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Shin, Hye-Won
Schwindt, Christina D
Aledia, Anna S
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Exercise-induced bronchoconstriction alters airway nitric oxide exchange in a pattern distinct from spirometry

Hye-Won Shin,[1] Christina D. Schwindt,[3,4] Anna S. Aledia,[1,6] Christine M. Rose-Gottron,[4] Jennifer K. Larson,[3,4] Robert L. Newcomb,[4,5] Dan M. Cooper,[1,3,4] and Steven C. George[1,2]

[1]*Department of Biomedical Engineering,* [2]*Department of Chemical Engineering and Materials Science,* [3]*Department of Pediatrics,* [4]*General Clinical Research Center,* [5]*Center for Statistical Consulting,* and [6]*Department of Medicine, Division of Pulmonary and Critical Care, University of California, Irvine, California*

Shin, Hye-Won, Christina D. Schwindt, Anna S. Aledia, Christine M. Rose-Gottron, Jennifer K. Larson, Robert L. Newcomb, Dan M. Cooper, and Steven C. George. Exercise-induced bronchoconstriction alters airway nitric oxide exchange in a pattern distinct from spirometry. *Am J Physiol Regul Integr Comp Physiol* 291: R1741–R1748, 2006. First published July 13, 2006; doi:10.1152/ajpregu.00178.2006.—Exhaled nitric oxide (NO) is altered in asthmatic subjects with exercise-induced bronchoconstriction (EIB). However, the physiological interpretation of exhaled NO is limited because of its dependence on exhalation flow and the inability to distinguish completely proximal (large airway) from peripheral (small airway and alveolar) contributions. We estimated flow-independent NO exchange parameters that partition exhaled NO into proximal and peripheral contributions at baseline, postexercise challenge, and postbronchodilator administration in steroid-naive mild-intermittent asthmatic subjects with EIB (24–43 yr old, $n = 9$) and healthy controls (20–31 yr old, $n = 9$). The mean ± SD maximum airway wall flux and airway diffusing capacity were elevated and forced expiratory flow, midexpiratory phase (FEF_{25-75}), forced expiratory volume in 1 s (FEV_1), and FEV_1/forced vital capacity (FVC) were reduced at baseline in subjects with EIB compared with healthy controls, whereas the steady-state alveolar concentration of NO and FVC were not different. Compared with the response of healthy controls, exercise challenge significantly reduced FEV_1 ($-23 ± 15\%$), FEF_{25-75} ($-37 ± 18\%$), FVC ($-12 ± 12\%$), FEV_1/FVC ($-13 ± 8\%$), and maximum airway wall flux ($-35 ± 11\%$) relative to baseline in subjects with EIB, whereas bronchodilator administration only increased FEV_1 ($+20 ± 21\%$), FEF_{25-75} ($+56 ± 41\%$), and FEV_1/FVC ($+13 ± 9\%$). We conclude that mild-intermittent steroid-naive asthmatic subjects with EIB have altered airway NO exchange dynamics at baseline and after exercise challenge but that these changes occur by distinct mechanisms and are not correlated with alterations in spirometry.

asthma; model; inflammation

NITRIC OXIDE (NO) appears in the exhaled breath and performs many functions in the lungs such as smooth muscle relaxation, host defense, inhibition of platelet aggregation, and neurotransmission. Much research effort has focused on utilizing the concentration of NO in the exhaled breath at a constant exhalation flow (Ce_{NO}) as a noninvasive marker of inflammation in diseases such as asthma (2, 5). Changes in Ce_{NO} during and after exercise have been reported (6, 8, 20, 29, 30, 33, 51), and more recently, alterations in Ce_{NO} have been reported to play a role in the pathogenesis of both exercise-induced (EIB) and thermally-induced bronchoconstriction (9, 21–23, 27, 38, 50).

EIB is thought to be triggered by increased heat and water losses from the airways during exercise (31), leading to airway inflammation and bronchoconstriction. However, even well-controlled steroid-treated asthmatics can experience EIB (4). Furthermore, baseline spirometry (forced expiratory volume in 1 s, FEV_1) cannot predict the presence of EIB (19), and Ce_{NO} is elevated at baseline in asthmatics independent of the presence of EIB (7). Thus a complete physiological understanding of alterations in spirometry and Ce_{NO} observed in EIB remains unknown.

There is a growing body of evidence that suggests Ce_{NO} has both a proximal (i.e., large airway) and peripheral (i.e., small airway and alveolar) contribution (18, 28, 35, 44, 52). This contrasts sharply with other endogenously produced gases, such as carbon dioxide, which are excreted mainly in the alveolar region of the lungs. In healthy adults, $>50\%$ of Ce_{NO} arises from the large airways (lobar bronchi and larger) (10, 43). It has been postulated that the elevated Ce_{NO} observed in asthma is due to an upregulation of one or more of the nitric oxide (NO) synthase (NOS) isoforms [inducible NOS, endothelial NOS, or neuronal NOS (1, 15, 17, 47, 55, 57)] and a peripheral extension of NO-producing cells in the airways (44). Thus our limited understanding of the role of NO in EIB is due, in part, to the fact that Ce_{NO} cannot distinguish proximal and more peripheral contributions.

