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# Exercise-Induced Changes in Exhaled NO Differentiates Asthma With or Without Fixed Airway Obstruction From COPD With Dynamic Hyperinflation

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**Abstract:** Asthmatic patients with fixed airway obstruction (FAO) and patients with chronic obstructive pulmonary disease (COPD) share similarities in terms of irreversible pulmonary function impairment. Exhaled nitric oxide (eNO) has been documented as a marker of airway inflammation in asthma, but not in COPD.

To examine whether the basal eNO level and the change after exercise may differentiate asthmatics with FAO from COPD, 27 normal subjects, 60 stable asthmatics, and 62 stable COPD patients were studied. Asthmatics with FAO ( $n=29$ ) were defined as showing a postbronchodilator FEV<sub>1</sub>/forced vital capacity (FVC)  $\leq 70\%$  and FEV<sub>1</sub> less than 80% predicted after inhaled salbutamol (400  $\mu\text{g}$ ). COPD with dynamic hyperinflation ( $n=31$ ) was defined as a decrease in inspiratory capacity ( $\Delta\text{IC}\%$ ) after a 6 minute walk test (6MWT).

Basal levels of eNO were significantly higher in asthmatics and COPD patients compared to normal subjects. The changes in eNO after 6MWT were negatively correlated with the percent change in IC ( $r=-0.380$ ,  $n=29$ ,  $P=0.042$ ) in asthmatics with FAO. Their levels of basal eNO correlated with the maximum mid-expiratory flow (MMEF % predicted) before and after 6MWT. In COPD patients with air-trapping, the percent change of eNO was positively correlated to  $\Delta\text{IC}\%$  ( $r_s=0.404$ ,  $n=31$ ,  $P=0.024$ ).

We conclude that asthma with FAO may represent residual inflammation in the airways, while dynamic hyperinflation in COPD may retain NO in the distal airspace. eNO changes after 6MWT may differentiate the subgroups of asthma or COPD patients and will help toward delivery of individualized therapy for airflow obstruction.

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**Abbreviations:** 6MWT = 6 minute walk test, AHR = airway hyperresponsiveness, COPD = chronic obstructive pulmonary disease, DH = dynamic hyperinflation, eNO = exhaled nitric oxide, FAO = fixed airway obstruction, FEV<sub>1</sub> = forced expiratory volume in 1 second, FVC = forced vital capacity, IC = inspiratory capacity, ICS = inhaled corticosteroid, MMEF = maximum mid-expiratory flow.

## INTRODUCTION

Asthma is defined as a disease of reversible airflow obstruction but it is known that many patients with asthma also can end up with irreversible airflow obstruction.<sup>1</sup> In contrast to asthma, chronic obstructive pulmonary disease (COPD) is characterized by the presence of airflow limitation that is not fully reversible and is usually progressive, regardless of adequate therapy.<sup>2</sup> Distinctive patterns of airway inflammation and structural changes underlie the pathology of the 2 diseases.<sup>3,4</sup> However, there are limited methods to differentiate patients with severe asthma from patients with COPD. The phenotypic differences between COPD and asthma can be difficult to differentiate and recently the term “asthma-COPD overlap syndrome” has been used.<sup>5</sup> Both static (resulting from decreased elasticity of the lung parenchyma) and dynamic effects (occurring when patients commence inhalation before full exhalation has been achieved) of breathing contribute to lung hyperinflation in COPD,<sup>6,7</sup> which contributes to airflow obstruction. A proportion of adult asthmatic patients with poor response to treatment develops persistent airflow limitation despite appropriate bronchodilator therapy.<sup>1</sup> One of the earliest approaches to phenotyping asthma in a clinically relevant manner was to use the inflammatory cell composition of induced sputum.<sup>8,9</sup> Patients may be separated into eosinophilic or noneosinophilic phenotypes according to the analysis of sputum cells. Although inflammatory processes have been associated with the presence of airway hyperresponsiveness (AHR) in subjects with asthma, a positive response to the bronchodilators was found to be predictive of AHR in patients with persistent allergic asthma.<sup>10</sup> Some asthmatics with persistent airway obstruction demonstrate impaired bronchodilator response presumed to be secondary to structural changes of airway remodeling which may result from persistent airway inflammation.<sup>11</sup> This group of patients may clinically mimic COPD patients who also demonstrate airflow limitation and reduced bronchodilator response.<sup>5</sup>

