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Airway diffusing capacity of nitric oxide and steroid therapy in asthma

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Shin, Hye-Won, Christine M. Rose-Gottron, Dan M. Cooper, Robert L. Newcomb, and Steven C. George. Airway diffusing capacity of nitric oxide and steroid therapy in asthma. *J Appl Physiol* 96: 65–75, 2004. First published September 5, 2003; 10.1152/japplphysiol.00575.2003.—Exhaled nitric oxide (NO) concentration is a noninvasive 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-naive asthma ($n = 8$) or steroid-treated asthma ($n = 12$) and in 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/s) and airway tissue concentration [parts/billion (ppb)] of NO were lower in steroid-treated asthmatic subjects compared with steroid-naive asthmatic subjects (1,195 ± 836 pl/s and 143 ± 66 ppb compared with 2,693 ± 1,687 pl/s and 438 ± 312 ppb, respectively). In contrast, the airway diffusing capacity for NO ($pl \cdot s^{-1} \cdot ppb^{-1}$) was elevated in both asthmatic groups compared with healthy controls, independent of steroid therapy (11.8 ± 11.7, 8.71 ± 5.74, and 3.13 ± 1.57 $pl \cdot s^{-1} \cdot ppb^{-1}$ for steroid treated, steroid naive, and healthy controls, respectively). In addition, the airway diffusing capacity was inversely correlated with both forced expired volume in 1 s and forced vital capacity (%predicted), whereas the airway tissue concentration was positively correlated with forced vital capacity. Consistent with previously reported results from Silkoff et al. (Silkoff PE, Sylvester JT, Zamel N, and Permutt S, *Am J Respir Crit Med* 161: 1218–1228, 2000) that used 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.

model; airways; alveoli; inflammation

NITRIC OXIDE (NO) WAS FIRST detected in the exhaled breath of humans more than a decade ago (19) and remains a promising noninvasive index of lung pathophysiology. Substantial evidence suggests that both the airway and alveolar regions are significant sources of exhaled NO [fraction of exhaled NO (F_{ENO})] (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_{ENO} measurements might lead to specific insights about pathophysiology throughout the respiratory tract. Guidelines for characterizing F_{ENO} by the American Thoracic Society (ATS) and the European Respiratory Society (ERS) include only the plateau concentration in phase III ($C_{NO,plat}$) at a constant exhalation flow rate ($\dot{V}_E$) (2, 29). However, a single measurement of $C_{NO,plat}$ 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 analytic 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 (C_{exh}) (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), whereas 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 NO synthase (NOS) and glutaminase, which impact NO metabolism (3, 23, 43). Consequently, corticosteroids, which attenuate the inflammatory process, also reduce the concentration of NO 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 ($D_{aw,NO}$) 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 $D_{aw,NO}$ was elevated in asthma independent of steroid therapy by measuring multiple $C_{NO,plat}$ at small flow rates (<50 ml/s). However, values for the flow-independent NO exchange parameters may depend on the breathing maneuver and analytic technique utilized. Thus the goal of the present study was to apply our alternate breathing and analytic technique (20-s preexpiratory breath hold followed by a decreasing flow-rate maneuver) in asthma to confirm the results of Silkoff et. al. and potentially provide additional insight into the pathophysiology that marks chronic asthma.

METHODS

Subjects. Twenty-four healthy adults and 20 subjects with a clinical history of asthma (8 steroid naive and 12 steroid treated) participated in

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Table 1. *Physical characteristics of subjects*

Subject No.	Gender	Age, yr	Height, in.	Weight, lb.	Iwgt, lb.	V_{air}, ml	FVC		FEV_1		FEV_1/FVC	
							liters	%pred	liters	%pred	liters	%pred
Healthy adults												
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
Steroid-naive adults with asthma												
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*
Steroid-treated adults with asthma												
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*

F, female; M, male; Iwgt, ideal body weight; V_{air}, air volume; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s, %pred, % predicted; FEV_1/FVC, normalized FEV_1 by FVC. Statistically different from *healthy controls and †steroid-naive asthmatic subjects (*t*-test with $P<0.05$).

this study. Inclusion criteria for the healthy subjects was a forced expiratory volume in 1 s (FEV_1)-to-forced vital capacity (FVC) ratio (FEV_1/FVC) >0.80; exclusion criteria were 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 two groups: *1*) steroid naive 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 7 days (27, 28). Subject characteristics are presented in Tables 1 and 2, 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 (2). The first maneuver was five repetitions of a 20-s preexpiratory