Our group has previously described a two-compartment (airway and alveolar regions) model of NO exchange (52) and a single-breath technique (53) to estimate flow-independent NO exchange parameters: global maximum airway flux of NO ($J'aw_{NO}$), global airway diffusing capacity (Daw_{NO}), airway wall concentration of NO (Caw_{NO}), and steady-state alveolar concentration of NO (C_{ANO}) (14). The flow-independent NO exchange parameters can partition Ce_{NO} into proximal ($J'aw_{NO}$, Daw_{NO}, and Caw_{NO}) and peripheral (C_{ANO}) contributions and thus potentially provide insight into the mechanisms of NO exchange in EIB. The goal of the present study was to *1*) characterize regional (proximal and peripheral) NO exchange dynamics in steroid-naive mild asthmatic subjects with EIB at baseline, after exercise challenge, and after bronchodilator administration, and *2*) determine whether dynamic changes in NO exchange are correlated with standard indexes of spirometry.

Address for reprint requests and other correspondence: S. C. George, Dept. of Biomedical Engineering, 3120 Natural Sciences II, Univ. of California, Irvine, CA 92697-2715 (e-mail: scgeorge@uci.edu).

METHODS

Glossary

$A_{I,II}$	Area under the curve in phases I and II of exhaled NO profile (ppb·ml)
$C_{A_{NO}}$	Mixed or average fractional concentration of NO in gas phase of alveolar region (ppb; a steady-state concentration is achieved for breathhold or exhalation times >10 s)
Caw_{NO}	Airway wall concentration of NO (ppb)
$C_{E_{NO},i}$	Exhaled NO concentration at the mouth at constant exhalation flow i (ppb)
$Cobs_{NO}$	Exhaled NO concentration (ppb) observed by analytical instrument
$Cpeak_{NO}$	Maximum or peak exhaled NO concentration (ppb) observed by analytical instrument
Daw_{NO}	Diffusing capacity (ml/s) of NO in entire airway tree, expressed as volume of NO per second per fractional concentration of NO in gas phase [ml NO·s^{-1}·(ml NO/ml gas)$^{-1}$] and equivalent to pl·s^{-1}·ppb^{-1}
$J'aw_{NO}$	Maximum total volumetric flux of NO from airways (ppb·ml·s^{-1} or pl/s)
$V_{I,II}$	Exhaled volume in phases I and II of exhalation profile (ml)
V_{air}	Volume of airway tree (ml), defined by the subject's ideal body weight (lb) plus age in yr (53), which is very similar to the cumulative volume of airway generations 0–17 based on Weibel (56)
$\dot{V}_E$	Exhalation flow (ml/s)
W_{50}	Volume (or width) in phases I and II of exhaled NO signal, calculated by taking the volume at which the exhaled concentration is $>50\%$ of $Cpeak_{NO}$

Subjects

Nine steroid-naive mild-intermittent atopic asthmatic adults with a clinical history of EIB (ages 24–43 yr) and nine healthy adult controls (ages 20–31 yr) participated in this study. Inclusion criteria for the EIB group were a clinical history of mild-intermittent asthma, EIB, and a $>10\%$ decrease in FEV_1 following a 10-min exercise challenge (3). Exclusion criteria included a history of smoking, pulmonary diseases other than asthma, cardiovascular or neurological disease, current or previous use of a corticosteroid to manage asthmatic symptoms, or use of a bronchodilator in the 6- to 24-h (depending on the specific bronchodilator) preceding exercise testing. For the healthy adult group, inclusion criteria included no history of heart disease, lung disease, or smoking and normal standard spirometry [FEV_1/forced vital capacity (FVC) $> 80\%$ predicted]. The protocol was approved by the Institutional Review Board at the University of California, Irvine, and written informed consent was obtained.

Protocol

Subjects refrained from exercise and food for 72 and 3 h, respectively, before the study. Baseline exhaled NO measurements and spirometry were obtained from each subject. Spirometry included FVC, FEV_1, and forced expiratory flow, midexpiratory phase ($FEF_{25–75}$) measured in triplicate (V_{max} 229; Sensormedics, Yorba Linda, CA) according to American Thoracic Society (ATS) guidelines (3). Each subject then completed 10 min of exercise challenge (target intensity of 80% of the predicted maximum heart rate at room temperature and ~50% relative humidity) according to ATS guidelines (3), 5 min of recovery, measurement of exhaled NO and spirometry, inhalation of a bronchodilator (3 puffs of Combivent with spacer; Boehringer Ingelheim, Ridgefield, CT), 10 min of rest, and a final measurement of exhaled NO and spirometry. Each puff of Combivent delivers ~18 μg of ipratropium bromide and 103 μg of albuterol sulfate. Five of the nine subjects with EIB participated in a second control visit in which the exercise period was replaced with a rest period to determine whether spirometry impacted the NO exchange dynamics (24, 45, 49).

