor (Hans Rudolph, Kansas City, MO) with a variable resistance was used to progressively decrease the flow rate during the exhalation. Following breathhold, the exhalation valve was opened allowing the patient to expire. A schematic of the experimental apparatus has been previously presented (53). The second maneuver was a vital capacity maneuver performed in triplicate to collect plateau NO concentration based on the ATS guidelines (3). We also included an exhalation flow rate of 250 milliliters/second (ATS guideline is 50 milliliters/second) consistent with the guidelines of the ERS (29). After measuring the indices of NO exchange dynamics, general spirometry such as forced vital capacity (FVC), and forced expiratory volume in 1 second normalized by forced vital capacity ($FEV_1$/FVC), were measured in all subjects (Vmax229; Sensormedics, Yorba Linda, CA) by using the best performance (see Table 1) from three consecutive maneuvers.

*Airstream Analysis*. A chemiluminesence NO analyzer (NOA280, Sievers, Inc., Boulder, CO) was used to measure the exhaled NO concentration. The instrument was calibrated on a daily basis using a certified NO gas (45 ppm in $N_2$, Sievers, Inc., Boulder, CO). The zero point calibration was performed with a NO filter (Sievers, Inc., Boulder, CO) immediately prior to the collection of a profile. The flow rate and pressure signals were measured using a pneumotachometer (RSS100, Hans Rudolph Inc., Kansas City, MO). The pneumotachometer was calibrated daily and was set to provide the flow in units of STPD.

*Data Analysis and Parameter Estimation.* Experimental single exhalation profiles with the 20-second pre-expiratory breathhold were characterized by the peak concentration in phase I and II, $C_{NOpeak}$, the peak width, $W_{50}$, in phase I and II defined as the exhaled volume in which the NO concentration was greater than 50% of $C_{NOpeak}$, and the total volume of phase I and II, $V_{I,II}$, defined as the inflection point (zero slope or $dC_{exh}/dV = 0$) in the exhalation profile (53) (Fig. 1). The constant flow rate single exhalations were characterized by the plateau concentration in phase III, $C_{NOplat}$, as previously described by the ATS and the ERS (3, 29).

A previously described two-compartment model was used to estimate four flow-independent NO exchange parameters: 1) maximum flux of NO from the airways, $J'_{awNO}$, $pl \cdot s^{-1}$; 2) diffusing capacity of NO in the airways, $D_{awNO}$, $pl \cdot s^{-1} \cdot ppb^{-1}$; 3) steady state alveolar concentration, $C_{alv,ss}$, ppb; and 4) mean airway tissue NO concentration, $C_{awNO}$, ppb, (equal to the ratio of $J'_{awNO}/D_{awNO}$). A simple schematic of the two-compartment model and flow-independent parameters are presented in Fig. 2, and a detailed description of the mathematical estimation of the parameters has been previously described (53).

The source of NO from the airways can be described by the instantaneous flux of NO from the airways, $J_{awNO}$ (pl/s). $J_{awNO}$ depends on the flow-independent parameters, and is expressed as a linear function of the airway gas phase concentration, $C_{air}$, by the following:

$$J_{awNO} = J'_{awNO} - D_{awNO} C_{air} \quad (1)$$

or

$$J_{awNO} = D_{awNO}\left(C_{awNO} - C_{air}\right) \quad (2)$$

$J'_{awNO}$ is the maximum flux of NO from the airway tissue equal to the product $D_{awNO}$*$C_{awNO}$ (Eq. 2). Conceptually, $J'_{awNO}$ approaches $J_{awNO}$ as the product $D_{awNO}$*$C_{air}$ approaches zero. $D_{awNO}$ is the conductance for mass transfer (transfer factor or airway diffusing capacity) of NO between the airway tissue and the gas phase. The alveolar region is characterized by the steady state alveolar gas concentration, $C_{alv,ss}$, which is equivalent to the alveolar tissue concentration (25, 52). Fig. 3 illustrates the independent (i.e., all other parameters are held constant) impact of $D_{awNO}$, $J'_{awNO}$, and $C_{alv,ss}$ on the single exhalation profile with a 20-second pre-expiratory breathhold and a decreasing exhalation flow rate.

Once the flow-independent parameters are known, the two-compartment model can be used to predict $C_{NOplat}$ at any constant exhalation flow, and thus there is no loss of information in characterizing NO exchange with the flow-independent NO parameters (53):

$$C^*_{NOplat} = C_{awNO} + \left(C_{alv,ss} - C_{awNO}\right) \bullet \exp\left(-D_{awNO} / \dot{V}_E\right) \quad (3)$$

$\dot{V}_E$ is the constant exhalation flow rate, and $C^*_{NOplat}$ is the plateau concentration of NO predicted by the model using the flow-independent parameters. We have previously demonstrated that $C^*_{NOplat}$ is not different than the experimentally measured $C_{NOplat}$ in healthy adults (44, 53), with the advantage that inter-subject and inter-population

variations in flow rate can be accounted for by calculating $C_{NOplat}$ at a precise desired flow rate (e.g., 50 ml/s).

*Statistics.* To detect differences among the three groups of subjects, data were analyzed using One-way Analysis of Variance (ANOVA) and post-hoc paired comparisons of treatment means. In those instances where Levene's test rejected homogeneity of variance, tests for group differences relied upon Welch's ANOVA or Satterthwaite's method to adjust the test to account for this problem. To detect significant relationships between the parameters which characterize nitric oxide exchange and either asthma symptoms or standard indices of lung function (e.g., FEV1), we utilized first and second order partial correlation coefficients, respectively. For example, to determine the relationship between NO parameters and lung function for all subjects, the second order partial correlation coefficient factors out the effect of having asthma or being treated with steroids by subtracting the group mean from each individual score. As to the question of Normality, in addition to screening variables for excessive skewness, all tests of group differences were rerun using a log transformation of the dependent variables. Because the log transformation of each variable did not impact the results, all statistical tests were reported using the untransformed data. Finally, a p-value $< 0.05$ was considered statistically significant, and all results were produced using the GLM procedure of SAS.

## RESULTS

FVC, $FEV_1$, $FEV_1$/FVC, and the clinical history of the subjects with asthma are presented in Table 1. $FEV_1$/FVC was more reproducible that $FEV_1$ alone. The mean maximum variability (defined as the difference between the maximum and minimum value normalized by the mean of the three repeated maneuvers) for $FEV_1$/FVC was 5.8% (range 1.5-10.2) and 2.9% (range 0-10.2) for steroid-naïve and steroid-treated asthma subjects, respectively. For $FEV_1$ alone, the mean maximum variability was slightly higher for each group -- 8.9% (range 0.7-20.6) and 5.2% (range 1.5-17.9) for steroid-naïve and steroid-treated asthma subjects, respectively. $FEV_1$/FVC was significantly lower in both groups of subjects with asthma compared to healthy adults. However, there was no difference in $FEV_1$/FVC or clinical symptoms (as assessed by the composite score on the asthma control questionnaire) among the two groups of subjects with asthma.

Of the 20 subjects with asthma, three of the steroid-treated (subject #2, #10 and #12) were not able to complete the 20-second breathhold, and thus we utilized a 10-second breathhold which may increase the confidence interval of $D_{awNO}$ (44, 53). To highlight differences amongst groups in exhaled concentrations, a composite exhalation profile for each group was attained (Fig. 4A and 4B) by taking the mean exhaled concentration at equivalent exhaled volume intervals for each of the three groups. The three asthmatic subjects who were not able to complete the 20-second breathhold were excluded from the composite exhalation profile. Steroid-naïve subjects with asthma had an increased concentration of NO in all phases of the

exhalation profile when compared to both steroid-treated subjects with asthma and healthy controls. Although the NO exhalation profile for steroid-treated subjects with asthma and healthy controls is similar (Fig. 4B), there are important differences that reflect alterations in the flow-independent NO parameters. Steroid-treated subjects with asthma have elevated NO in phase III that is reflected in a steeper phase III slope. This steeper slope reflects a greater airway wall flux ($J'_{awNO}$) as opposed to an elevated alveolar concentration ($C_{alv,ss}$) which would cause a uniform increase in NO concentration over phase III (52, 53). The elevated $J'_{awNO}$ would result in a much larger $C_{NOpeak}$ than actually observed, and this results in an elevated $D_{awNO}$ as described below.

Mean (SD) $C_{NOpeak}$ for steroid-naïve, steroid-treated, and healthy subjects were 192 (127) ppb, 82 (42) ppb, and 67 (29) ppb, respectively. $C_{NOpeak}$ for steroid-naïve subjects with asthma was statistically larger than the other two groups. Mean (SD) $W_{50}$ for steroid-naïve, steroid-treated, and healthy subjects were 189 (60), 171 (49), and 190 (51) ml, and were not different amongst groups. Mean (SD) $V_{I,II}$ for steroid-naïve, steroid-treated, and healthy subjects were 657 (98), 604 (127), and 668 (142) ml, and were also not different amongst the groups.

As shown in Fig. 5, $J'_{awNO}$ and $D_{awNO}$, are elevated in steroid-naïve subjects with asthma relative to healthy controls. The use of corticosteroids does not impact $D_{awNO}$, but is associated with a significantly lower $J'_{awNO}$ and $C_{awNO}$ that are equivalent to healthy adults. $C_{alv,ss}$ is not different amongst the three groups.

