EXERCISE-INDUCED BRONCHOCONSTRICTION ALTERS AIRWAY NITRIC OXIDE EXCHANGE IN A PATTERN DISTINCT FROM SPIROMETRY

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Running Title: Exercise-Induced Bronchoconstriction and Exhaled NO

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ABSTRACT

Exhaled nitric oxide is altered in asthmatic subjects with exercise-induced bronchoconstriction (EIB). However, the physiological interpretation of exhaled nitric oxide is limited due to its dependence on exhalation flow, and inability to distinguish completely proximal (large airway) from peripheral (small airway and alveolar) contributions. We estimated flow-independent nitric oxide exchange parameters that partition exhaled nitric oxide into proximal and peripheral contributions at baseline, post-exercise challenge, and post-bronchodilator administration in steroid naïve mild-intermittent asthmatic subjects with exercise-induced bronchoconstriction (24-43 years old, n=9) and healthy controls (20-31 years old, n=9). The mean±SD maximum airway wall flux (pl·s⁻¹) and the airway diffusing capacity (pl·s⁻¹·ppb⁻¹), were elevated while FEF_{25-75} (liters), FEV₁ (% predicted), and FEV₁/FVC were reduced at baseline in subjects with EIB compared to healthy controls (2050±1286, 8.4±7.3, 2.2±1.2, 75.7±18.8, and 69±8.5 compared to 484±227, 3.1±1.3, 4.0±0.7, 101±9.4, and 86±3.0, respectively) whereas the steady state alveolar concentration of nitric oxide, and FVC were not different. Compared to the response of healthy controls, exercise challenge significantly reduced FEV₁ (-23±15%), FEF_{25-75} (-37±18%), FVC (-12±12%), FEV₁/FVC (-13±8%) and the maximum airway wall flux (-35±11%), relative to baseline in subjects with EIB while bronchodilator administration only increased FEV₁ (+20±21%), FEF_{25-75} (+56±41%), and FEV₁/FVC (+13±9%). We conclude that mild-intermittent steroid naïve asthmatic subjects with EIB have altered airway nitric oxide exchange dynamics at baseline and following exercise challenge, but these changes occur by distinct mechanisms, and are not correlated with alterations in spirometry.

Keywords: NO, asthma, model, inflammation
INTRODUCTION

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 ($\text{CE}_{\text{NO}}$) as a non-invasive marker of inflammation in diseases such as asthma (2, 5). Changes in $\text{CE}_{\text{NO}}$ during and after exercise have been reported (6, 8, 20, 29, 30, 33, 51), and more recently alterations in $\text{CE}_{\text{NO}}$ 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 ($\text{FEV}_1$) cannot predict the presence of EIB (19), and $\text{CE}_{\text{NO}}$ is elevated at baseline in asthmatics independent of the presence of EIB (7). Thus, a complete physiological understanding of alterations in spirometry and $\text{CE}_{\text{NO}}$ observed in EIB remain unknown.

There is a growing body of evidence that suggests $\text{CE}_{\text{NO}}$ 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, more than 50% of $\text{CE}_{\text{NO}}$ arises from the large airways (lobar bronchi and larger) (10, 43). It has been postulated that the elevated $\text{CE}_{\text{NO}}$ observed in asthma is due to an
upregulation of one or more of the nitric oxide synthase isoforms (1, 15, 17, 47, 55, 57) (iNOS, eNOS or nNOS) 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.

We have 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 (D_{aw NO}), airway wall concentration of NO (C_{aw NO}), and steady state alveolar concentration of NO (C_{A NO}) (14). The flow-independent NO exchange parameters can partition CE_{NO} into proximal (J'_{aw NO}, D_{aw NO}, and C_{aw NO}) and peripheral (C_{A NO}) 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 naïve mild asthmatic subjects with EIB at baseline, post-exercise challenge, and post-bronchodilator administration, and 2) determine if dynamic changes in NO exchange are correlated with standard indices of spirometry.