Table 2. *Clinical history of adults with asthma*

Subject No.	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	

breath hold 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 cmH_2O was maintained to prevent nasal contamination during the breath hold (2), and a Starling resistor (Hans Rudolph, Kansas City, MO) with a variable resistance was used to progressively decrease the flow rate during the exhalation. After breath hold, 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 (2). We also included an exhalation flow rate of 250 ml/s (ATS guideline is 50 ml/s) consistent with the guidelines of the ERS (29). After measuring the indexes of NO exchange dynamics, general spirometry, such as FVC, and FEV_1 normalized by FVC (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, Boulder, CO) was used to measure the C_{exh}. The instrument was calibrated on a daily basis by using a certified NO gas (45 parts/million in N_2; Sievers, Boulder, CO). The zero-point calibration was performed with a NO filter (Sievers) immediately before the collection of a profile. The flow rate and pressure signals were measured by using a pneumotachometer (RSS100, Hans Rudolph, 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-s preexpiratory breath hold were characterized by the peak concentration in phases I and II ($C_{NO,peak}$); the peak width (W_{50}) in phases I and II, defined as the exhaled volume in which the NO concentration was >50% of $C_{NO,peak}$; and the total volume of phases I and II ($V_{I,II}$), defined as the point of zero slope ($dC_{exh}/dV = 0$, where V is volume) in the exhalation profile (53) (Fig. 1). The constant-flow-rate single exhalations were characterized by the $C_{NO,plat}$, as previously described by the ATS and the ERS (2, 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 ($J'_{aw,NO}$; pl/s); *2*) $D_{aw,NO}$ [$pl \cdot s^{-1} \cdot$parts per billion $(ppb)^{-1}$]; *3*) steady-state alveolar concentration ($C_{alv,ss}$; ppb); and *4*) mean airway tissue NO concentration ($C_{aw,NO}$; ppb; equal to the ratio of $J'_{aw,NO}$ to $D_{aw,NO}$). A simple schematic of the two-compartment model and flow-independent parameters is 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 ($J_{aw,NO}$; pl/s). $J_{aw,NO}$ depends on the flow-independent parameters and is expressed as a linear function of the airway gas-phase concentration (C_{air}) by the following

$$J_{aw,NO} = J'_{aw,NO} - D_{aw,NO}C_{air} \quad (1)$$

or

$$J_{aw,NO} = D_{aw,NO}(C_{aw,NO} - C_{air}) \quad (2)$$

$J'_{aw,NO}$ is equal to the product $D_{aw,NO} * C_{aw,NO}$ (*Eq. 2*). Conceptually, $J'_{aw,NO}$ approaches $J_{aw,NO}$ as the product $D_{aw,NO} * C_{air}$ approaches zero. $D_{aw,NO}$ 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 $C_{alv,ss}$, 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 $D_{aw,NO}$, $J'_{aw,NO}$, and $C_{alv,ss}$ on the single-exhalation profile with a 20-s preexpiratory breath hold and a decreasing exhalation flow rate.

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

$$C^*_{NO,plat} = C_{aw,NO} + (C_{alv,ss} - C_{aw,NO}) \cdot \exp(-D_{aw,NO}/\dot{V}_E) \quad (3)$$

where $C^*_{NO,plat}$ is the plateau concentration of NO predicted by the model using the flow-independent parameters. Our laboratory (44, 53) has previously demonstrated that $C^*_{NO,plat}$ is not different than the experimentally measured $C_{NO,plat}$ in healthy adults, with the advan-

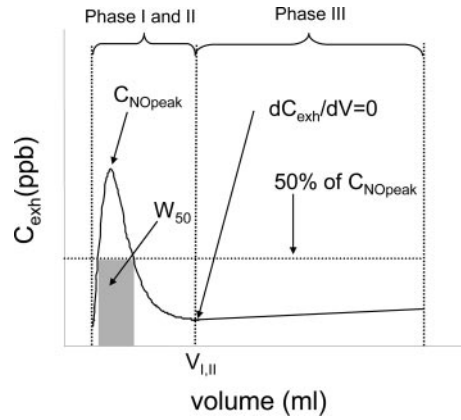

Fig. 1. Definition of $C_{NO,peak}$, W_{50}, and $V_{I,II}$ are presented by a schematic of a representative exhalation nitric oxide (NO) profile using the single-breath technique with a preexpiratory breath hold and a decreasing exhalation flow rate. $C_{NO,peak}$ is the maximum concentration of NO in phases I and II; W_{50} is the width of the phase I and II peak calculated by taking the volume (V) at which the exhaled concentration (C_{exh}) is >50% of $C_{NO,peak}$; and $V_{I,II}$ is the volume of phases I and II. The distinction between phases I and II and phase III is the point of zero slope in the exhalation profile, as previously described (53). ppb, Parts per billion.