The experimental values of $C_{NOplat}$ at the target flow rates of 50 ml/s and 250 ml/s, respectively are presented in Table 2 (A: healthy adults and B: subjects with asthma)

along with the model-predicted $C_{NOplat}$ (Eq. 3, $C^*_{NOplat}$) at exhalation flow rates of exactly 50 ml/s and 250 ml/s. $C^*_{NOplat}$ and $C_{NOplat}$ were not statistically different from each other with the exception of the steroid naïve group of asthmatic subjects at 250 ml/s (see Table 2B). Statistical differences between the groups did not depend on the choice of $C_{NOplat}$ or $C^*_{NOplat}$. Thus, to control for small variations in the exhalation flow rate between groups (e.g., mean exhalation flow rate at the target of 50 ml/s was 62 ml/s and 55 ml/s for steroid-naïve and steroid-treated groups, respectively), statistical differences between groups are presented using $C^*_{NOplat}$ (Fig. 6). Mean (SD) $C^*_{NOplat}$ was 13.0 (5.97) ppb and 5.17 (2.97) ppb for healthy adults, 53.9 (33.0) ppb and 16.1 (9.46) ppb for steroid-naïve adults with asthma, and 23.2 (14.3) ppb and 7.76 (5.34) ppb for steroid-treated adults with asthma at flow rates of 50 ml/s and 250 ml/s, respectively. $C^*_{NOplat}$ at 50 ml/s is significantly higher for both groups of subjects with asthma when compared to healthy controls (Fig. 6), whereas only the steroid-naïve subjects with asthma have a higher $C^*_{NOplat}$ at 250 ml/s.

$D_{awNO}$ was inversely correlated with both $FEV_1$ (% predicted) and FVC (% predicted) (Fig. 7A and B). In contrast, $C_{awNO}$ was positively correlated with FVC (% predicted). $J'_{awNO}$ and $C_{alv,ss}$ were not correlated with any lung function indices. $C^*_{NOplat}$ at either constant exhalation flow rate was not correlated with indices of lung function, but $C_{NOplat}$ was inversely correlated with $FEV_1$/FVC (% predicted) (Fig. 8). The asthma control questionnaire composite score was not correlated with any of the NO exchange parameters.

## DISCUSSION

In the current study, we estimate flow-independent NO exchange parameters with a single exhalation breathing technique, and plateau exhaled NO concentrations following ATS and ERS guidelines in a group of subjects with a low $FEV_1$ ($FEV_1/FVC < 0.75$) and a clinical history of asthma. We found that the use of corticosteroids was associated with a decrease in the plateau exhaled NO concentrations at flow rates of 50 ml/s and 250 ml/s as well as a decrease in the flow-independent parameters that reflect airway tissue concentration ($J'_{awNO}$ and $C_{awNO}$), respectively. In contrast, $D_{awNO}$ was elevated in both groups of asthmatic subjects, and was independent of the use of corticosteroids. These findings are in good agreement with previously published data by Silkoff et al (48) despite using a different breathing maneuver and analytical technique to estimate the flow-independent NO exchange parameters. In addition, we found that $D_{awNO}$ is inversely correlated with both $FEV_1$ and FVC (% predicted) independent of the presence of asthma and steroid use. Thus, we confirm that $D_{awNO}$ may reflect physiological changes in the lungs that impact lung function independent of the use of corticosteroids.

Since the initial reports that $FE_{NO}$ in asthma was elevated (1, 30), subsequent studies have focused on exploring the correlation between exhaled NO concentration and other inflammatory markers (i.e., eosinophils), clinical interventions such as corticosteroids, and standard indices of lung function (i.e., $FEV_1/FVC$). Corticosteroid treatment significantly decreases $C_{NOplat}$ in subjects with asthma (31, 40, 41), and the dose of steroid is inversely related to $C_{NOplat}$ (32). In addition, an increase in $C_{NOplat}$ has

recently been shown to be equally effective as sputum eosinophils and airway hyperresponsiveness to hypertonic saline as a predictor for loss of asthma control (26). However, the current study as well as that of Silkoff et.al. (48) demonstrate the presence of steroid-independent factors (i.e., $D_{awNO}$) that can also contribute to the elevated levels of NO in the exhaled breath of asthmatics.

We are also now aware of disease states in which exhaled concentration of NO is in the normal range only because abnormalities in the flow-independent determinants of NO concentration balance each other. For example, in scleroderma, the alveolar concentration of NO is elevated while the airway wall flux of NO is reduced (15). In cystic fibrosis, the airway diffusing capacity of NO (transfer factor) is elevated, but the airway wall concentration is reduced leading to an exhaled NO concentration that is similar to healthy controls (45).

Silkoff et al. (48) first reported that $D_{awNO}$ is 4-fold higher in subjects with asthma, and that this increase is independent of steroid treatment while $J'_{awNO}$ decreases. Lehtimaki (38) then demonstrated that steroid treatment reduces $J'_{awNO}$ in newly diagnosed asthma subjects (previously steroid-naïve) by utilizing multiple constant flow rate maneuvers (52, 54). Most recently, Hogman et. al (22) also recently demonstrated that $D_{awNO}$ is increased 1.5 fold in a group of atopic asthmatic subjects. Although we utilized a different breathing maneuver and technique to estimate the flow-independent NO parameters, our results are consistent with previously reported trends (22, 38, 48), and also demonstrates that $D_{awNO}$ is inversely correlated with $FEV_1$ and FVC (% predicted) and $C_{awNO}$ is positively correlated with FVC. The positive correlation of $C_{awNO}$ with FVC is likely due to the fact that it is inversely related to $D_{awNO}$

(i.e., $C_{awNO} = J'_{awNO}/D_{awNO}$). Of note is the fact that Silkoff et al. (48) reported that values of $J'_{awNO}$, $D_{awNO}$, and $C_{awNO}$ after steroid use in asthmatic subjects were all *positively* correlated with $FEV_1$/FVC (% predicted). These important differences may be due to differences in study design and the technique used to estimate the flow-independent NO parameters. Nonetheless, future studies will need to continue to investigate the relationship between NO flow-independent parameters and lung function.

Exhaled nitric oxide concentration necessarily reflects both the chemical and physical properties of the airway wall and alveoli as well as the endogenous production rate from nitric oxide synthase (NOS) isoforms in the airway and alveoli. Our ability to estimate the flow-independent NO parameters, which depend on these properties from the exhaled concentration signal, can be illustrated using the composite exhalation profile (Fig. 4A). We have previously demonstrated that only phase I and II are sensitive to changes in $D_{awNO}$ (if $D_{awNO}$ increases, less NO is exhaled in phase I and II), only phase III is sensitive to $C_{alv,ss}$ (if $C_{alv,ss}$ increases, there is a uniform increase across exhaled volume in phase III), and all three phases are sensitive to $J'_{awNO}$ (if $J'_{awNO}$ increases, there is more NO exhaled in all phases, and the impact on phase III is a steeper slope) (53) (see Fig. 3). Thus, the observed changes in the composite profile of each group are consistent with our reported values of the flow-independent parameters. For example, steroid-treated subjects with asthma have a steeper slope in phase III and a higher concentration (necessitating a larger $J'_{awNO}$), yet a similar amount of NO in phase I and II (necessitating a larger $D_{awNO}$ to balance the increased $J'_{awNO}$). Of note is the fact that amongst the parameters characterizing phase I and II of

the exhalation profile, only $C_{NOpeak}$ differs between the groups ($W_{50}$ and $V_{I,II}$ are not different between the three groups). This is consistent with altered NO production and transport in the airway wall during the breathhold, but also suggests that the volume accumulating NO during the breathhold and subsequently eliminated during exhalation is similar between the three groups.

We have previously reported analytical expressions for the flow-independent parameters that approximate the functional dependence on the surface area emitting nitric oxide ($A_i$, $cm^2$, where subscript "i" refers to the airways, "aw", or the alveoli, "alv"), solubility (partition coefficient, $\lambda_{t:air}$), molecular diffusion (molecular diffusivity, $\mathcal{D}_{t,NO}$, $cm^2/s$), chemical consumption (lumped first order rate reaction constant, k, $s^{-1}$), thickness of the tissue layer ($L_{t,i}$, cm), and chemical production (airway and alveolar production rate per unit volume, $S_{awNO}$ and $S_{alvNO}$, respectively, ml $NO \cdot s^{-1} \cdot cm^{-3}$) (45, 52). The analytical expressions are summarized in Appendix B, and provide a level of quantitative insight into the mechanism of the observed changes in the flow-independent parameters.

$D_{awNO}$ is independent of $S_{awNO}$, is a positive function of $A_{aw}$, $\lambda_{t:air}$, $\mathcal{D}_{t,NO}$, and k, and is an inverse function of $L_{t,aw}$ (Eq. B2 in Appendix B). Thus, the increase in $D_{awNO}$ may be due to alterations in any of these parameters. The airway wall in asthma is generally considered to be thicker than in healthy controls due to remodeling processes such as subepithelial fibrosis and increased mucous production (56) . The thicker airway wall would tend to increase the diffusion distance for NO, and the mucous tends to be more viscous which would decrease the "ease" at which NO can diffuse (i.e., decrease $\mathcal{D}_{t,NO}$) (4). Both of these observations would *decrease* $D_{awNO}$ and

contrast with our experimental observation, as well as that of Silkoff et. al (48), of an elevated $D_{awNO}$. Enhanced chemical consumption, primarily with superoxide (9), can increase $D_{awNO}$ due to an increase in the radial concentration gradient (4, 45). However, $D_{awNO}$ remains elevated following steroid treatment, which has been reported to suppress superoxide release (10).