**Glossary**

A_{I,II}: area under the curve in phase I and II of the exhaled NO profile (ppb·ml).

C_{A NO}: mixed or average fractional concentration of NO in the gas phase of the alveolar region (ppb). A steady state concentration is achieved for breathhold or exhalation times > 10 seconds.

C_{aw NO}: airway wall concentration of NO (ppb).
$C_{\text{E}_{\text{NO,i}}}$: exhaled NO concentration at the mouth (ppb) at constant exhalation flow “i”.

$C_{\text{obs}_{\text{NO}}}$: exhaled NO concentration (ppb) observed from the analytical instrument.

$C_{\text{peak}_{\text{NO}}}$: the maximum or peak exhaled NO concentration (ppb) observed by the analytical instrument.

$D_{\text{aw}_{\text{NO}}}$: diffusing capacity (ml/s) of NO in the entire airway tree, which is expressed as the volume of NO per second per fractional concentration of NO in the gas phase (ml NO s$^{-1}$ (ml NO/ml gas)$^{-1}$), and is equivalent to pl s$^{-1}$ ppb$^{-1}$.

$J'_{\text{aw}_{\text{NO}}}$: maximum total volumetric flux (ppb·ml s$^{-1}$ or pl/s) of NO from the airways.

$V_{I,II}$: exhaled volume in phase I and II of the exhalation profile (ml).

$V_{\text{air}}$: volume (ml) of the airway tree defined by the subjects ideal body weight (lbs) plus age in years (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).

$W_{50}$: the volume (or width) of phase I and II of the exhaled NO signal calculated by taking the volume at which the exhaled concentration is larger than 50% of $C_{\text{peak}_{\text{NO}}}$.

**METHODS**

*Subjects.* Nine steroid naïve mild intermittent atopic asthmatic adults with a clinical history of EIB (ages 24-43), and nine healthy adult controls (ages 20–31) 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-minute exercise challenge (3). Exclusion criteria included a history of smoking, pulmonary diseases other than
asthma, cardiovascular or neurologic disease, current or previous use of a corticosteroid to manage asthmatic symptoms, or use of a bronchodilator in the 6-24 hours (depending on the specific bronchodilator) prior to exercise testing. For the healthy adult group, inclusion criteria included no history of heart disease, lung disease, or smoking, and normal standard spirometry (FEV$_1$/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 hours, respectively, prior to the study. Each subject performed baseline exhaled NO measurements (see below) and spirometry. Spirometry included FVC, FEV$_1$, and FEF$_{25-75}$ measured in triplicate (Vmax229, Sensormedics, Yorba Linda, CA) according to ATS guidelines (3). Each subject then completed a 10-minute exercise challenge (target intensity of 80% of the predicted maximum heart rate and at room temperature, and approximately 50% relative humidity) according to ATS guidelines (3), 5 minutes of recovery, exhaled NO measurements and spirometry, inhalation of a bronchodilator (three puffs of Combivent with spacer; Boeringer Ingelheim, Ridgefield, CT), 10 minutes of rest, and a final measurement of exhaled NO and spirometry. Each puff of Combivent delivers approximately 18 µg and 103 µg of ipratropium bromide and albuterol sulfate, respectively. 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, Inc., Boulder, CO) and pneumotachometer (Hans Rudolph Inc., Kansas City, MO), were used to record NO, pressure, and flow for five repetitions in each subject of a 20-second pre-expiratory breathhold followed by a decreasing exhalation flow maneuver (41, 53). The profiles were characterized by the peak concentration in phase I and II, $C_{\text{peak}NO}$, the volume (or width) of phase I and II, $W_{50}$, and the total mass of NO, $A_{I,II}$ (Fig. 1). In addition, using a two-compartment model (14, 52), the profiles were used to determine the flow-independent NO exchange parameters ($J'_{awNO}$, $D_{awNO}$, $C_{A_{NO}}$, and $C_{awNO}$) as previously defined (14). The flow-independent parameters were then used to predict $C^{*}_{ENO}$ (* denotes calculated) at a constant exhalation flow ($\dot{V}_E$) of 50 ml/s using the relatively simple expression (53):

$$
C^*_{ENO,\dot{V}} = C_{awNO} + (C_{A_{NO}} - C_{awNO}) \cdot \exp\left(-D_{awNO} / \dot{V}_E\right)
$$