Nitric oxide (NO), while produced by airway epithelial cells, circulatory endothelial cells, or inflammatory cells, has been reported to be involved in the bronchoconstriction,

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inflammation, and remodeling airway changes in asthmatics.<sup>12</sup> Measurements of exhaled nitric oxide (eNO) levels are considered to be a valuable marker of airway inflammation<sup>12</sup> and elevated in the exhaled breath of asthmatic patients.<sup>13</sup> The level of eNO has been reported to correlate with bronchoconstrictor response to methacholine,<sup>14</sup> and to be associated with the degree of decrease in forced expiratory volume in 1 second (FEV<sub>1</sub>) during exercise.<sup>15,16</sup> These data suggest that eNO level is likely to reflect both inflammation, as well as airflow limitations.<sup>17</sup>

Previously, quantification of dynamic hyperinflation (DH) due to airflow limitation has been derived from the complex use of on-line measurement of inspiratory capacity (IC) from flow-volume loops.<sup>18</sup> The 6 minute walk test (6MWT) now has been widely used as an objective measurement of functional status in patients with respiratory impairment.<sup>19</sup> During the 6MWT, incremental exercise causes increased minute ventilation primarily as a function of increases in tidal volume, and the increase in respiratory frequency at higher levels of exercise has been used to estimate DH in patients.<sup>18,20</sup>

However, since the levels of eNO before and after exercise in asthmatic and COPD patients remain to be poorly characterized,<sup>12</sup> we determined whether this could be used to detect DH. The aim of this study was to examine whether measurements of eNO prior to or after the 6MWT could be used as a marker of DH in COPD and asthma patients, and help to differentiate these 2 groups of patients in order to inform on treatment strategy.

## METHODS

### Study Population

This was a control, prospective study. Asthmatic and COPD patients, aged between 40 and 80 years and who are not current smokers or exsmokers, were recruited from outpatient clinics of Chang Gung Memorial Hospital, Linkou Medical Center in Taiwan. Patients who were previously diagnosed with bronchiectasis, cystic fibrosis, upper airways obstruction, bronchiolitis related to systemic disease, recent upper respiratory tract infection, or use of antibiotics within the last 6 weeks were excluded.

Sixty patients with asthma (aged  $57.8 \pm 2.1$  years, 21 women and 39 men) were documented with a clinical history of reversible airways obstruction with an increase in FEV<sub>1</sub> of  $\geq 12\%$  or more than 200 mL.<sup>21</sup> Patients were clinically stable for at least 3 months and were either on inhaled corticosteroids (ICSs) alone, or combination of ICS and inhaled long-acting  $\beta_2$  agonist, or combination of ICS, long-acting  $\beta_2$  agonist, and inhaled long-acting muscarinic antagonist. Patients discontinued their regular medicine for 24 hours, prior to performing spirometry before and after bronchodilator inhalation, and a 6MWT. 6MWT was done 2 days after spirometry. Asthmatic patients were divided into 2 groups: the fixed airway obstruction (FAO) group was defined as having postbronchodilator FEV<sub>1</sub>/FVC  $\leq 0.70$  and FEV<sub>1</sub> (% predicted) less than normal limit (FAO group,  $n = 29$ ) and those with no fixed airflow obstruction had FEV<sub>1</sub>/FVC  $> 0.70$  and FEV<sub>1</sub> within normal limit (non-FAO group,  $n = 31$ ). These definitions were irrespective of the magnitude of FEV<sub>1</sub> changes after administration of 400  $\mu\text{g}$  salbutamol.<sup>22</sup>