An increase in $A_{aw}$ is a plausible mechanism for the increase in $D_{awNO}$. Silkoff et. al (48) postulated that extension of the nitric oxide producing non-adrenergic non-cholinergic (NANC) nerves from the large airways into the small airways may increase the surface area emitting nitric oxide, which is supported both directly and indirectly by several studies (6, 7, 16, 17, 39, 55). Expression of iNOS in the airways of subjects with asthma has been demonstrated (12, 36, 50) which could potentially increase $A_{aw}$; however, this possible mechanism would likely be sensitive to corticosteroid therapy which is not the observation.

$J'_{awNO}$ has a similar functional dependence on the physical and chemical parameters of the airways ($A_{aw}$, $\mathcal{D}_{t,NO}$, and $L_{t,aw}$) (see Appendix B) as $D_{awNO}$. However, in contrast to $D_{awNO}$, $J'_{awNO}$ is inversely related to k, and is a positive function of an additional parameter, $S_{awNO}$. An increase in $S_{awNO}$ by an increase in nNOS expression from NANC nerves (6, 7, 16, 17, 39, 55) or prokaryotic denitrification (13) may increase the exhaled concentration of NO, and thus contribute to the observed increase in $J'_{awNO}$ for both steroid-naïve and steroid-treated subjects with asthma. Other enzymatic and non-enzymatic chemical events in the airways such as increased iNOS expression in the epithelium (18), nitrite reduction to NO at lower pH (23, 24, 35), and GSNO

catabolism (5, 11, 14, 49) could also increase $S_{awNO}$, and contribute to the increase in $J'_{awNO}$ for steroid-naïve subjects with asthma.

Steroid treatment dramatically decreases the exhaled NO concentration (see Fig 5), which corresponds to observed decreases in $J'_{awNO}$ and $C_{awNO}$ as well as $C_{NOplat}$ at both 50 ml/s and 250 ml/s flow rates. As previously discussed, steroid therapy decreases superoxide production which would correspond to a reduced consumption rate and an *increase* in $J'_{awNO}$, which is not observed. The decrease in $J'_{awNO}$ in steroid-treated subjects with asthma may be related to: 1) the reduced iNOS activity in the epithelial and inflammatory cells in the airways (12, 34, 36, 50, 57), 2) reduced nitrite to NO reduction due to normalized airway pH (23, 24, 35), 3) decreased prokaryotic colonization (13), and 4) inhibition of arginase up-regulation (33). The decrease in $C_{awNO}$ (a ratio of $J'_{awNO}$ over $D_{awNO}$) for steroid-treated subjects with asthma is due to the decrease in $J'_{awNO}$ while $D_{awNO}$ is not changed.

In summary, we have estimated both flow-independent NO exchange parameters and plateau exhaled NO concentrations following ATS guidelines in subjects with low $FEV_1$/FVC and a clinical history of asthma. $D_{awNO}$ is elevated independent of corticosteroid use, whereas $J'_{awNO}$, $C_{awNO}$, and $C_{NOplat}$ (at both 50 and 250 milliliters per second) are all reduced by the use of steroids. In addition, $D_{awNO}$ is inversely correlated with pulmonary function independent of the presence of asthma and steroid use. In agreement with Silkoff et. al (48), we conclude that $D_{awNO}$ may reflect changes in the lungs that impact function that are not impacted by steroid therapy, and thus may provide clinical information not available from exhaled NO concentration alone.

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## APPENDIX A: Asthma Control Questionnaire

The following six questions are from a previously published and validated asthma control questionnaire (27, 28).

1. On average, during the past week, how often were you woken by your asthma during the night?
2. On average, during the past week, how bad were your asthma symptoms when you woke up in the morning?
3. In general, during the past week, how limited were you in your activities because of your asthma?
4. In general, during the past week, how much shortness of breath did you experience because of your asthma?
5. In general, during the past week, how much of the time did you wheeze?
6. On average, during the past week, how many puffs of short-acting bronchodilator (e.g., Ventolin) have you used each day?

Each question is answered by the subject on a scale of 0 to 6 representing the absence (score of 0) to severe (score of 6) symptoms. The composite score is then the mean of the six scores. Thus, a higher composite score reflects more asthmatic symptoms. The questionnaire has been shown to have improved discriminative and evaluative measurement properties than an asthma control diary (27).

**APPENDIX B: Mathematical description of flow-independent NO parameters**

The following analytical expressions for the steady state values of $J'_{awNO}$, $D_{awNO}$ and $C_{alv,ss}$ have been previously derived (52) and presented in a slightly different form (45, 51):

$$D_{awNO} = \frac{A_{aw}\lambda_{t:air}D_{t,NO}}{L_{t,aw}}\left[\frac{\xi_{aw}}{\tanh(\xi_{aw})}\right] \quad \text{(B1)}$$

$$J'_{awNO} = S_{awNO}A_{aw}L_{t,aw} \bullet \left[\frac{1-\exp(-\xi_{aw})-\tanh(\xi_{aw})\exp(-\xi_{aw})}{\xi_{aw}\tanh(\xi_{aw})}\right] \quad \text{(B2)}$$

$$C_{alv,ss} = \frac{S_{alvNO}L_{t,alv}^2}{\lambda_{t:air}D_{t,NO}} \bullet \left[\frac{1-\exp(-\xi_{alv})-\tanh(\xi_{aw})\exp(-\xi_{alv})}{\xi_{alv}^2}\right] \quad \text{(B3)}$$

where $S_{awNO}$ and $S_{alvNO}$ are the production rate of NO per unit volume of airway and alveolar tissue (milliliters NO·second$^{-1}$·centimeter$^{-3}$), respectively, $\lambda_{t,air}$ is the tissue:air partition coefficient of NO, k (second$^{-1}$) is the first order rate constant which characterizes the rate of chemical consumption by substrates such as superoxide, $A_i$ (centimeter$^2$) is the surface area available for diffusion (subscript "i" is either "aw" or "alv" for airway or alveolar compartment, respectively), $\mathcal{D}_{t,NO}$ (centimeter$^2$/second) is the molecular diffusivity of NO in the tissue, $\xi_i = L_{t,i}\Big/\sqrt{\mathcal{D}_{t,NO}/k}$, and $L_{t,i}$ (centimeter) is the thickness of the tissue layer. $\xi_i$ represents the ratio of the rate of chemical

consumption (k, second$^{-1}$) to the rate of molecular diffusion ($\mathcal{D}_{t,NO}/L_i^2$, second$^{-1}$) for NO. The hyperbolic tangent (tanh) is bounded between –1 and 1, and is a monotonically increasing function of its argument. Eq. B1 provides units of milliliters/second for $D_{awNO}$ that are equivalent in magnitude to picoliter·second$^{-1}$·part per billion$^{-1}$.

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Table 1. *Physical Characteristics of Subjects*

(A) Healthy Adults

| Sub | Gen | Age (yrs) | Hgt (in) | Wgt (Lb) | Iwgt (Lb) | $V_{air}$ (ml) | FVC (l) | FVC (%pred) | $FEV_1$ (l) | $FEV_1$ (%pred) | $FEV_1$/FVC | $FEV_1$/FVC (%pred) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 21 | 68 | 153 | 152 | 173 | 4.69 | 96 | 4.35 | 104 | 93 | 109 |
| 2 | F | 24 | 70 | 150 | 147 | 171 | 4.76 | 111 | 3.83 | 105 | 81 | 95 |
| 3 | F | 22 | 61 | 125 | 117 | 139 | 3.31 | 100 | 2.74 | 94 | 83 | 94 |
| 4 | M | 23 | 66 | 157 | 145 | 168 | 4.21 | 89 | 3.72 | 92 | 88 | 103 |
| 5 | F | 23 | 61 | 117 | 119 | 142 | 2.53 | 76 | 2.14 | 73 | 85 | 97 |
| 6 | M | 37 | 70 | 165 | 160 | 197 | 4.66 | 91 | 4.03 | 95 | 86 | 104 |
| 7 | F | 26 | 65 | 139 | 130 | 156 | 4.67 | 127 | 3.72 | 118 | 80 | 93 |
| 8 | M | 24 | 72 | 175 | 167 | 191 | 5.51 | 97 | 4.71 | 96 | 87 | 101 |
| 9 | M | 27 | 65 | 166 | 141 | 168 | 4.54 | 105 | 3.76 | 103 | 83 | 98 |
| 10 | M | 27 | 71 | 183 | 162 | 189 | 5.44 | 97 | 4.39 | 94 | 82 | 98 |
| 11 | F | 23 | 62 | 120 | 120 | 143 | 3.33 | 98 | 3.00 | 102 | 90 | 104 |
| 12 | F | 31 | 68 | 124 | 141 | 172 | 4.39 | 111 | 3.76 | 113 | 86 | 102 |
| 13 | F | 22 | 68 | 179 | 141 | 163 | 4.89 | 119 | 4.02 | 115 | 82 | 96 |
| 14 | F | 28 | 66 | 144 | 134 | 162 | 3.97 | 105 | 3.41 | 106 | 87 | 102 |
| 15 | F | 26 | 63 | 112 | 124 | 150 | 3.06 | 88 | 2.56 | 85 | 84 | 97 |
| 16 | F | 20 | 64 | 140 | 128 | 148 | 3.67 | 102 | 3.17 | 98 | 89 | 99 |
| 17 | F | 25 | 65 | 114 | 130 | 155 | 3.46 | 94 | 3.08 | 100 | 89 | 106 |
| 18 | F | 33 | 59 | 101 | 111 | 144 | 3.41 | 118 | 2.88 | 115 | 85 | 98 |
| 19 | M | 35 | 69 | 145 | 155 | 190 | 4.60 | 95 | 3.76 | 93 | 82 | 98 |
| 20 | F | 31 | 61 | 97 | 117 | 148 | 3.00 | 99 | 2.52 | 95 | 84 | 96 |
| 21 | M | 22 | 66 | 145 | 145 | 167 | 4.71 | 102 | 4.03 | 101 | 86 | 100 |
| 22 | M | 29 | 66 | 145 | 145 | 174 | 4.06 | 90 | 3.57 | 94 | 88 | 105 |
| 23 | M | 35 | 67 | 140 | 148 | 183 | 4.40 | 97 | 3.88 | 103 | 88 | 106 |
| 24 | F | 20 | 64 | 128 | 128 | 148 | 3.34 | 90 | 3.02 | 90 | 90 | 100 |
| **Mean** | | **26.4** | **65.7** | **140** | **138** | **164** | **4.11** | **99.9** | **3.50** | **99.3** | **86** | **100** |