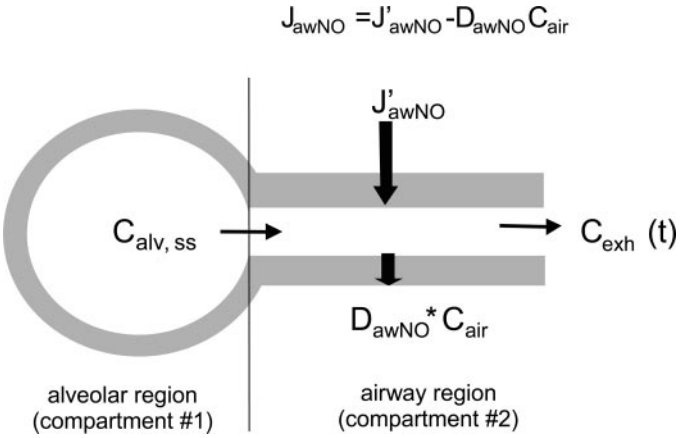

Fig. 2. Schematic of 2-compartment model used to describe NO exchange dynamics. C_{exh} is the sum of two contributions, the alveolar region and the airway region, which depends on 3 flow-independent parameters: maximum total volumetric flux of NO from the airway wall ($J'_{aw,NO}$; pl/s), diffusing capacity of NO in the airways ($D_{aw,NO}$; $pl \cdot s^{-1} \cdot ppb^{-1}$), and steady-state alveolar concentration ($C_{alv,ss}$; ppb). $J_{aw,NO}$ is the total flux (pl/s) 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: $J'_{aw,NO} - D_{aw,NO} * C_{air}$ (airway gas phase concentration). If $D_{aw,NO}$ increases while $J'_{aw,NO}$ is held constant (note that this necessitates a decrease in the wall concentration, $C_{aw,NO}$, as $J'_{aw,NO}$ is the product of $D_{aw,NO} * C_{aw,NO}$), then $J_{aw,NO}$ decreases (see text for details). If exhalation flow rate is held constant (i.e., 50 ml/s as suggested by the American Thoracic Society), then C_{exh} approaches a constant value in phase III of the exhalation profile and is equivalent to $C_{NO,plat}$ (NO plateau concentration in phase III). *t*, Time.

tage that intersubject and interpopulation variations in flow rate can be accounted for by calculating $C_{NO,plat}$ at a precise desired flow rate (e.g., 50 ml/s).

Statistics. To detect differences among the three groups of subjects, data were analyzed by using ANOVA and post hoc paired comparisons of treatment means. In those instances in which Levene's test rejected homogeneity of variance, tests for group differences relied on Welch's ANOVA or Satterthwaite's method to adjust the test to account for this problem. To detect significant relationships between the parameters that characterize NO exchange and either asthma symptoms or standard indexes of lung function (e.g., FEV_1), 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 by 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 by using the untransformed data. Finally, a P value <0.05 was considered statistically significant, and all results were produced by 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 Tables 1 and 2, respectively. FEV_1/FVC was more reproducible than 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-naive 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-naive and steroid-treated asthma subjects, respectively. FEV_1/FVC was significantly lower in both groups of subjects with asthma compared with healthy adults. However, there was no difference in FEV_1/FVC or clinical symptoms (as assessed by the composite score on the

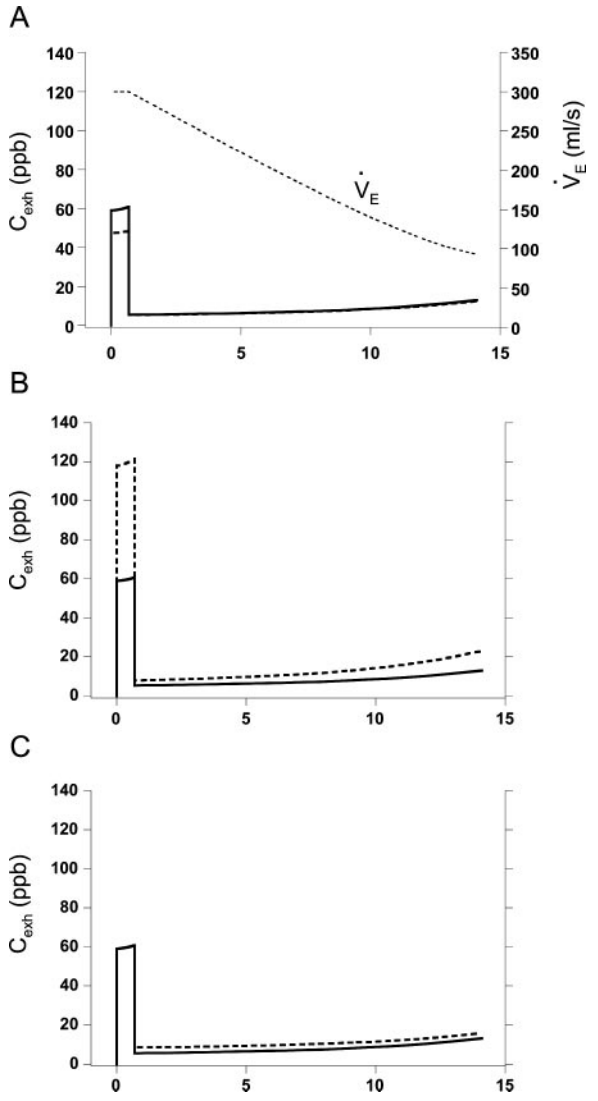

Fig. 3. The 2-compartment model prediction of the exhaled NO profile is shown for the single-exhalation maneuver with a 20-s preexpiratory breath hold. Representative values for lung volumes of a healthy adult have been used, and the "control" values for the flow-independent parameters are as follows: $D_{aw,NO} = 5\ pl \cdot s^{-1} \cdot ppb^{-1}$ (*A*); $J'_{aw,NO} = 750\ pl \cdot s^{-1}$ (*B*); $C_{alv,ss} = 3$ ppb (*C*). 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). *A*: the decreasing $\dot{V}_E$ is also shown on the *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 $D_{aw,NO}$ primarily impacts phases I and II, $C_{alv,ss}$ impacts primarily phase III, whereas $J'_{aw,NO}$ impacts all 3 phases. In addition, note that an increase in $D_{aw,NO}$ (while holding $J'_{aw,NO}$ and $C_{alv,ss}$) decreases the NO concentration in phases I and II if $J'_{aw,NO}$ (*Eq. 2*) and $C_{alv,ss}$ are held constant, but would increase the concentration in phases I and II if $C_{aw,NO}$ (*Eq. 2*) and $C_{alv,ss}$ were held constant (*Eq. 2*). In the former case, $C_{aw,NO}$ must be decreased to hold $J'_{aw,NO}$ constant (product of $D_{aw,NO} * C_{aw,NO}$), whereas, in the later case, $J'_{aw,NO}$ would increase as $C_{aw,NO}$ is constant.

asthma control questionnaire) between the two groups of subjects with asthma.