Exhaled NO Measurement

A chemiluminescence NO analyzer (Sievers, Boulder, CO) and pneumotachometer (Hans Rudolph, Kansas City, MO) were used to record NO, pressure, and flow for five repetitions in each subject of a 20-s preexpiratory breathhold followed by a decreasing exhalation flow maneuver (41, 53). The profiles were characterized by the peak concentration in phases I and II, $Cpeak_{NO}$, the volume (or width) of phases I and II, W_{50}, and the total mass of NO, $A_{I,II}$ (Fig. 1). In addition, with a two-compartment model (14, 52), the profiles were used to determine the flow-independent NO exchange parameters ($J'aw_{NO}$, Daw_{NO}, $C_{A_{NO}}$, and Caw_{NO}) as previously defined (14). The flow-independent parameters were then used to predict $C*E_{NO}$ (asterisk denotes calculated value) at a constant exhalation flow ($\dot{V}_E$) of 50 ml/s by using the relatively simple expression (53):

$$C*_{E_{NO},\dot{V}} = Caw_{NO} + (C_{A_{NO}} - Caw_{NO})\cdot\exp(-Daw_{NO}/\dot{V}_E) \quad (1)$$

Statistics

Untransformed data were analyzed using repeated-measures analysis of variance to detect differences between groups and within subjects over time. Log transformation did not improve the normality of the data distribution. In addition, contrast analyses were performed to assess differences between baseline and postexercise scores, between baseline and postbronchodilator scores, and between postexercise and postbronchodilator scores for each of the dependent variables. Paired comparison t-tests were employed to evaluate the differences between subjects' baseline scores and their scores at each of the other two time points over time. Statistical significance was considered at $P < 0.05$.

RESULTS

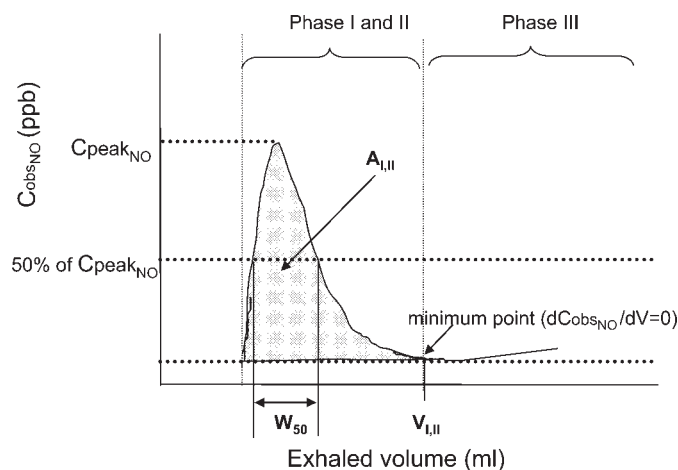The physical characteristics of the subjects, such as age, height, weight, and ideal body weight, were not different

Fig. 1. Model-independent parameters characteristic of the exhalation profile in phases I and II are defined schematically. $Cobs_{NO}$ is the exhaled NO concentration observed experimentally from the analytical instrument (ppb, parts per billion); $Cpeak_{NO}$ 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 at which the exhaled concentration is $>50\%$ of $Cpeak_{NO}$; $V_{I,II}$ is the volume of phases I and II; and $A_{I,II}$ is the total mass of NO (area under the curve, shown as a shaded region) in phases I and II. The distinction between phases I and II and phase III is the point of zero slope (minimum point) in the exhalation profile as previously described (53).

between subjects with healthy controls and EIB (Table 1). All subjects were able to complete the 10 min of targeted intensity exercise without complication.

FVC, FEV_1, FEF_{25-75}, and FEV_1/FVC at baseline, postexercise challenge, and postbronchodilator administration are presented in Table 2. At baseline, subjects with EIB had lower FEV_1 (%predicted), FEF_{25-75}, and FEV_1/FVC compared with the healthy controls. FEV_1 (liters) and FVC (liters, %predicted) were not significantly different between healthy controls and subjects with EIB. Postexercise, all four indexes of lung function (FVC, FEV_1, FEF_{25-75}, and FEV_1/FVC) were reduced in the subjects with EIB compared with the response of the healthy adults. Postbronchodilator, FEV_1, FEF_{25-75} and FEV_1/FVC were elevated relative to baseline compared with the response of the healthy controls.

All the subjects were able to complete the single breath maneuver with a 20-s preexpiratory breathhold. Composite NO exhalation profiles for subjects with EIB (baseline, postexercise challenge, and postbronchodilator administration) and healthy controls (only presented baseline) were generated by taking the mean exhaled concentration at equivalent exhaled volume intervals of all subjects in a given group and condition (e.g., EIB postexercise) and are presented in Fig. 2. The exercise challenge and bronchodilator administration did not impact the exhalation NO profile for healthy controls. In contrast, subjects with EIB had an increased concentration of NO in all phases of the exhalation profile. For subjects with EIB, significantly less NO was exhaled postexercise challenge, which can be seen by the reduced peak height in phases I and II, smaller area under the curve ($A_{I,II}$), and lower NO concentrations in phase III of the exhalation profile. In addition, bronchodilator administration increased the NO in all phases of the exhalation profile.

Mean (SD) $Cpeak_{NO}$ values for subjects with EIB at baseline, postexercise challenge, and postbronchodilator administration were 136 (104), 94 (53), and 112 (65) parts per billion (ppb), respectively. Mean (SD) $A_{I,II}$ values for subjects with EIB at baseline, postexercise challenge, and postbronchodilator administration were 32,600 (22,400), 24,300 (15,500), and 31,900 (19,600) ppb·ml, respectively. Both $Cpeak_{NO}$ and $A_{I,II}$ values are significantly smaller postexercise challenge compared with the baseline ($P < 0.05$). Mean (SD) W_{50} for subjects with EIB at baseline was 206 (52) and was not impacted by the exercise challenge or bronchodilator. Mean (SD) $Cpeak_{NO}$, W_{50}, and $A_{I,II}$ values for healthy controls at baseline were 72 (32) ppb, 190 (48) ppb, and 12,822 (6,571) ppb/ml respectively, and were not impacted by the exercise challenge or bronchodilator (see Fig. 2).