Sub: subject, Gen: gender, Hgt: height, Wgt: body weight, Iwgt: ideal body weight, FVC: forced vital capacity; $FEV_1$: forced expiratory volume in 1 second, l: liter, %pred: % predicted $FEV_1$/FVC: normalized forced expiratory volume in 1 second by forced vital capacity.

(B) Steroid-naïve Adults with Asthma

| Sub | Gen | Age (yrs) | Hgt (in) | Wgt (Lb) | Iwgt (Lb) | $V_{air}$ (ml) | FVC (l) | FVC (%pred) | $FEV_1$ (l) | $FEV_1$ (%pred) | $FEV_1$/FVC (%pred) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 29 | 64 | 138 | 128 | 157 | 3.70 | 104 | 2.35 | 77 | 64 | 75 |
| 2 | M | 21 | 76 | 202 | 183 | 204 | 7.77 | 127 | 4.68 | 88 | 62 | 71 |
| 3 | M | 26 | 74 | 198 | 174 | 200 | 5.35 | 85 | 3.65 | 70 | 69 | 83 |
| 4 | M | 36 | 70 | 227 | 158 | 194 | 3.41 | 67 | 1.88 | 45 | 55 | 67 |
| 5 | M | 43 | 65 | 193 | 142 | 185 | 3.98 | 101 | 2.95 | 90 | 74 | 89 |
| 6 | F | 26 | 63 | 155 | 123 | 149 | 3.09 | 90 | 2.17 | 73 | 70 | 81 |
| 7 | F | 43 | 63 | 149 | 125 | 168 | 2.07 | 64 | 1.35 | 50 | 65 | 78 |
| 8 | M | 37 | 70 | 196 | 160 | 197 | 3.72 | 72 | 2.41 | 57 | 65 | 79 |
| **Mean** | | **32.6*** | **68.1** | **182*** | **149** | **182** | **4.14** | **88.8*** | **2.68*** | **68.8*** | **66*** | **78*** |

Sub: subject, Gen: gender, Hgt: height, Wgt: body weight, Iwgt: ideal body weight, FVC: forced vital capacity; $FEV_1$: forced expiratory volume in 1 second, l: liter, %pred: % predicted $FEV_1$/FVC: normalized forced expiratory volume in 1 second by forced vital capacity.

*statistically different from healthy controls (t-test with $p < 0.05$)

(C) Steroid-treated Adults with Asthma

| Sub | Gen | Age (yrs) | Hgt (in) | Wgt (Lb) | Iwgt (Lb) | $V_{air}$ (ml) | FVC (l) | FVC (%pred) | $FEV_1$ (l) | $FEV_1$ (%pred) | $FEV_1$/FVC (%pred) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 40 | 64 | 180 | 138 | 178 | 3.63 | 96 | 2.65 | 84 | 73 | 87 |
| 2 | F | 35 | 61 | 184 | 117 | 152 | 2.15 | 69 | 1.37 | 52 | 64 | 76 |
| 3 | M | 29 | 67 | 158 | 147 | 176 | 4.63 | 100 | 3.33 | 86 | 73 | 87 |
| 4 | M | 18 | 69 | 119 | 155 | 173 | 4.27 | 90 | 2.76 | 67 | 65 | 75 |
| 5 | M | 40 | 70 | 149 | 160 | 200 | 5.69 | 113 | 4.12 | 99 | 73 | 88 |
| 6 | F | 39 | 68 | 187 | 141 | 180 | 4.54 | 119 | 3.33 | 105 | 74 | 89 |
| 7 | F | 36 | 68 | 124 | 140 | 176 | 4.15 | 108 | 2.92 | 91 | 71 | 85 |
| 8 | F | 28 | 62 | 110 | 120 | 148 | 2.69 | 81 | 1.84 | 64 | 68 | 79 |
| 9 | F | 30 | 65 | 122 | 130 | 160 | 4.56 | 126 | 3.29 | 107 | 72 | 85 |
| 10 | F | 29 | 64 | 123 | 126 | 155 | 2.4 | 69 | 1.61 | 53 | 67 | 77 |
| 11 | F | 44 | 60 | 122 | 113 | 157 | 2.69 | 96 | 1.77 | 74 | 66 | 77 |
| 12 | F | 30 | 63 | 179 | 125 | 155 | 2.87 | 83 | 2.04 | 69 | 71 | 83 |
| **Mean** | | **33.2*** | **65.1** | **146#** | **134** | **168** | **3.69** | **95.8** | **2.59*** | **79.3*** | **70*** | **83*** |

Sub: subject, Gen: gender, Hgt: height, Wgt: body weight, Iwgt: ideal body weight, FVC: forced vital capacity; $FEV_1$: forced expiratory volume in 1 second, l: liter, %pred: % predicted $FEV_1$/FVC: normalized forced expiratory volume in 1 second by forced vital capacity.

*statistically different from healthy controls (t-test with $p < 0.05$)

#staistically different from steroid-naïve asthmatics (t-test with $p < 0.05$)

(D) Clinical History of Adults with Asthma

| Subject | Questionnaire score | Therapies |
|---|---|---|
| Steroid-naive | | |
| 1 | 1.17 | Albuterol, Salmeterol |
| 2 | 2.00 | none |
| 3 | 1.33 | Albuterol |
| 4 | 3.17 | Albuterol |
| 5 | 1.83 | Albuterol |
| 6 | 1.67 | Albuterol |
| 7 | 0.83 | Albuterol |
| 8 | 2.33 | Albuterol, Primatene |
| Mean (SD) | 1.79 (0.73) | |
| Steroid-treated | | |
| 1 | 2.17 | Zafirlukast, Salmeterol, Albuterol, Beclomethasone |
| 2 | 3.00 | Albuterol, Triamcinolone, Prednisone |
| 3 | 0.50 | Fluticasone, Salmeterol, Albuterol |
| 4 | 1.33 | Fluticasone, Albuterol, Loratadine |
| 5 | 0.67 | Beclomethasone, Flonase, Loratadine, Albuterol |
| 6 | 0.00 | Fluticasone |
| 7 | 1.50 | Triamcinolone, Albuterol |
| 8 | 0.33 | Salmeterol, Fluticasone |
| 9 | 1.00 | Albuterol, Fluticasone |
| 10 | 2.17 | Albuterol, Fluticasone |
| 11 | 1.83 | Flunisolide, Albuterol |
| 12 | 3.67 | Montelukast sodium, Fluticasone/almeterol, Albuterol |
| Mean (SD) | 1.51 (1.11) | |