Of the 20 subjects with asthma, three of the steroid treated (*subjects 2*, *10*, and *12*) were not able to complete the 20-s breath hold, and thus we utilized a 10-s breath hold, which may increase the confidence interval of $D_{aw,NO}$ (44, 53). To highlight differences among groups in exhaled concentrations, a composite exhalation profile for each group was attained (Fig. 4) 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-s breath hold were excluded from the composite exhalation profile. Steroid-naive subjects with asthma had an increased concentration of NO in all phases of the exhalation profile compared with 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. 4*B*), 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 ($J'_{aw,NO}$) as opposed to an elevated $C_{alv,ss}$, which would cause a uniform increase in NO concentration over phase III (52, 53). The elevated $J'_{aw,NO}$ would result in a much larger $C_{NO,peak}$ than actually observed, and this results in an elevated $D_{aw,NO}$ as described below.

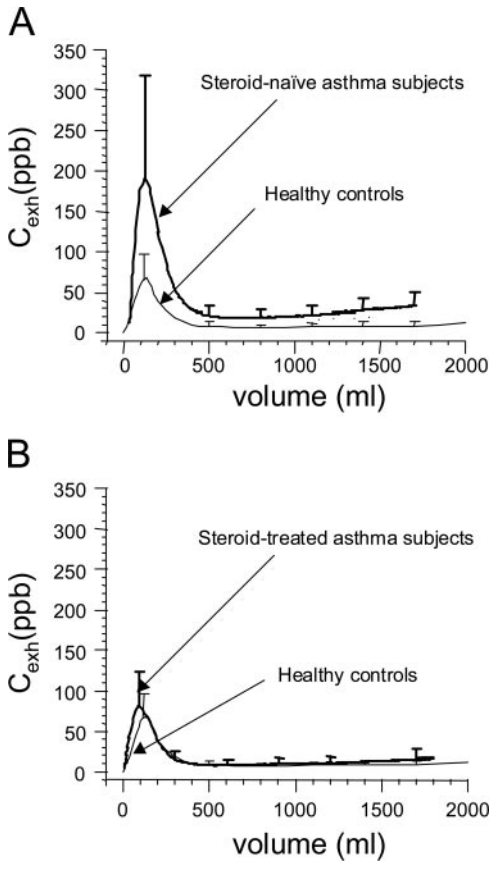

Fig. 4. *A*: composite experimental NO exhalation profiles are presented for the 20-s breath hold followed by a decreasing flow rate maneuver for steroid-naive (SN) asthma subjects (thick line) and healthy adults (HA) (thin line). *B*: composite experimental NO exhalation profiles are presented for the 20-s breath hold followed by a decreasing flow rate maneuver for steroid-treated (ST) asthma subjects (thick line) and HA (thin line). Values are means ± SD.

Mean (±SD) $C_{NO,peak}$ for steroid-naive, steroid-treated, and healthy subjects was 192 ± 127, 82 ± 42, and 67 ± 29 ppb, respectively. $C_{NO,peak}$ for steroid-naive subjects with asthma was statistically larger than that for the other two groups. W_{50} for steroid-naive, steroid-treated, and healthy subjects was 189 ± 60, 171 ± 49, and 190 ± 51 ml, respectively, and was not different among groups. $V_{I,II}$ for steroid-naive, steroid-treated, and healthy subjects was 657 ± 98, 604 ± 127, and 668 ± 142 ml, respectively, and was also not different among the groups.

As shown in Fig. 5, $J'_{aw,NO}$ and $D_{aw,NO}$ are elevated in steroid-naive subjects with asthma relative to healthy controls. The use of corticosteroids does not impact $D_{aw,NO}$, but is associated with a significantly lower $J'_{aw,NO}$ and $C_{aw,NO}$ that are equivalent to those in healthy adults. $C_{alv,ss}$ is not different among the three groups.

The experimental values of $C_{NO,plat}$ at the target flow rates of 50 and 250 ml/s, respectively, are presented in Table 3 (for healthy adults and subjects with asthma) along with the model-predicted $C_{NO,plat}$ (*Eq. 3*, $C^*_{NO,plat}$) at exhalation flow rates of exactly 50 and 250 ml/s. $C^*_{NO,plat}$ and $C_{NO,plat}$ were not statistically different from each other, with the exception of the steroid-naive group of asthmatic subjects at 250 ml/s (see Table 3). Statistical differences between the groups did not depend on the choice of $C_{NO,plat}$ or $C^*_{NO,plat}$. 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-naive and steroid-treated groups, respectively), statistical differences between groups are presented by using $C^*_{NO,plat}$ (Fig. 6). $C^*_{NO,plat}$ was 13.0 ± 5.97 and 5.17 ± 2.97 ppb for healthy adults, 53.9 ± 33.0 and 16.1 ± 9.46 ppb for steroid-naive adults with asthma, and 23.2 ± 14.3 and 7.76 ± 5.34 ppb for steroid-treated adults with asthma at flow rates of 50 and 250 ml/s, respectively. $C^*_{NO,plat}$ at 50 ml/s is significantly higher for both groups of subjects with asthma compared with healthy controls (Fig. 6), whereas only the steroid-naive subjects with asthma have a higher $C^*_{NO,plat}$ at 250 ml/s.