(1)

Statistics. Untransformed data were analyzed using repeated measure Analysis of Variance (ANOVA) 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 post-exercise scores, between baseline and post-bronchodilator scores, and between post-exercise and post-bronchodilator 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 $p < 0.05$. 
RESULTS

The physical characteristics of the subjects such as age, height, weight, and ideal body weight were not different between healthy controls and subjects with EIB (Table 1). All subjects were able to complete the 10 minutes of targeted intensity exercise without complication.

FVC, FEV$_1$, FEF$_{25-75}$ and FEV$_1$/FVC at baseline, post-exercise challenge, and post-bronchodilator administration are presented in Table 2. At baseline, subjects with EIB had a lower FEV$_1$ (% predicted), FEF$_{25-75}$ and FEV$_1$/FVC compared to the healthy controls. FEV$_1$ (liter) and FVC (liter, % predicted) were not significantly different between healthy controls and subjects with EIB. Post-exercise, all four indices of lung function (FVC, FEV$_1$, FEF$_{25-75}$, and FEV$_1$/FVC) were reduced in the subjects with EIB when compared to the response of the healthy adults. Post-bronchodilator, FEV$_1$, FEF$_{25-75}$ and FEV$_1$/FVC were elevated relative to baseline compared to the response of the healthy controls.

All the subjects were able to complete the single breath maneuver with a 20-second pre-expiratory breathhold. Composite NO exhalation profiles for subjects with EIB (baseline, post-exercise challenge and post-bronchodilator 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 post-exercise), 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 post-exercise challenge, which can be seen by the reduced peak height in phase 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) $C_{\text{peak NO}}$ for subjects with EIB at baseline, post-exercise challenge, and post-bronchodilator administration were 136 (104) ppb, 94 (53) ppb, and 112 (65) ppb, respectively. Mean (SD) $A_{I,II}$ for subjects with EIB at baseline, post-exercise challenge, and post-bronchodilator administration were 32,600 (22,400) ppb·ml, 24,300 (15,500) ppb·ml, and 31,900 (19,600) ppb·ml, respectively. Both $C_{\text{peak NO}}$ and $A_{I,II}$ are significantly smaller post-exercise challenge compared to 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) $C_{\text{peak NO}}$, $W_{50}$, and $A_{I,II}$ for healthy controls at baseline were 72 (32) ppb, 190 (48) ppb, and 12822 (6571) 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. $J'_{\text{aw NO}}$ and $D_{\text{aw NO}}$ were statistically elevated at baseline in subjects with EIB while $C_{\text{A NO}}$ and $C_{\text{aw NO}}$ were not different when compared to healthy controls (Fig. 3). For healthy controls, none of the flow-independent NO exchange parameters were significantly different following the exercise challenge or bronchodilator administration compared with baseline (Fig. 4A, C, E, and G). $J'_{\text{aw NO}}$ was significantly decreased (mean change of -
35%) following the exercise challenge relative to baseline in all nine subjects with EIB (Fig. 4B). Administration of the bronchodilator significantly increased $J'$awNO relative to post-exercise, but the difference relative to baseline was not statistically significant when compared to healthy controls. $D_{awNO}$, $C_{NO}$ and $C_{awNO}$ were not statistically altered following exercise or bronchodilator administration (Fig. 4D, F, and H). For the five subjects who participated in the second visit, the mean (SD) change in FEV$_1$ and $J'$awNO following the 10 minute rest period was -1.4 (1.3) % and -3.5 (18) % and did not represent a significant change from baseline.