Table 2. *Model-predicted and experimental $C_{NOplat}$ of Subjects*

(A) Healthy Adults

| Subject | $\dot{V}_E$ and $C_{NOplat}$ (experimental data) | | | | $C^*_{NOplat}$ (model-predicted) | |
|---|---|---|---|---|---|---|
| | (ml/s) | (ppb) | (ml/s) | (ppb) | 50 ml/s | 250 ml/s |
| 1 | 47.9 | 12.9 | 251 | 4.03 | 13.0 | 4.60 |
| 2 | 57.6 | 20.8 | 269 | 6.67 | 19.9 | 7.04 |
| 3 | 63.8 | 1.87 | 230 | 0.65 | 3.73 | 1.20 |
| 4 | 49.2 | 13.0 | 248 | 3.44 | 13.1 | 4.76 |
| 5 | 45.2 | 5.14 | 197 | 1.74 | 4.02 | 1.53 |
| 6 | 58.8 | 2.17 | 254 | 0.92 | 2.75 | 0.93 |
| 7 | 54.1 | 17.9 | 254 | 6.64 | 17.9 | 7.62 |
| 8 | NC | NC | NC | NC | 6.79 | 2.59 |
| 9 | NC | NC | NC | NC | 8.07 | 3.83 |
| 10 | NA | NA | 259 | 10.5 | 23.4 | 12.9 |
| 11 | 51.7 | 8.44 | 265 | 2.29 | 7.56 | 3.34 |
| 12 | 59.2 | 14.8 | NA | NA | 17.2 | 9.82 |
| 13 | 57.4 | 17.0 | 244 | 5.35 | 16.4 | 5.46 |
| 14 | 59.5 | 8.16 | 244 | 3.17 | 9.57 | 3.10 |
| 15 | 56.0 | 24.8 | 217 | 12.4 | 24.6 | 11.0 |
| 16 | 63.2 | 6.84 | 271 | 3.29 | 6.78 | 2.35 |
| 17 | 58.5 | 9.67 | 231 | 4.31 | 12.5 | 4.13 |
| 18 | 50.3 | 8.89 | 251 | 4.14 | 11.0 | 4.38 |
| 19 | 92.5 | 9.25 | 266 | 2.52 | 14.6 | 5.03 |
| 20 | 55.9 | 15.5 | 192 | 5.30 | 16.3 | 5.48 |
| 21 | 64.4 | 13.1 | 249 | 4.34 | 17.5 | 5.22 |
| 22 | 60.0 | 14.2 | 253 | 4.58 | 13.9 | 5.00 |
| 23 | 55.3 | 19.4 | 208 | 6.94 | 17.8 | 6.93 |
| 24 | 62.7 | 14.4 | 253 | 7.66 | 12.7 | 5.91 |
| **Mean** | **58.2** | **12.3** | **243** | **4.80** | **13.0** | **5.17** |

NC: data not collected
NA: not able to complete the maneuver

(B) Subjects with Asthma

| Subject | $\dot{V}_E$ and $C_{NOplat}$ (experimental data) | | | | $C^*_{NOplat}$ (model-predicted) | |
|---|---|---|---|---|---|---|
| | (ml/s) | (ppb) | (ml/s) | (ppb) | 50 ml/s | 250 ml/s |
| Steroid-naive | | | | | | |
| 1 | 58.7 | 96.0 | 258 | 26.2 | 100 | 28.2 |
| 2 | 58.6 | 92.9 | 273 | 23.3 | 93.6 | 27.0 |
| 3 | 55.8 | 38.8 | 221 | 11.2 | 41.8 | 15.6 |
| 4 | NA | NA | NA | NA | 32 | 8.23 |
| 5 | 66.3 | 36.7 | 253 | 7.41 | 38.9 | 11.1 |
| 6 | 61.1 | 21.0 | 234 | 7.03 | 20.2 | 6.92 |
| 7 | 71.9 | 17.5 | 260 | 4.5 | 21.2 | 5.77 |
| 8 | 58.0 | 91.6 | 274 | 22.6 | 83.2 | 25.4 |
| **Mean** | **61.5** | **56.3*** | **253** | **14.6*** | **53.9*** | **16.1*§** |
| Steroid-treated | | | | | | |
| 1 | 66.3 | 15.9 | 230 | 5.56 | 18.5 | 5.34 |
| 2 | 54.1 | 7.35 | 211 | 2.69 | 7.43 | 2.18 |
| 3 | 57.1 | 7.74 | 254 | 2.55 | 8.14 | 2.67 |
| 4 | 48.9 | 59.7 | 237 | 16.0 | 49.9 | 17.7 |
| 5 | 53.2 | 16.6 | 249 | 6.15 | 16.1 | 7.73 |
| 6 | 52.8 | 19.7 | 273 | 5.16 | 20.8 | 5.45 |
| 7 | 45.2 | 38.8 | 262 | 11.6 | 38.9 | 13.2 |
| 8 | 50.3 | 11.7 | 239 | 2.58 | 11.8 | 3.42 |
| 9 | 53.4 | 8.61 | 270 | 1.90 | 8.95 | 2.13 |
| 10 | 54.5 | 34.1 | 197 | 17.8 | 38.7 | 13.7 |
| 11 | 55.4 | 22.1 | 171 | 6.34 | 23.1 | 6.34 |
| 12 | 73.3 | 31.5 | 271 | 11.0 | 35.7 | 13.2 |
| **Mean** | **55.4** | **22.8 #** | **239** | **7.44#** | **23.2*#** | **7.76#** |

*****statistically different from healthy controls (t-test with $p < 0.05$)
#statistically different from steroid-naïve asthmatics (t-test with $p < 0.05$)
§statistically different from $C_{NOplat}$ at 250 ml/s.
NA: not able to complete the maneuver

## FIGURE LEGENDS

Fig. 1: Definition of $C_{NOpeak}$, $W_{50}$, and $V_{I,II}$ are presented using a schematic of a representative exhalation NO profile using the single breath technique with a pre-expiratory breathhold and a decreasing exhalation flow rate. $C_{NOpeak}$ is the maximum concentration of NO in phase I and II, $W_{50}$ is the width of the Phase I and II peak calculated by taking the volume of at which the exhaled concentration is larger than 50% of $C_{NOpeak}$, and $V_{I,II}$ is the volume of phase I and II. The distinction between phase I and II and phase III is the point of zero slope (inflection point) in the exhalation profile as previously described (53).

Fig. 2: Schematic of two-compartment model used to describe NO exchange dynamics. Exhaled NO concentration, $C_{exh}$, is the sum of two contributions – the alveolar region and the airway region – which depends on three flow-independent parameters: maximum total volumetric flux of NO from the airway wall ($J'_{awNO,}$ picoliters$\cdot$second$^{-1}$), diffusing capacity of NO in the airways ($D_{awNO,}$ picoliter$\cdot$second$^{-1}\cdot$part per billion$^{-1}$), and steady state alveolar concentration ($C_{alv,ss,}$ part per billion). $J_{awNO}$ is the total flux (picoliters$\cdot$second$^{-1}$) of NO between the tissue and gas phase in the airway, and is an inverse function of the exhalation flow rate, $\dot{V}_E$, and is the sum of two terms: $J'_{awNO}$ minus $D_{awNO}*C_{air}$. If $D_{awNO}$ increases while $J'_{awNO}$ is held constant (note this necessitates a decrease in the wall concentration, $C_{awNO}$, as $J'_{awNO}$ is the product $D_{awNO}*C_{awNO}$), then $J_{awNO}$ decreases (see text for details). If exhalation flow rate is held

constant (i.e., 50 ml/s as suggested by the ATS), then $C_{exh}$ approaches a constant value in phase III of the exhalation profile and is equivalent to $C_{NOplat}$.

Fig. 3: The two-compartment model prediction of the exhaled NO profile is shown for the single exhalation maneuver with a 20-second pre-expiratory breathhold. Representative values for lung volumes of a healthy adult have been used, and the "control" values for the flow-independent parameters are: $D_{awNO}$ = 5 pl•s$^{-1}$•ppb$^{-1}$; $J'_{awNO}$ = 750 pl•s$^{-1}$; $C_{alv,ss}$ = 3 ppb. In each panel, the control profile (solid line) is shown together with the exhaled profile when one of the flow-independent parameters is doubled (dashed line). In panel A, the decreasing exhalation flow rate is also shown on the second y-axis. This informal sensitivity analysis demonstrates graphically which part of the profile is impacted by each parameter. It can be seen that each parameter uniquely impacts the exhaled profile and can thus be uniquely determined. Note that $D_{awNO}$ primarily impacts phase I and II, $C_{alv,ss}$ impacts primarily phase III, whereas $J'_{awNO}$ impacts all three phases. In addition, note that an increase in $D_{awNO}$ (while holding $J'_{awNO}$ and $C_{alv,ss}$) decreases the NO concentration in phase I and II if $J'_{awNO}$ (Eq. 2) and $C_{alv,ss}$ are held constant, but would increase the concentration in phase I and II if $C_{awNO}$ (Eq. 2) and $C_{alv,ss}$ were held constant (Eq. 2). In the former case, $C_{awNO}$ must be decreased to hold $J'_{awNO}$ constant (product $D_{awNO}$*$C_{awNO}$) whereas in the later case $J'_{awNO}$ would increase as $C_{awNO}$ is constant.

Fig. 4: (A) Composite experimental NO exhalation profiles are presented for the 20 second breathhold followed by a decreasing flow rate maneuver for steroid-naïve (SN)

asthma subjects (dark solid line with standard deviation as the error bar) and healthy adults (HA) (solid line). (B) Composite experimental NO exhalation profiles are presented for the 20 second breathhold followed by a decreasing flow rate maneuver for steroid-treated (ST) asthma subjects (dark solid line with standard deviation as the error bar) and healthy adults (HA) (solid line). Error bar represents the standard deviation.

Fig. 5: Individual and population mean (solid bar) values of four flow-independent parameters (A: $J'_{awNO}$, B: $D_{awNO,}$ C: $C_{alv,ss}$, D: $C_{awNO}$) for steroid-naïve (SN), steroid-treated (ST) asthma subjects (closed circle and open circle, respectively) and healthy controls (HA) (open diamond). The mean (SD) $J'_{awNO}$, $D_{awNO,}$ $C_{alv,ss}$, and $C_{awNO}$, respectively, for healthy controls (HA), steroid-naïve (SN) asthma subjects, and steroid-treated (ST) asthma subjects are: HA: 530(234), 3.13(1.57), 3.08(2.39), 220(177); SN: 2693(1687), 8.71(5.74), 5.68(3.22), 438(312); ST: 1196(837), 11.8(11.7), 3.30(2.74), 143(66). * Statistically different from healthy adults ($p<0.05$) and # statistically different from steroid-naïve subjects with asthma ($p<0.05$).