$D_{aw,NO}$ was inversely correlated with both FEV_1 (%predicted) and FVC (%predicted) (Fig. 7, *A* and *B*). In contrast, $C_{aw,NO}$ was positively correlated with FVC (%predicted). $J'_{aw,NO}$ and $C_{alv,ss}$ were not correlated with any lung function indexes. $C^*_{NO,plat}$ at either $\dot{V}_E$ was not correlated with indexes of lung function, but $C_{NO,plat}$ 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 present study, we estimate flow-independent NO exchange parameters with a single exhalation breathing technique and plateau C_{exh} by 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 $C_{NO,plat}$ at flow rates of 50 and 250 ml/s, as well as a decrease in the flow-independent parameters that reflect airway tissue concentration ($J'_{aw,NO}$ and $C_{aw,NO}$, respectively). In contrast, $D_{aw,NO}$

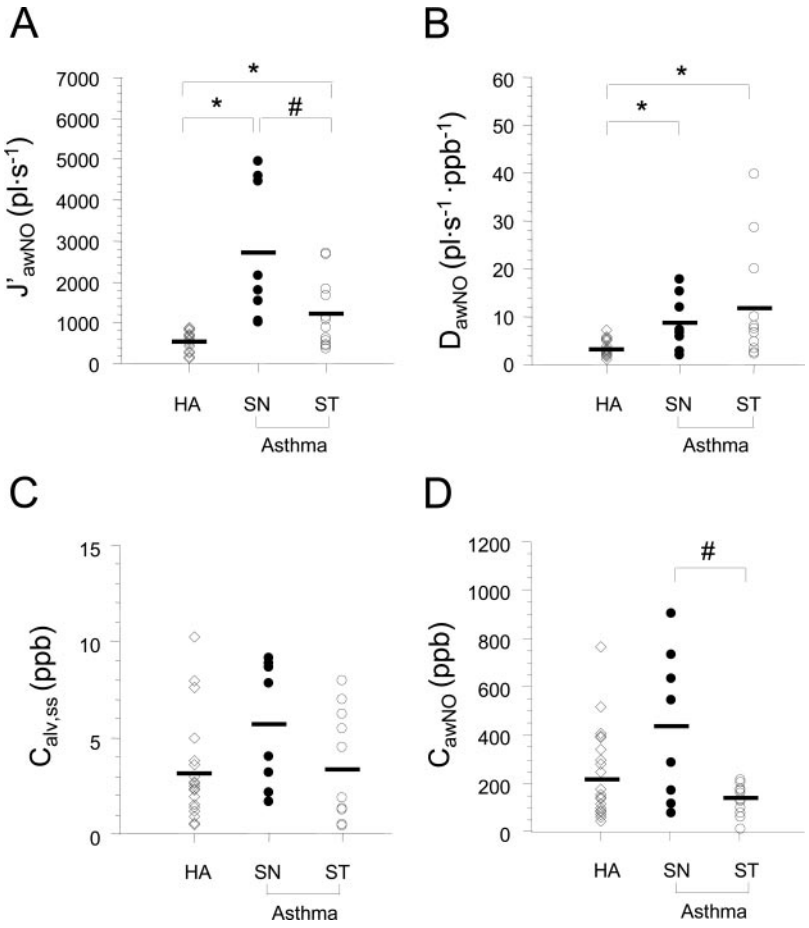

Fig. 5. Individual and population mean (solid bar) values of 4 flow-independent parameters (*A*: $J'_{aw,NO}$; *B*: $D_{aw,NO}$; *C*: $C_{alv,ss}$; *D*: $C_{aw,NO}$) for SN (●) and ST (○) asthma subjects and HA (◇). The mean ± SD $J'_{aw,NO}$, $D_{aw,NO}$, $C_{alv,ss}$, and $C_{aw,NO}$ for HA, SN, and ST are as follows: HA: 530 ± 234 pl/s, 3.13 ± 1.57 pl·s^{-1}·ppb^{-1}, 3.08 ± 2.39 ppb, and 220 ± 177 ppb; SN: 2,693 ± 1,687 pl/s, 8.71 ± 5.74 pl·s^{-1}·ppb^{-1}, 5.68 ± 3.22 ppb, and 438 ± 312 ppb; ST: 1,196 ± 837 pl/s, 11.8 ± 11.7 pl·s^{-1}·ppb^{-1}, 3.30 ± 2.74 ppb, and 143 ± 66 ppb, respectively. Statistically different from *HA and #SN subjects with asthma: $P < 0.05$.

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 analytic technique to estimate the flow-independent NO exchange parameters. In addition, we found that $D_{aw,NO}$ is inversely correlated with both FEV_1 and FVC (%predicted), independent of the presence of asthma and steroid use. Thus we confirm that $D_{aw,NO}$ may reflect physiological changes in the lungs that impact lung function independent of the use of corticosteroids.