We have previously demonstrated that experimental values of $C_{ENO}$ were not different from model-predicted (Eq. 1) $C*_{ENO}$ for healthy adults (41, 42) and steroid-naïve asthmatics (40) at an exhalation flow of 50 ml/s. Thus, $C*_{ENO}$ for subjects with EIB is presented at baseline in Fig. 3B and as function of time in Fig. 4I (Healthy Adults) and 4J (EIB). Relative to healthy controls, the trend for the changes in $C*_{ENO}$ in the subjects with EIB were the same as that for $J'$awNO. For healthy controls, $C*_{ENO}$ was not impacted by the exercise challenge or bronchodilator. None of the observed changes in $J'$awNO and $C*_{ENO}$ post-exercise and post-bronchodilator administration had any significant correlation with changes in spirometric indices (FVC, FEV$_1$, FEF$_{25-75}$ and FEV$_1$/FVC) post-exercise and post-bronchodilator administration for healthy control or EIB subjects.

**DISCUSSION**

This is the first study to examine the dynamic relationship in EIB between spirometric indices 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
\( J'_{\text{awNO}} = D_{\text{awNO}} \times C_{\text{awNO}} \) of NO from the airway wall. EIB caused a significant decrease in
\( J'_{\text{awNO}} \) without significant changes in \( D_{\text{awNO}} \) or \( C_{\text{awNO}} \). Bronchodilation following EIB
returned \( J'_{\text{awNO}} \) to near baseline but this response did not differ significantly from
healthy controls. In addition, changes in spirometric indices did not correlate with the
airway NO parameters altered in EIB – \( J'_{\text{awNO}} \) and \( D_{\text{awNO}} \). We conclude that: 1)
steroid naïve 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 post-
exercise and post-bronchodilator which impact spirometry (FVC, FEV\(_1\) and FEF\(_{25-75}\),
and FEV\(_1\)/FVC) do not correlate with changes in the NO exchange parameters.

Prior to the current study, the relationship between exercise and exhaled NO has
been investigated primarily using either exhaled concentration or the product of exhaled
concentration and flow (elimination rate). In healthy subjects, exercise either causes no
change, or a small decrease in the exhaled concentration post-exercise, 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 minutes) post-exercise, 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 asthma 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 is only one other study examining region specific alterations in NO dynamics following exercise; however, this study focused on healthy subjects and a more intense exercise challenge (20 minutes at 90% of the maximum heart rate) (39). In this case, \( \text{Daw}_{\text{NO}} \) acutely (3 minutes post-exercise) increased, both \( \text{Caw}_{\text{NO}} \) and \( \text{J'aw}_{\text{NO}} \) decreased, and there was no change in spirometry or \( \text{CA}_{\text{NO}} \). The decrease in \( \text{Caw}_{\text{NO}} \) 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 minutes post-exercise.

At baseline, our data demonstrate that \( \text{CA}_{\text{NO}} \) in subjects with EIB is not different from healthy controls. In contrast, \( \text{J'aw}_{\text{NO}} \) is approximately five-fold higher in subjects with EIB. Most of this increase can be attributed to an increase (~3-fold) in \( \text{Daw}_{\text{NO}} \) and a modest increase in \( \text{Caw}_{\text{NO}} \). Both \( \text{J'aw}_{\text{NO}} \) and \( \text{Daw}_{\text{NO}} \) are proportional to the airway surface area emitting NO (39, 41). Thus, the increase in \( \text{J'aw}_{\text{NO}} \) and \( \text{Daw}_{\text{NO}} \) at baseline may be due to the peripheral extension of non-adrenergic non-cholinergic nerves from the large airways into the smaller airways (44) or the enhanced expression of iNOS 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
DAwNO and J'awNO, with no change in CawNO and CA NO) at baseline is the same to that previously reported in steroid naïve asthmatic subjects (40, 44).