The experimentally observed changes in exhaled NO concentration in EIB are reflected in changes in the flow-independent NO exchange parameters. $J'aw_{NO}$ and Daw_{NO} were statistically elevated at baseline in subjects with EIB, whereas C_{ANO} and Caw_{NO} were not different compared with healthy controls (Fig. 3). For healthy controls, none of the flow-independent NO exchange parameters were significantly different after the exercise challenge or bronchodilator administration compared with baseline (Fig. 4, A, C, E, and G). $J'aw_{NO}$ was significantly decreased (mean change of -35%) after the exercise challenge relative to baseline in all nine subjects with EIB (Fig. 4B). Administration of the bronchodilator significantly increased $J'aw_{NO}$ relative to postexercise, but the difference relative to baseline was not statistically significant compared with healthy controls. Daw_{NO}, C_{ANO}, and Caw_{NO} were not statistically altered after exercise or bronchodilator administration (Fig. 4, D, F, and H). For the five subjects who participated in the second visit, the mean (SD) changes in

Table 1. *Physical characteristics of subjects*

Subject	Sex	Age, yr	Height, m	Weight, kg	BMI, kg/m^2	IBW, kg	V_{air}, ml	Therapies
				Healthy adults				
1	M	24	1.83	79.4	23.7	76	191	
2	M	27	1.65	75.3	27.7	64	168	
3	M	27	1.80	83.0	25.6	73	189	
4	F	23	1.57	54.4	22.1	54	143	
5	F	31	1.73	56.2	18.8	64	172	
6	F	22	1.73	81.2	27.1	64	163	
7	F	28	1.68	65.3	23.1	61	162	
8	F	26	1.60	50.8	19.8	56	150	
9	F	20	1.63	63.5	23.9	58	148	
Mean		25.3	1.69	67.7	23.5	63.4	165	
SD		3.39	0.09	12.4	3.02	7.26	17.0	
				Adults with EIB				
1	F	29	1.63	62.6	23.6	58.1	157	Albuterol, salmeterol
2	M	36	1.78	103	32.5	71.7	194	Albuterol
3	F	26	1.60	70.3	27.5	55.8	149	Albuterol
4	F	43	1.60	67.6	26.4	56.7	168	Albuterol
5	M	26	1.88	89.8	25.4	78.9	200	Albuterol
6	M	24	1.75	71.7	23.4	69.9	178	Albuterol
7	M	25	1.83	77.6	23.2	75.8	192	Albuterol
8	F	31	1.75	69.9	22.8	64.4	173	Albuterol
9	M	27	1.78	92.5	29.2	71.7	185	Albuterol
Mean		29.7	1.73	78.3	26.0	67.0	177	
SD		6.20	0.10	13.6	3.28	8.58	17.3	

EIB, exercise-induced bronchoconstriction (asthma); M, male; F, female; BMI, body mass index; IBW, ideal body weight; V_{air}, volume of the airway compartment estimated in ml as the sum of the subject's ideal body weight (lb.) plus age (yr.) (53).

Table 2. *Spirometry at baseline, postexercise challenge, and postbronchodilator*