Because the initial reports that F_{ENO} in asthma was elevated (1, 30), subsequent studies have focused on exploring the correlation between C_{exh} and other inflammatory markers (i.e., eosinophils), clinical interventions such as corticosteroids, and standard indexes of lung function (i.e., FEV_1/FVC). Corticosteroid treatment significantly decreases $C_{NO,plat}$ in subjects with asthma (31, 40, 41), and the dose of steroid is inversely related to $C_{NO,plat}$ (32). In addition, an increase in $C_{NO,plat}$ 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 present study, as well as that of Silkoff et al. (48), demonstrates the presence of steroid-independent factors (i.e., $D_{aw,NO}$) that can also contribute to the elevated levels of NO in the exhaled breath of asthmatic subjects.

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, whereas the airway wall flux of NO is reduced (15). In cystic fibrosis, the $D_{aw,NO}$ (transfer factor) is elevated, but the airway wall concentration is reduced, leading to an C_{exh} that is similar to that of healthy controls (45).

Silkoff et al. (48) first reported that $D_{aw,NO}$ is fourfold higher in subjects with asthma and that this increase is independent of steroid treatment, whereas $J'_{aw,NO}$ decreases. Lehtimaki et al. (38) then demonstrated that steroid treatment reduces $J'_{aw,NO}$ in newly diagnosed asthma subjects (previously steroid naive) by utilizing multiple constant-flow rate maneuvers (52, 54). Most recently, Hogman et al. (22) also recently demonstrated that $D_{aw,NO}$ 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 $D_{aw,NO}$ is inversely correlated with FEV_1 and FVC (%predicted) and $C_{aw,NO}$ is positively correlated with FVC. The positive correlation of $C_{aw,NO}$ with FVC is likely due to the fact that it is inversely related to $D_{aw,NO}$ (i.e., $C_{aw,NO} = J'_{aw,NO}/D_{aw,NO}$). Of note is the fact that Silkoff et al. (48) reported that values of $J'_{aw,NO}$, $D_{aw,NO}$, and $C_{aw,NO}$ 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

Table 3. *Model-predicted and experimental $C_{NO,plat}$ of subjects*

	$\dot{V}_E$ and $C_{NO,plat}$ Experimental Data				$C^*_{NO,plat}$ Model Predicted	
Subject No.	ml/s	ppb	ml/s	ppb	50 ml/s	250 ml/s
			Healthy adults			
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
			Steroid-naïve adults with asthma			
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 adults with asthma			
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†

$\dot{V}_E$, constant exhalation flow rate; $C_{NO,plat}$, nitric oxide plateau concentration in phase III; $C^*_{NO,plat}$, plateau concentration of nitric oxide predicted by the model; ppb, parts per billion; NC, data not collected; NA, not able to complete the maneuver. Statistically different from *healthy controls, †steroid-naive asthmatic subjects, and ‡$C_{NO,plat}$ at 250 ml/s (*t*-test with $P<0.05$).

investigate the relationship between NO flow-independent parameters and lung function.

C_{exh} necessarily reflects both the chemical and physical properties of the airway wall and alveoli, as well as the endogenous production rate from 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 by using the composite exhalation profile (Fig. 4*A*). Our laboratory (53) has previously demonstrated that only phases I and II are sensitive to changes in $D_{aw,NO}$ (if $D_{aw,NO}$ increases, less NO is exhaled in phases 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 $J'_{aw,NO}$ (if $J'_{aw,NO}$ increases, there is more NO exhaled in all phases, and the impact on phase III is a steeper slope) (see Fig. 3). Thus the observed

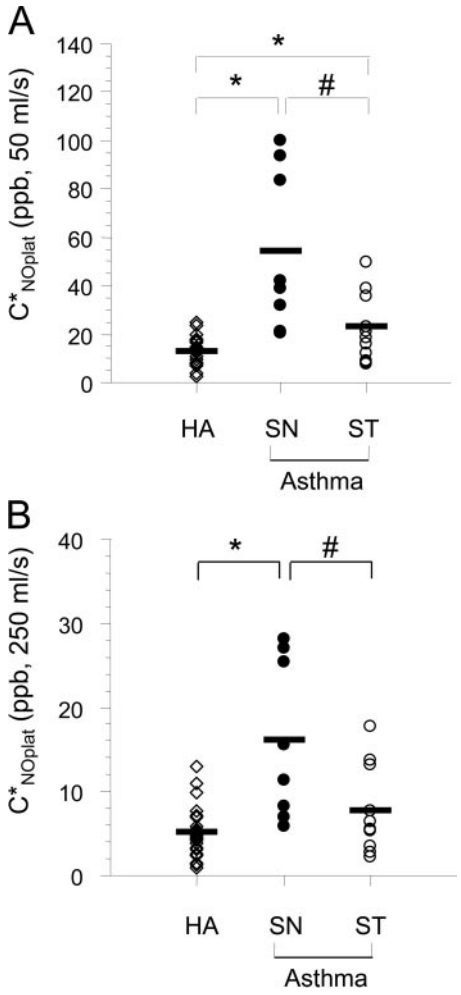

Fig. 6. Individual and population mean (solid bar) values of the plateau exhaled concentration for nitric oxide as predicted by the model ($C^*_{NO,plat}$; *Eq. 3*) using the flow-independent parameters for each subject. *A*: exhalation flow rate of exactly 50 ml/s; *B*: exhalation flow rate of exactly 250 ml/s. ●, SN; ○, ST; ◇, HA. Statistically different from *HA and #SN subjects with asthma: $P < 0.05$.