Following an exercise challenge, all subjects with EIB demonstrate a marked decrease in only J'awNO and no significant change in CA NO, D awNO, or C awNO. This is consistent with a 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 D awNO and C awNO (Fig. 3B and D), some demonstrated an increase. Thus, the total airway flux of NO is decreased due to a combination of changes in D awNO and C awNO, and a washout of NO in the airway wall tissue cannot fully explain the observations. The observation that D awNO 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 different mechanisms than 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, FEV1, FEF25-75, and FEV1/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'awNO is altered post-exercise and post-bronchodilator, when both J'awNO and D awNO 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 nitric oxide synthase (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 nitric oxide, but also the molecular diffusivity (the relative ease at 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 indices 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 has been demonstrated that bronchoconstriction and air-trapping in asthma is 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 the airway volume, $V_{air}$, 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 $D_{aw,NO}$ is significantly impacted (an overestimation in $V_{air}$ results in an
overestimation in $D_{aw NO}$) by the estimate of $V_{air}$. Thus, this interaction could potentially increase the estimated value of $D_{aw NO}$ following EIB. The mean value that we reported for $D_{aw NO}$ following EIB was slightly lower compared to baseline, but this change was not significant. However, if $V_{air}$ was overestimated at this time point, $D_{aw NO}$ 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 indices. 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 following 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 which alter spirometric indices (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 anatomical and metabolic determinants of NO exchange.
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49. **Tee AK and Hui KP.**


51. **Trolin G, Anden T, and Hedenstierna G.**

52. **Tsoukias NM and George SC.**


Table 1. Physical Characteristics of Subjects

(A) Healthy adults

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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

(B) Adults with exercise-induced asthma

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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

Gen: gender; Hgt: height; Wgt: body weight; BMI: body mass index; lwgt: ideal body weight; V_{air}: volume of the airway compartment estimated in ml as the sum of the subjects ideal body weight (lbs) plus age (yrs) (53)
Table 2. Spirometry at baseline, post-exercise challenge, and post-bronchodilator.

(A) Healthy adults

<table>
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## (B) Adults with exercise-induced asthma

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### FEF<sub>25-75</sub> FEV₁/FVC

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<tr>
<td><strong>SD</strong></td>
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<td><strong>17.7</strong></td>
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</table>

EX: post-exercise challenge, BD: post-bronchodilator 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)
FIGURE LEGENDS

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

Fig. 2: Composite experimental NO exhalation profiles are presented for the 20 second breathhold followed by a decreasing flow rate maneuver for subjects with asthma (EIB, n=9) at baseline, post-exercise challenge, and post-bronchodilator administration and at baseline for healthy controls (HA, n=9).

Fig. 3: (A) Mean values of $J'_{awNO}$, $D_{awNO}$, $C_{ANO}$, $C_{awNO}$ with individual data points and (B) mean experimental values of $C^{*}_{ENO}$ at exhalation flow of 50 ml/s with individual data points at baseline are presented in the subjects with exercise induced bronchoconstriction (solid gray bars with open circles) and healthy adults (open bars with open squares). * Statistically different at baseline from healthy controls.
Fig. 4: Percent change 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 post-exercise challenge and at post-bronchodilator administration relative to baseline (% change) are shown in nine healthy subjects (left panels -- A, C, E, G, and I) and in nine subjects with exercise-induced bronchoconstriction (EIB, right panels -- B, D, F, H, and J). The light gray rectangles indicate the window of time for either the exercise challenge or the delivery of the bronchodilator. The mean value at each time point is shown by the solid dark bar. # Difference between scores relative to baseline is statistically different from healthy controls. ‡ Difference between scores relative to post-exercise challenge is statistically different from healthy controls.
Fig 1

Exhaled volume (ml)

$C_{\text{obs,NO}}$ (ppb)

$C_{\text{peak,NO}}$

50% of $C_{\text{peak,NO}}$

$W_{50}$

$V_{I,II}$

Phase I and II

Phase III

minimum point ($dC_{\text{obs,NO}}/dV=0$)

$A_{I,II}$
Fig 2
Fig 3

A

- ○ subjects with EIB
- □ healthy adult subjects

B

Fig 3
Fig 4

A Healthy Adults

exercise bronchodilator

B EIB

exercise bronchodilator

C

D

E

F

Fig 4
Fig 4