	FVC, liters			FVC, % predicted			FEV$_1$, liters			FEV$_1$, % predicted			FEF$_{25-75}$, liters			FEV$_1$/FVC		
Subject	Base, liters	EX, %Δ	BD, %Δ	Base, %	EX, %Δ	BD, %Δ	Base, liters	EX, %Δ	BD, %Δ	Base, %	EX, %Δ	BD, %Δ	Base, liters	EX, %Δ	BD, %Δ	Base	EX, %Δ	BD, %Δ
Healthy adults																		
1	5.5	0.4	3.4	97	0.0	3.1	4.7	−0.8	2.5	96	−1.0	3.1	4.9	1.4	10.9	87	−1.1	−1.1
2	4.5	−0.2	0.0	105	−1.0	0.0	3.8	−1.3	−0.5	103	−1.9	−1.0	3.9	−4.3	−7.1	83	−1.2	2.4
3	5.4	0.4	1.7	97	0.0	2.1	4.4	−0.5	4.6	94	0.0	4.3	4.3	−2.6	10.9	82	−1.2	1.2
4	3.3	3.3	5.1	98	3.1	5.1	3.0	3.3	7.0	102	2.9	6.9	4.4	−2.5	13.5	90	0.0	3.3
5	4.4	0.7	0.7	111	0.9	0.9	3.8	1.6	4.5	113	1.8	4.4	4.3	−0.2	8.9	86	1.2	3.5
6	4.9	−1.0	−1.0	119	−0.8	−0.8	4.0	−3.7	1.0	115	−3.5	0.9	4.1	−11.1	0.7	82	−2.4	2.4
7	4.0	4.0	4.3	105	3.8	3.8	3.4	2.1	0.9	106	1.9	0.9	4.1	−10.0	−13.1	87	−2.3	−4.6
8	3.1	4.6	4.6	88	4.5	4.5	2.6	1.2	10.5	85	2.4	10.6	2.5	−5.1	30.0	84	−2.4	6.0
9	3.7	1.1	0.8	102	1.0	1.0	3.2	−8.8	−8.2	98	−9.2	−8.2	3.5	−28.2	−22.1	89	−12.4	−10.1
Mean	4.3	1.5	2.2	102	1.3	2.1	3.6	−0.8	2.5	101	−0.7	2.4	4.0	−7.0	3.6	86	−2.4	0.3
SD	0.9	2.0	2.2	9.0	2.0	2.2	0.7	3.7	5.3	9.4	3.8	5.3	0.7	9.0	15.8	3.0	3.9	4.9
Adults with EIB																		
1	3.7	−0.8	12.7	104	−1.0	12.5	2.4	−11.9	38.7	77	−11.7	39.0	1.4	−19.6	106	64	−12.5	21.9
2	3.4	−19.9	29.9	67	−19.4	29.9	1.9	−27.7	60.1	45	−28.9	60.0	1.0	−33.7	95.9	55	−7.3	23.6
3	3.1	−37.5	−2.9	90	−37.8	−2.2	2.2	−56.7	0.9	73	−56.2	1.4	1.5	−74.3	8.1	70	−30.0	4.3
4	2.1	−11.1	17.9	64	−10.9	18.8	1.4	−20.0	33.3	50	−20.0	32.0	0.8	−34.2	76.3	65	−9.2	13.8
5	5.4	−16.4	−1.3	85	−16.5	−1.2	3.7	−31.0	18.9	70	−31.4	18.6	2.5	−51.8	75.1	69	−20.3	18.8
6	5.1	−2.1	3.3	114	−2.6	3.5	3.3	−18.2	12.9	93	−18.3	12.9	2.0	−36.5	28.1	63	−15.9	9.5
7	5.6	−3.9	2.5	98	−4.1	2.0	4.1	−10.0	23.8	83	−10.8	22.9	3.1	−14.8	91.6	73	−6.8	20.5
8	3.8	−1.3	−0.5	94	−1.1	0.0	3.0	−11.7	2.3	89	−11.2	2.2	2.8	−39.0	19.1	80	−11.3	2.5
9	5.5	−12.7	−9.1	103	−12.6	−8.7	4.5	−17.1	−6.7	101	−17.8	−6.9	4.6	−27.9	2.0	82	−6.1	2.4
Mean	4.2	−11.8†	5.8	91.0	−11.8†	6.1	2.9	−22.7†	20.5†	75.7*	−22.9†	20.2†	2.2*	−36.9†	55.8†	69*	−13.3†	13.1†
SD	1.3	11.9	12.1	16.7	11.9	12.1	1.1	14.6	21.2	18.8	14.5	21.1	1.2	17.7	41.0	8.5	7.8	8.6

FVC, forced vital capacity; FEV$_1$, forced expiratory volume in 1 s; FEF$_{25-75}$, forced expiratory flow, midexpiratory phase; EX, postexercise challenge; BD, postbronchodilator administration; %Δ, percent change from baseline. *Statistically different from healthy controls at baseline ($P < 0.05$). †Difference between scores relative to baseline is statistically different from healthy controls ($P < 0.05$).

FEV$_1$ and J'aw$_{NO}$ after the 10-min rest period were −1.4 (1.3)% and −3.5 (18)% and did not represent significant changes from baseline.

Our group previously demonstrated that experimental values of C$_{E_{NO}}$ were not different from model-predicted (*Eq. 1*) C*E$_{NO}$ for healthy adults (41, 42) and steroid-naive asthmatics (40) at an exhalation flow of 50 ml/s. Thus C*E$_{NO}$ for subjects with EIB is presented at baseline in Fig. 3*B* and as a function of time in Fig. 4, *I* (healthy adults) and *J* (EIB). Relative to healthy controls, the trend for the changes in C*E$_{NO}$ in the subjects with EIB was the same as that for J'aw$_{NO}$. For healthy controls, C*E$_{NO}$ was not
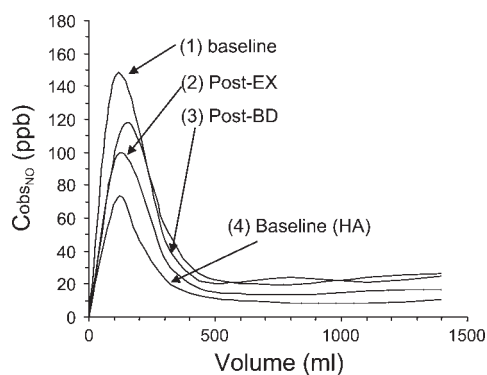