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 $J'_{aw,NO}$), yet a similar amount of NO in phases I and II (necessitating a larger $D_{aw,NO}$ to balance the increased $J'_{aw,NO}$). Of note is the fact that, among the parameters characterizing phases I and II of the exhalation profile, only $C_{NO,peak}$ differs among the groups (W_{50} and $V_{I,II}$ are not different among the three groups). This is consistent with altered NO production and transport in the airway wall during the breath hold, but also suggests that the volume accumulating NO during the breath hold and subsequently eliminated during exhalation is similar among the three groups.

Our laboratory has previously reported analytic expressions for the flow-independent parameters that approximate the functional dependence on the surface area emitting NO [A_i, where i is either airways (aw) or alveoli (alv); cm^2], solubility [partition coefficient ($\lambda_{t:air}$)], molecular diffusion [molecular diffusivity ($D_{t,NO}$); 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 ($S_{aw,NO}$) and alveolar ($S_{alv,NO}$) production rate per unit volume; ml $NO \cdot s^{-1} \cdot cm^{-3}$] (45, 52). The analytic 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.

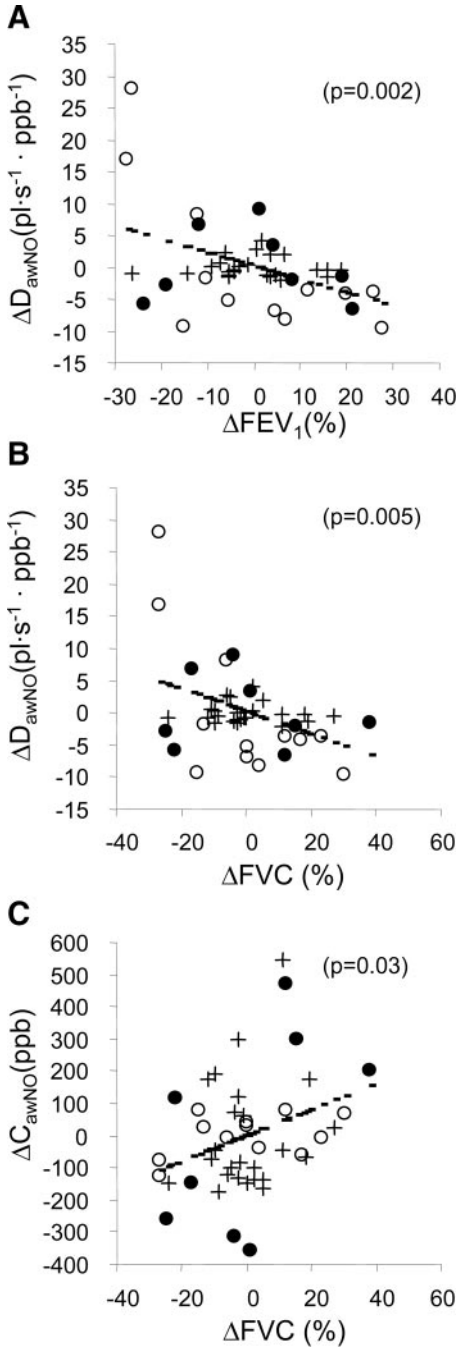

Fig. 7. Second-order partial correlation analysis demonstrates a significant inverse relationship between $D_{aw,NO}$ and forced expiratory volume in 1 s (FEV_1; %predicted) (*A*), $D_{aw,NO}$ and forced vital capacity (FVC; %predicted) (*B*), and a positive relationship between $C_{aw,NO}$ and FVC (%predicted) (*C*) in a total of 44 subjects. Δ, Difference between the individual score of each subject and the group mean value to which each subject belongs. +, HA (n = 24); ○, ST (n = 12); ●, SN (n = 8).

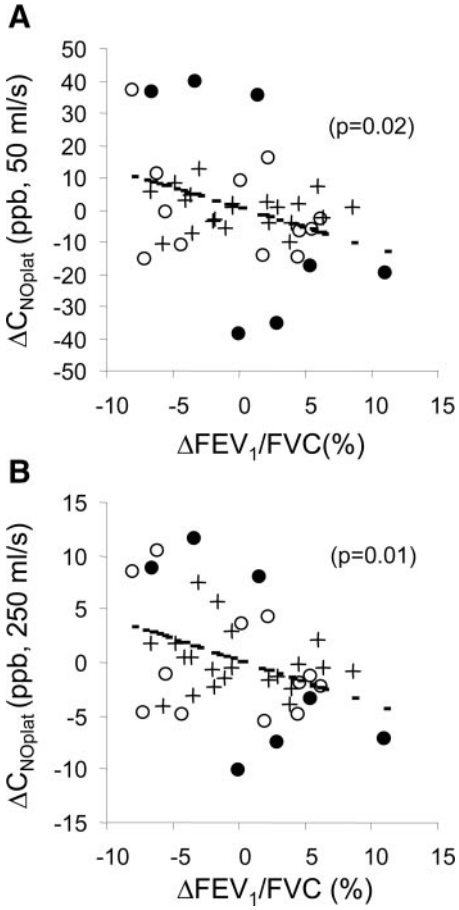

Fig. 8. Second-order partial correlation analysis demonstrates a significant inverse relationship between $C_{NO,plat}$ and ratio of FEV_1 to FVC (FEV_1/FVC; %predicted) at an exhalation flow rate of 50 ml/s (*A*) and 250 ml/s (*B*) in a total of 44 subjects. +, HA ($n = 24$); ○, ST ($n = 12$); ●, SN ($n = 8$).