Fig. 6: Individual and population mean (solid bar) values of the plateau exhaled concentration for nitric oxide as predicted by the model (Eq. 3, $C^*_{NOplat}$) using the flow-independent parameters for each subject. A) exhalation flow rate of exactly 50 milliliters/second, B) exhalation flow rate of exactly 250 milliliters/second. SN - steroid-naïve (closed circles), ST - steroid-treated (open circles), HA - healthy adults (open

diamond). * Statistically different from healthy adults ($p<0.05$) and # statistically different from steroid-naïve subjects with asthma ($p<0.05$).

Fig. 7: Second order partial correlation analysis demonstrates a significant inverse relationship between $D_{awNO}$ and $FEV_1$ (% predicted) (A), $D_{awNO}$ and FVC (% predicted) (B), and a positive relationship between $C_{awNO}$ and FVC (% predicted) (C) in a total of 44 subjects. $\Delta$ indicates the difference between the individual score of each subject and the group mean value to which each subject belongs. Plus sign (+) represents healthy adults (n=24), open and closed circles represent steroid-treated (n=12) and steroid-naïve (n=8) asthma subjects, respectively.

Fig. 8: Second order partial correlation analysis demonstrates a significant inverse relationship between $C_{NOplat}$ and $FEV_1$/FVC (% predicted) at an exhalation flow rate of 50 ml/s (A) and 250 ml/s (B) in a total of 44 subjects. $\Delta$ indicates the difference between the individual score of each subject and the group mean value to which each subject belongs. Plus sign (+) represents healthy adults (n=24), open and closed circles represent steroid-treated (n=12) and steroid-naïve (n=8) asthma subjects, respectively.

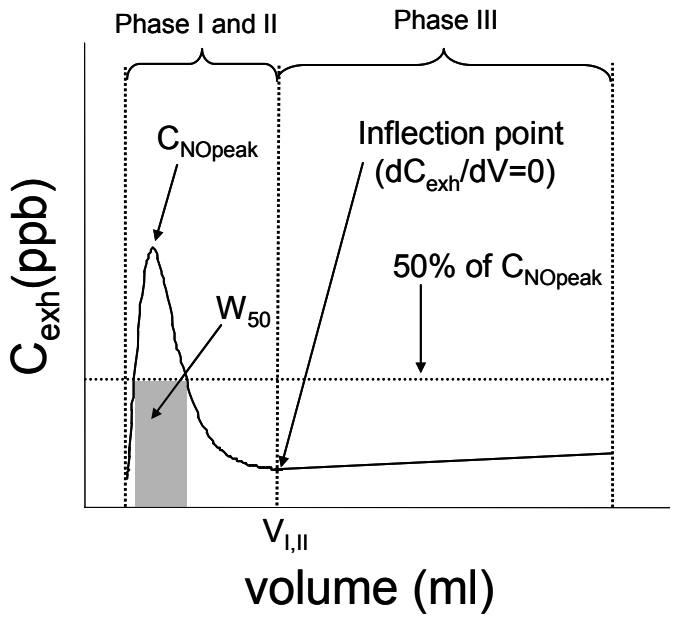

Phase I and II
Phase III
$C_{NOpeak}$
Inflection point
($dC_{exh}/dV=0$)
50% of $C_{NOpeak}$
$W_{50}$
$C_{exh}$(ppb)
$V_{I,II}$
volume (ml)

Fig 1

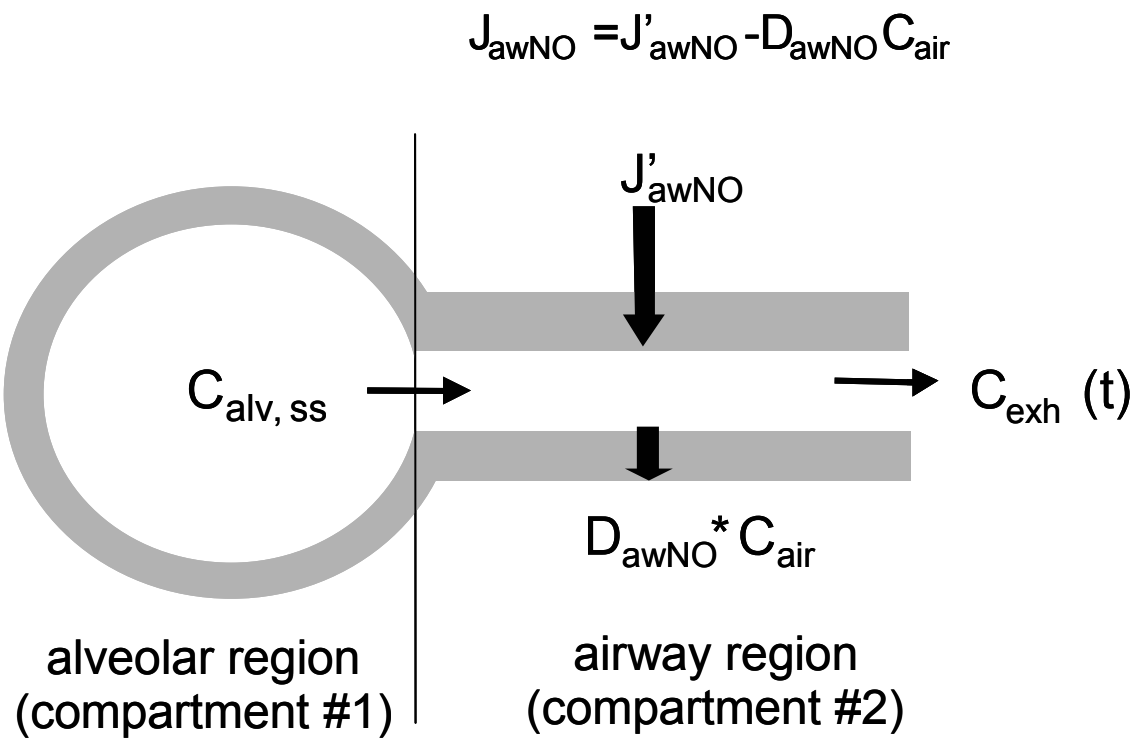

$J_{awNO} = J'_{awNO} - D_{awNO} C_{air}$
$J'_{awNO}$
$C_{alv, ss}$
$C_{exh}$ (t)
$D_{awNO} * C_{air}$
alveolar region
(compartment #1)
airway region
(compartment #2)