Fig. 2. Composite experimental NO exhalation profiles are presented for the 20-s breathhold followed by a decreasing flow rate maneuver for subjects with asthma [exercise-induced bronchoconstriction (EIB), $n = 9$] at baseline, postexercise (Post-EX) challenge, and postbronchodilator (Post-BD) administration and for healthy controls [healthy adults (HA), $n = 9$] at baseline.
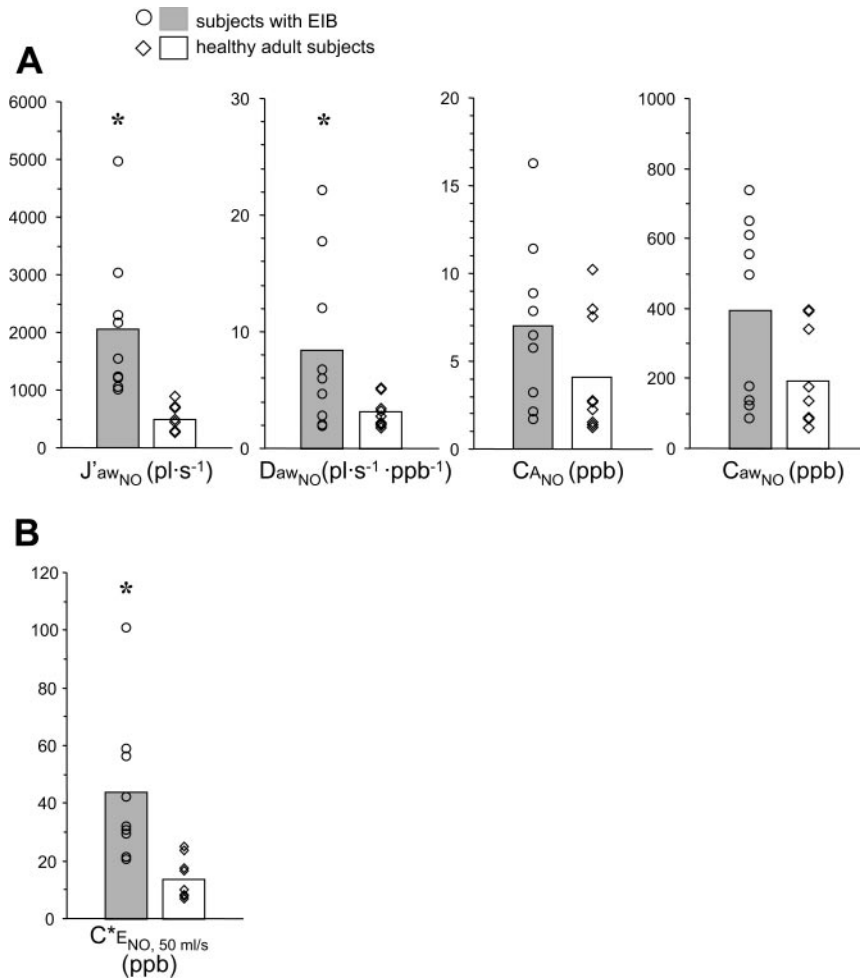
impacted by the exercise challenge or bronchodilator. None of the observed changes in J'aw$_{NO}$ and C*E$_{NO}$ postexercise and postbronchodilator administration had any significant correlation with changes in spirometric indexes (FVC, FEV$_1$, FEF$_{25-75}$, and FEV$_1$/FVC) postexercise and postbronchodilator administration for healthy control or EIB subjects.

DISCUSSION

This is the first study to examine the dynamic relationship in EIB between spirometric indexes and proximal and peripheral NO exchange. We found that elevated baseline exhaled NO concentration in subjects with EIB was due primarily to an increase in the airway diffusing capacity of NO, resulting in a larger maximum flux (J'aw$_{NO}$ = Daw$_{NO}$ × Caw$_{NO}$) of NO from the airway wall. EIB caused a significant decrease in J'aw$_{NO}$ without significant changes in Daw$_{NO}$ or Caw$_{NO}$. Bronchodilation following EIB returned J'aw$_{NO}$ to near baseline, but this response did not differ significantly from that of healthy controls. In addition, changes in spirometric indexes did not correlate with the airway NO parameters altered in EIB: J'aw$_{NO}$ and Daw$_{NO}$. We conclude that *1*) steroid-naive subjects with EIB have no alterations in alveolar NO but have an elevated baseline airway wall diffusing capacity and maximum airway wall flux of NO; *2*) EIB acutely reduces the maximum airway wall flux of NO by a mechanism distinct from the altered baseline NO exchange dynamics; and *3*) changes in airway caliber postexercise and postbronchodilator that impact spirometry (FVC, FEV$_1$, FEF$_{25-75}$, and FEV$_1$/FVC) do not correlate with changes in the NO exchange parameters.

Fig. 3. Mean values of global maximum airway flux of NO ($J'aw_{NO}$), global airway diffusing capacity (Daw_{NO}), steady-state alveolar concentration of NO (C_{ANO}), and airway wall concentration of NO (Caw_{NO}) with individual data points (*A*) and mean experimental values of exhaled NO concentration at the mouth at constant exhalation flow ($C*E_{NO}$) determined at an exhalation flow of 50 ml/s with individual data points at baseline (*B*) are presented in subjects with EIB and HA. *Statistically different from healthy controls at baseline.

Before the current study was performed, the relationship between exercise and exhaled NO had been investigated primarily using either exhaled concentration or the product of exhaled concentration and flow (elimination rate). In healthy subjects, exercise causes either no change or a small decrease in the exhaled concentration postexercise, with a large increase in the elimination of NO during exercise due to the increase in ventilation rate (while concentration stays relatively constant) (6, 8, 9, 20, 29, 30, 33, 34, 46, 51). These observed changes last for only a short time (<5 min) postexercise, until ventilation rate returns to baseline. In contrast, exhaled NO concentration in asthma has been reported to be significantly reduced shortly after exercise (9, 48, 50), and the degree of EIB is significantly associated with atopy and baseline exhaled NO (37). These observations are consistent with our current findings in asthmatic and healthy subjects. A major difference between asthmatic and healthy subjects is the observed changes in spirometry following exercise and bronchodilator administration, which reflect changes in the caliber of the airways. Thus region-specific analysis of NO exchange can potentially provide mechanistic insight into the altered NO exchange dynamics between healthy and asthmatic subjects.