$D_{aw,NO}$ is independent of $S_{aw,NO}$; is a positive function of total surface area of airway space (A_{aw}), $\lambda_{t:air}$, $D_{t,NO}$, and k; and is an inverse function of the thickness of the airway tissue layer ($L_{t,aw}$) (*Eq. B2* in APPENDIX B). Thus the increase in $D_{aw,NO}$ 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 mucus tends to be more viscous, which would decrease the "ease" at which NO can diffuse (i.e., decrease $D_{t,NO}$) (4). Both of these observations would decrease $D_{aw,NO}$ and contrast with our experimental observation, as well as that of Silkoff et al. (48), of an elevated $D_{aw,NO}$. Enhanced chemical consumption, primarily with superoxide (9), can increase $D_{aw,NO}$ due to an increase in the radial concentration gradient (4, 45). However, $D_{aw,NO}$ remains elevated after steroid treatment, which has been reported to suppress superoxide release (10).

An increase in A_{aw} is a plausible mechanism for the increase in $D_{aw,NO}$. Silkoff et al. (48) postulated that extension of the NO-producing nonadrenergic noncholinergic nerves from the large airways into the small airways may increase the A_i, which is supported both directly and indirectly by several studies (6, 7, 16, 17, 39, 55). Expression of inducible NOS (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.

$J'_{aw,NO}$ has a similar functional dependence on the physical and chemical parameters of the airways (A_{aw}, $D_{t,NO}$, and $L_{t,aw}$) (see APPENDIX B) as $D_{aw,NO}$. However, in contrast to $D_{aw,NO}$, $J'_{aw,NO}$ is inversely related to k and is a positive function of an additional parameter, $S_{aw,NO}$. An increase in $S_{aw,NO}$ by an increase in neuronal NOS expression from nonadrenergic noncholinergic 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 $J'_{aw,NO}$ for both steroid-naive and steroid-treated subjects with asthma. Other enzymatic and nonenzymatic 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 *S*-nitrosoglutathione catabolism (5, 11, 14, 49), could also increase $S_{aw,NO}$ and contribute to the increase in $J'_{aw,NO}$ for steroid-naive subjects with asthma.

Steroid treatment dramatically decreases the C_{exh} (see Fig. 5), which corresponds to observed decreases in $J'_{aw,NO}$ and $C_{aw,NO}$ as well as $C_{NO,plat}$ at both 50 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 $J'_{aw,NO}$, which is not observed. The decrease in $J'_{aw,NO}$ 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 upregulation (33). The decrease in $C_{aw,NO}$ (a ratio of $J'_{aw,NO}$ over $D_{aw,NO}$) for steroid-treated subjects with asthma is due to the decrease in $J'_{aw,NO}$, whereas $D_{aw,NO}$ is not changed.

In summary, we have estimated both flow-independent NO exchange parameters and plateau C_{exh} following ATS guidelines, in subjects with low FEV_1/FVC and a clinical history of asthma. $D_{aw,NO}$ is elevated independent of corticosteroid use, whereas $J'_{aw,NO}$, $C_{aw,NO}$, and $C_{NO,plat}$ (at both 50 and 250 ml/s) are all reduced by the use of steroids. In addition, $D_{aw,NO}$ 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 $D_{aw,NO}$ may reflect changes in the lungs that impact function and that are not impacted by steroid therapy and thus may provide clinical information not available from C_{exh} alone.

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 0–6, representing the absence of symptoms (score of 0) to severe symptoms (score of 6). 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 analytic expressions for the steady-state values of $J'_{aw,NO}$, $D_{aw,NO}$, and $C_{alv,ss}$ have been previously derived (52) and presented in a slightly different form (45, 51)

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

$$J'_{aw,NO} = S_{aw,NO}A_{aw}L_{t,aw}\cdot\left[\frac{1-\exp(-\xi_{aw})-\tanh(\xi_{aw})\exp(-\xi_{aw})}{\xi_{aw}\tanh(\xi_{aw})}\right] \quad (B2)$$

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

where $\xi_i = L_{t,i}/\sqrt{D_{t,NO}/k[/rt]}$, where k (s^{-1}) is the first-order rate constant that characterizes the rate of chemical consumption by substrates such as superoxide. The ξ_i represents the ratio of the rate of chemical consumption (k; s^{-1}) to the rate of molecular diffusion ($D_{t,NO}/L^2$; s^{-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 per second for $D_{aw,NO}$ that are equivalent in magnitude to picoliters per second per ppb.

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