Fig 2

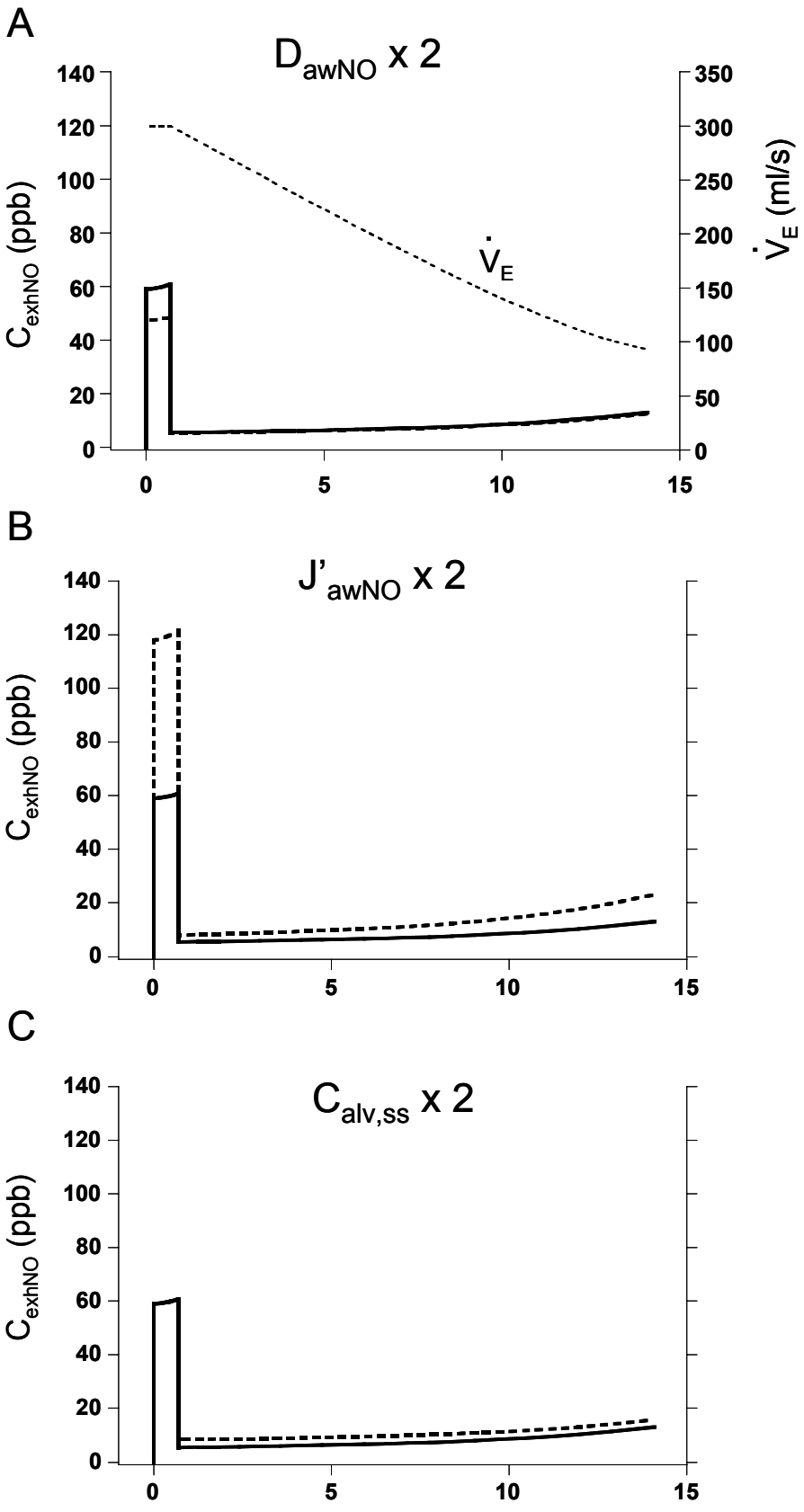
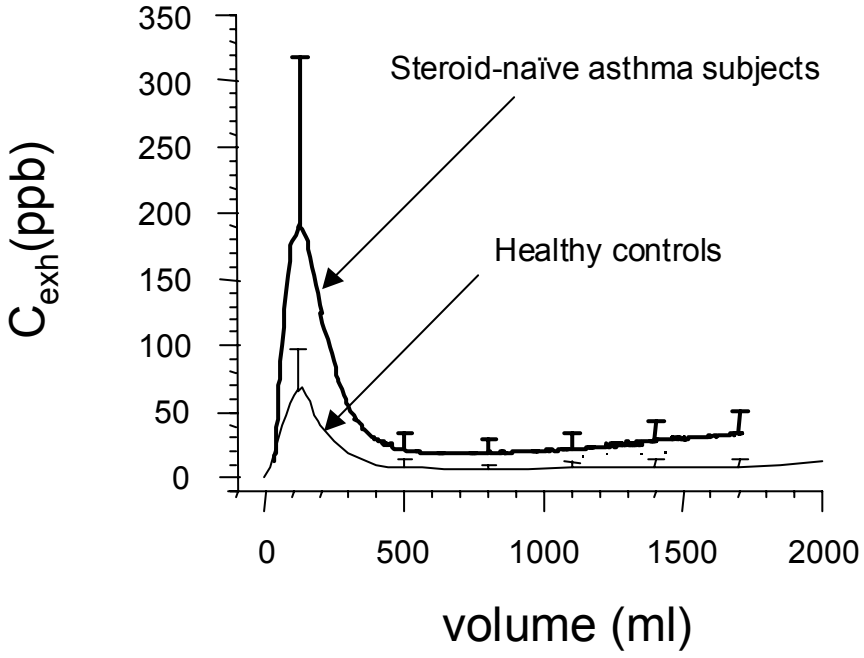

A
$D_{awNO}$ x 2
$C_{exhNO}$ (ppb)
$\dot{V}_E$
$\dot{V}_E$ (ml/s)
B
$J'_{awNO}$ x 2
$C_{exhNO}$ (ppb)
C
$C_{alv,ss}$ x 2
$C_{exhNO}$ (ppb)

Fig. 3

A


350
300
250
200
150
100
50
0
$C_{exh}$(ppb)
Steroid-naïve asthma subjects
Healthy controls
0
500
1000
1500
2000
volume (ml)

B

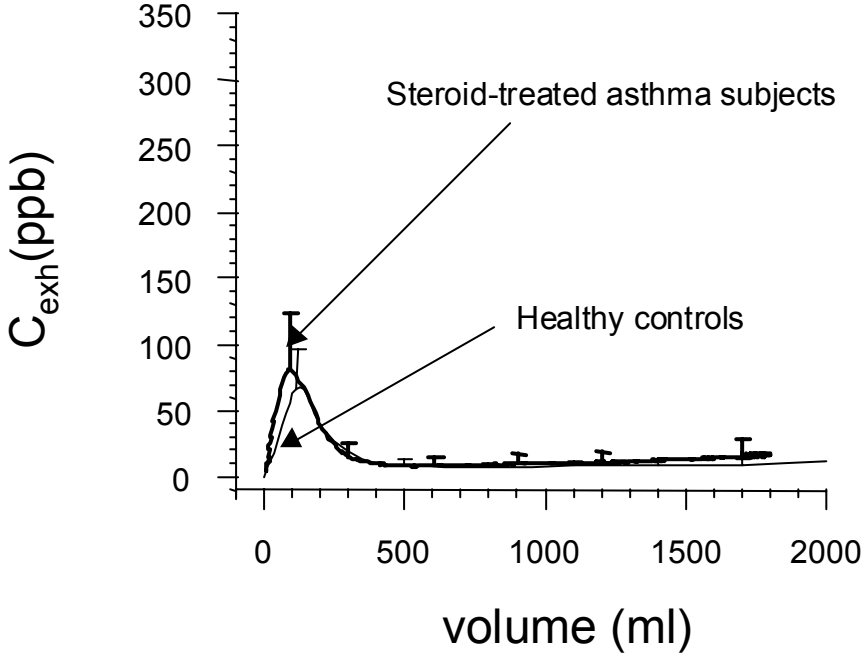

350
300
250
200
150
100
50
0
$C_{exh}$(ppb)
Steroid-treated asthma subjects
Healthy controls
0
500
1000
1500
2000
volume (ml)

Fig 4

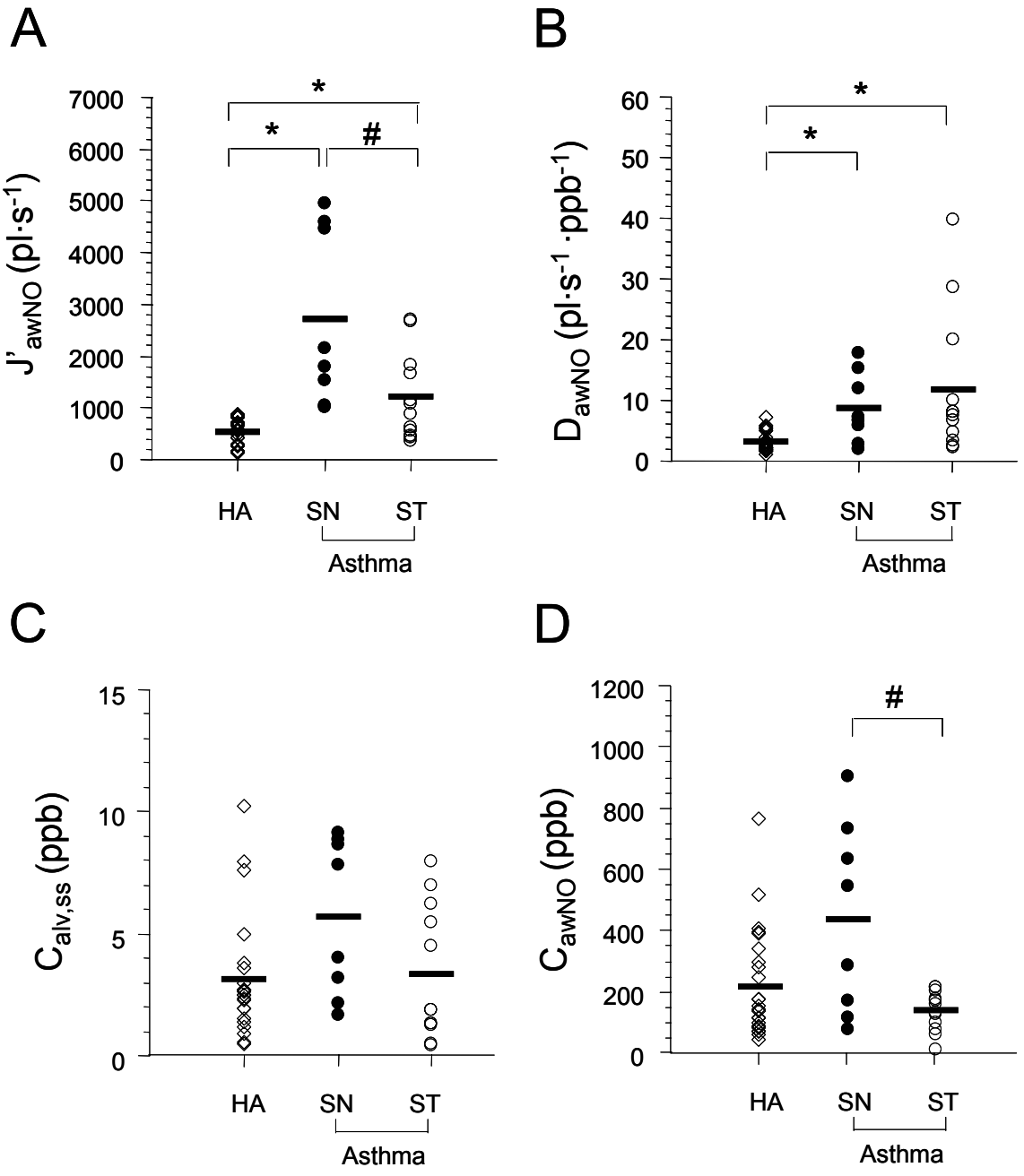

A
B
C
D
7000
6000
5000
4000
3000
2000
1000
0
J'awNO (pl·s-1)
60
50
40
30
20
10
DawNO (pl·s-1·ppb-1)
15
10
5
Calv,ss (ppb)
1200
1000
800
600
400
200
CawNO (ppb)
*
#
HA
SN
ST
Asthma