There has been only one other study examining region-specific alterations in NO dynamics following exercise; however, this study (39) focused on healthy subjects and a more intense exercise challenge (20 min at 90% of the maximum

heart rate). In this case, Daw_{NO} acutely (3 min postexercise) increased, both Caw_{NO} and $J'aw_{NO}$ decreased, and there was no change in spirometry or C_{ANO}. The decrease in Caw_{NO} was attributed to enhanced losses of NO in the exhaled air during the period of exercise, or in other words, a washout of tissue NO stores. These changes were short-lived, and there was no difference from baseline at 30 min postexercise.

At baseline, our data demonstrate that C_{ANO} in subjects with EIB is not different from that in healthy controls. In contrast, $J'aw_{NO}$ is approximately fivefold higher in subjects with EIB. Most of this increase can be attributed to an increase (~3-fold) in Daw_{NO} and a modest increase in Caw_{NO}. Both $J'aw_{NO}$ and Daw_{NO} are proportional to the airway surface area emitting NO (39, 41). Thus the increase in $J'aw_{NO}$ and Daw_{NO} at baseline may be due to the peripheral extension of nonadrenergic, noncholinergic nerves from the large airways into the smaller airways (44) or the enhanced expression of inducible NOS in the airway epithelium (16), both of which may increase the airway surface area emitting NO. This pattern in the flow-independent NO parameters (i.e., significant elevation of Daw_{NO} and $J'aw_{NO}$ with no change in Caw_{NO} and C_{ANO}) at baseline is the same as that previously reported in steroid-naive asthmatic subjects (40, 44).

After an exercise challenge, all subjects with EIB demonstrated a marked decrease in only $J'aw_{NO}$ and no significant change in C_{ANO}, Daw_{NO}, or Caw_{NO}. This finding is consistent with a

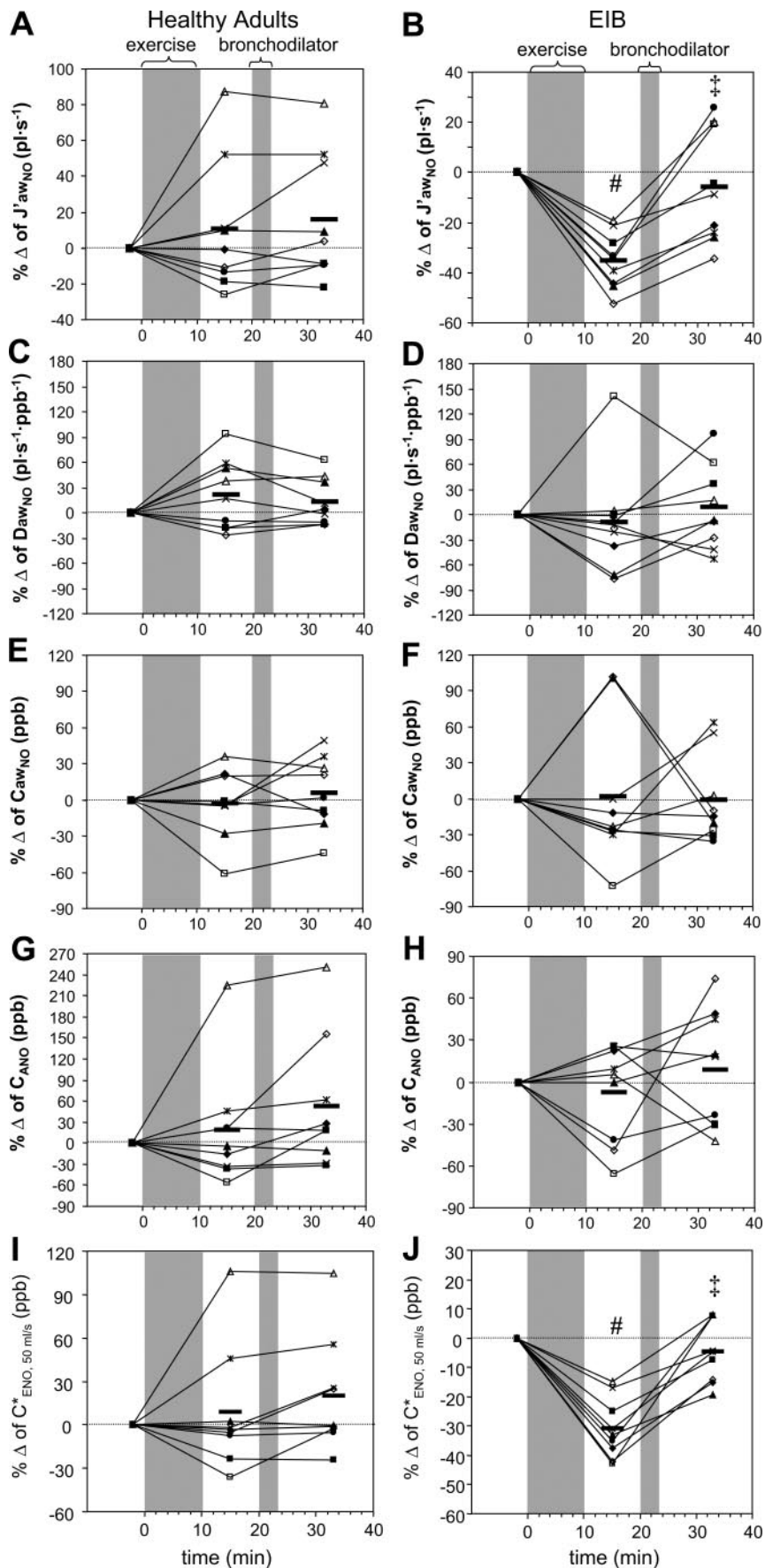Fig. 4. Percent changes of $J'aw_{NO}$ (A and B), Daw_{NO} (C and D), Caw_{NO} (E and F), C_{ANO} (G and H), and C^*E_{NO} (I and J; *Eq. 1*) at postexercise challenge and at postbronchodilator administration relative to baseline are shown in 9 healthy adult subjects (A, C, E, G, and I) and in 9 subjects with EIB (B, D, F, H, and J). Gray shading indicates the window of time for either the exercise challenge or the delivery of bronchodilator; horizontal solid bars indicate the mean value at each time point. #Difference between scores relative to baseline is statistically different from that of healthy controls ($P < 0.05$). ‡Difference between scores relative to postexercise challenge is statistically different from that of healthy controls ($P < 0.05$).

decrease in the airway (proximal) contribution of exhaled NO and no change in the alveolar (peripheral) contribution. Although most subjects demonstrated a mild decrease in Daw_{NO} and Caw_{NO} (Fig. 3, *B* and *D*), some demonstrated an increase. Thus the total airway flux of NO is decreased due to a combination of changes in Daw_{NO} and Caw_{NO}, and a washout of NO in the airway wall tissue cannot fully explain the observations. The observation that Daw_{NO} is altered at baseline but not in response to exercise leads us to conclude that alterations in NO exchange dynamics at baseline are due to mechanisms different from those that alter NO exchange following exercise.

As mentioned earlier, bronchoconstriction following exercise and bronchodilation following bronchodilator administration are major differences between the response of asthmatic and healthy subjects. These changes in airway caliber, which reflect the dynamic changes in FVC, FEV_1, FEF_{25-75}, and FEV_1/FVC, can impact exhaled NO in several ways. First, changes in the caliber of the airways impact the surface area of the airways (26). However, the observation that only $J'aw_{NO}$ is altered postexercise and postbronchodilator, when both $J'aw_{NO}$ and Daw_{NO} are proportional to surface area, suggests that determinants other than surface area are involved and that physical determinants of spirometry are decoupled with NO exchange dynamics.

Second, metabolic determinants may modulate exhaled NO and EIB, such as the production rate from NOS isoforms (9, 48), breakdown of *S*-nitrosothiols (11, 12, 36), the presence of eosinophils at sites of inflammation (25, 58), or altered mucus production and hydration (39). The later may impact not only the thickness of the diffusion barrier for NO but also the molecular diffusivity (the relative ease with which a molecule can diffuse through a medium), as previously described (39). Bronchoconstriction may occur preferentially at sites of greater inflammation (and thus higher NO production and release), leading to reduced ventilation or air trapping in these areas and a reduction in exhaled NO. This possibility is consistent with the observed mild decrease in FVC (and thus air trapping) following the exercise challenge.

Third, changes in airway caliber that lead to changes in spirometry are complex and only partially understood. Decreases in all of the spirometric indexes can reflect an increased resistance to expiratory air flow due to changes in either the proximal or peripheral airways without providing region-specific information regarding airflow limitation (13, 32). Most recently, it was demonstrated that bronchoconstriction and air trapping in asthma are the result of heterogeneous changes (contraction and dilation) in the airway caliber throughout the airway tree (26, 54). In contrast, exhaled NO may be predominantly from the larger airways (10, 43), thus weakening the relationship with spirometry.

Our two-compartment model makes a simplifying assumption that V_{air} does not change in response to EIB and the bronchodilator. Evidence supporting this assumption is the finding that W_{50} did not change in response to EIB or the bronchodilator. Nonetheless, we have previously reported (53) that only the estimation of Daw_{NO} is significantly impacted (an overestimation in V_{air} results in an overestimation in Daw_{NO}) by the estimate of V_{air}. Thus this interaction could potentially increase the estimated value of Daw_{NO} following EIB. The mean value that we reported for Daw_{NO} following EIB was slightly lower compared with baseline, but this change was not significant. However, if V_{air} was overestimated at this time point, Daw_{NO} would be overestimated and could potentially be further from the baseline.

In conclusion, we have quantified several flow-independent parameters characteristic of NO exchange in response to EIB and reported their dynamic relationship with spirometric indexes. The source of elevated exhaled NO at baseline in subjects with EIB is an elevated airway diffusing capacity that increases the airway flux of NO. There is a significant decrease in the airway wall flux of NO after EIB, and bronchodilation returns the airway wall flux to baseline without altering airway diffusing capacity and airway wall concentration. These observations do not correlate with changes in airway caliber. Thus structural changes in the airways that alter spirometric indexes (e.g., FEV_1) cannot completely account for changes in airway NO exchange dynamics. We conclude that elevated exhaled NO at baseline in subjects with EIB and reduced exhaled NO in acute EIB occur by distinct mechanisms, do not correlate with changes in spirometry, and thus reflect both anatomic and metabolic determinants of NO exchange.

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