Fig 5

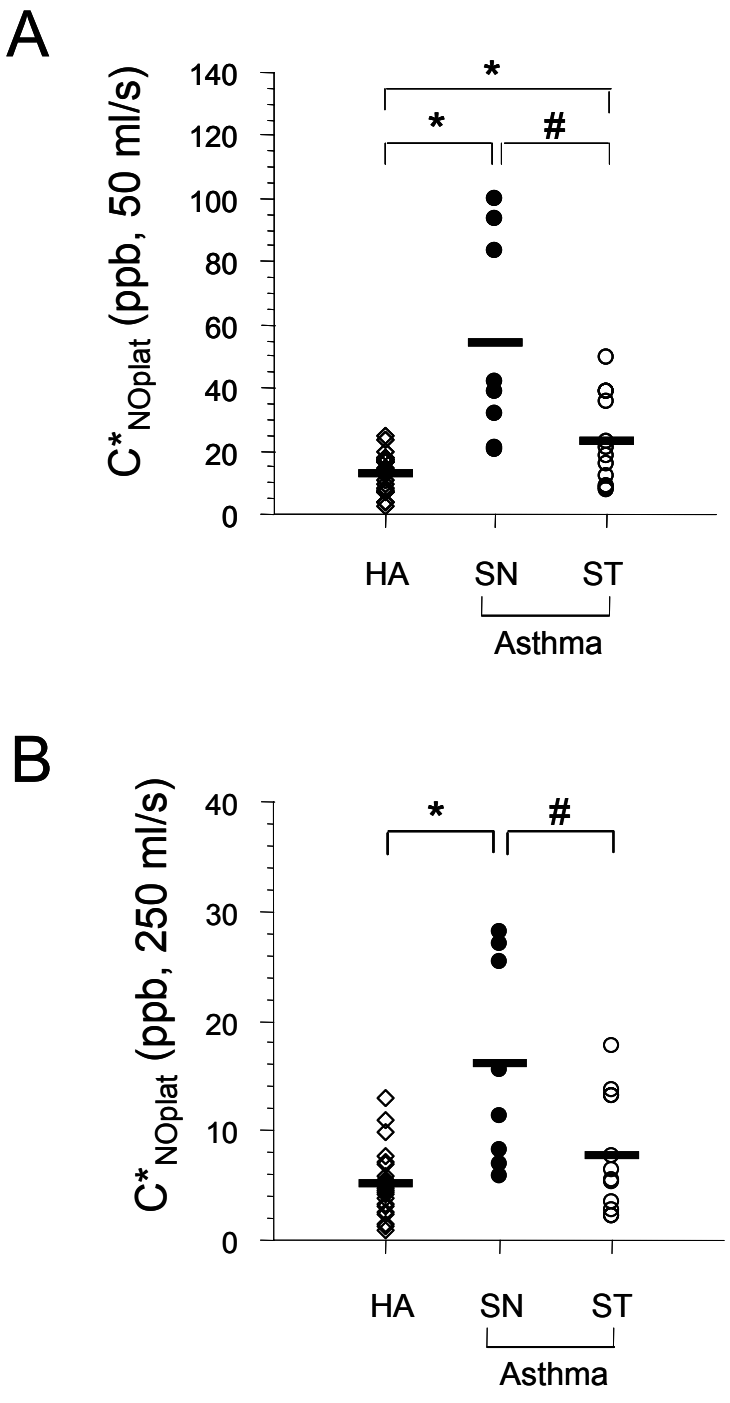
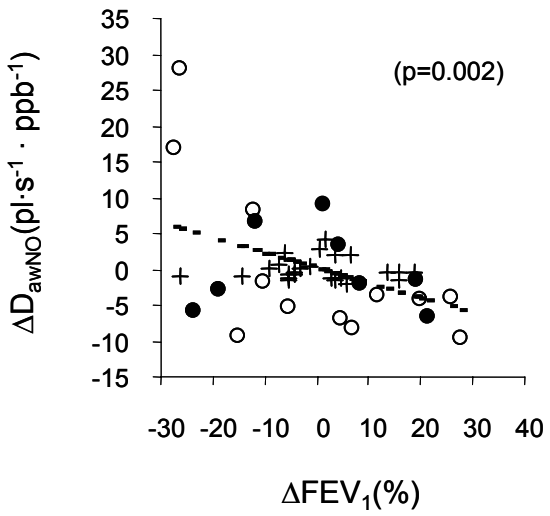
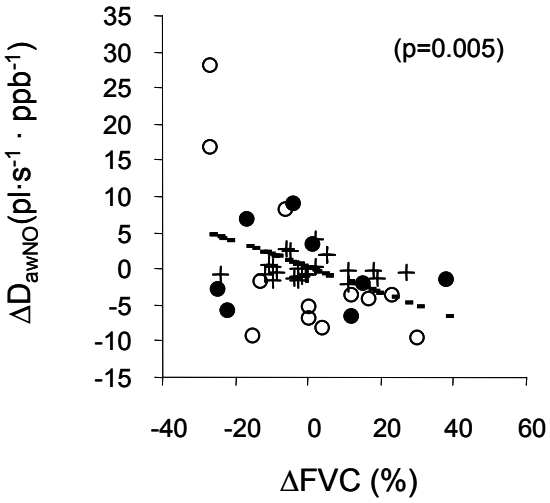



Fig 6

A


(p=0.002)
ΔD_awNO(pl·s^-1 · ppb^-1)
ΔFEV_1(%)

B


(p=0.005)
ΔD_awNO(pl·s^-1 · ppb^-1)
ΔFVC (%)

C

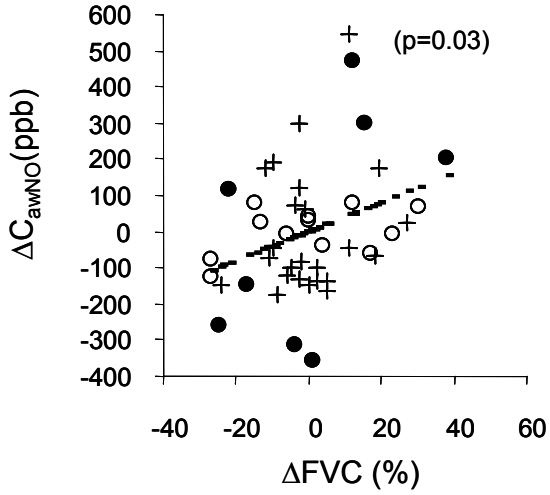
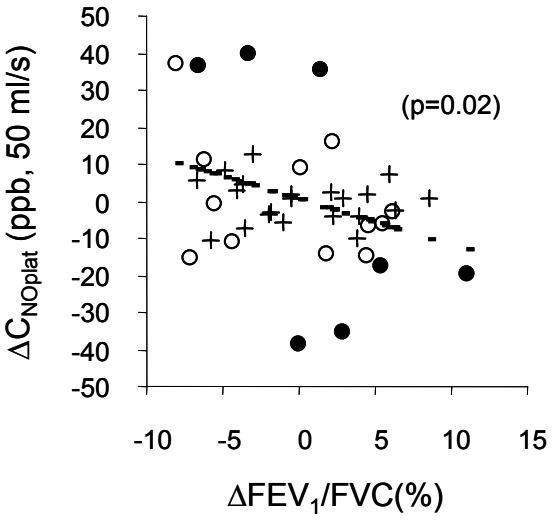

(p=0.03)
ΔC_awNO(ppb)
ΔFVC (%)

Fig. 7

A


50
40
30
20
10
0
-10
-20
-30
-40
-50
(p=0.02)
ΔC_NOplat (ppb, 50 ml/s)
-10
-5
0
5
10
15
ΔFEV1/FVC(%)

B

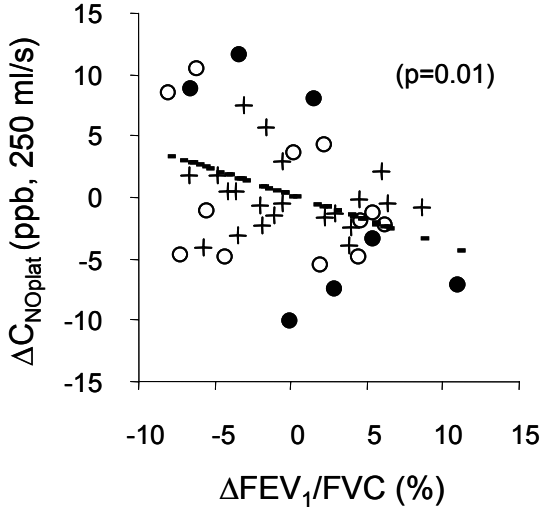

15
10
5
0
-5
-10
-15
(p=0.01)
ΔC_NOplat (ppb, 250 ml/s)
-10
-5
0
5
10
15
ΔFEV1/FVC (%)

Fig. 8