Sixty-two patients diagnosed with COPD (aged  $71.2 \pm 1.2$  years, all 62 men) had a postbronchodilator FEV<sub>1</sub> between 30% and 80% of predicted, with a postbronchodilator FEV<sub>1</sub>/FVC ratio of  $\leq 0.70$ , and a bronchodilator response of less than

200 mL or less than 12% change from baseline values.<sup>23</sup> Patients were stable for at least 3 months and were taking their regular medication (shown in Table 2) before entry into the study.

Twenty-seven healthy volunteers (aged  $51.2 \pm 2.9$  years, 15 women and 12 men) had normal lung function, negative methacholine challenge testing, negative serum specific IgE (Phadiatop), normal total IgE level, as well as blood eosinophil counts. Those with evidence of allergic rhinitis or asthma were excluded.

The institutional review board of Chang Gung Memorial Hospital approved the study. All participants gave their written informed consent.

### Exhaled NO Measurements

eNO was measured by NIOX MINO (Aerocrine AB, Sweden), a hand-held device, and can detect eNO as low as 5 ppb.<sup>24</sup> To exclude nasally produced NO, the participant exhaled against a fixed resistance resulting in closure of the velopharyngeal aperture. Ambient temperature and humidity were measured prior to testing. Participants received eNO measurements prior to and immediately after 6MWT.

### Six Minute Walk Test

6MWT was performed according to ATS guidelines.<sup>19</sup> Clinical staff educated all participants and explained the modified Borg dyspnea score before the test. Participants were asked to walk back and forth in a 35 m corridor. At each minute during the walk, a physiotherapist told participant the remaining time and gave encouragement. The participant stopped walk at the end of 6 minutes, and walking distance was recorded. Pulmonary function test, IC, and dyspnea score were recorded before and after walking. Heart rate and oxygen saturation were observed through the whole procedure. Spirometric parameters, including forced vital capacity (FVC), FEV<sub>1</sub>, maximum mid-expiratory flow (MMEF, FEF<sub>25%-75%</sub>), FEV<sub>1</sub>/FVC, IC, and together with eNO measurements, were performed before and at the end of 6MWT. DH after 6MWT was measured as change of IC ( $\Delta\text{IC}$ ).<sup>20</sup>

### Statistical Analysis

Data are reported as mean  $\pm$  SEM. Continuous variables were compared between groups using Student *t* test or the Mann–Whitney *U* test. Comparison between groups of category data was done using Fisher exact test, or the Chi-square method. Correlation between percent change of eNO and IC, or MMEF predicted value before and after 6MWT was calculated by Pearson or Spearman test. Statistical analysis was performed by using the Prism 6 software (GraphPad Software, Inc., La Jolla, CA). Significance was accepted at a *P* value less than 0.05.

## RESULTS

### Characteristics of Study Population

Twenty-seven normal subjects, 60 patients with asthma, and 62 COPD patients participated in this study (Table 1). Healthy control subjects and asthmatics were significantly younger than those of COPD. Patients with COPD had a lower body mass index, IC, and change of oxygen saturation after walking compared to healthy controls and asthmatics. FVC, FEV<sub>1</sub>, and MMEF (% predicted) were significantly different

TABLE 1. Demographic Data

	Normal Subjects	Asthma	COPD
Number	27	60	62
Gender, male <sup>†</sup>	12	39	62 <sup>**,*</sup>
Age, years	51.2 ± 2.9	57.8 ± 2.1	71.2 ± 1.2 <sup>**,*</sup>
BMI, kg/m <sup>2</sup>	25.1 ± 0.7	24.5 ± 0.4	22.8 ± 0.6 <sup>**,*</sup>
FVC, L	2.7 ± 0.2	2.6 ± 0.1	2.1 ± 0.1 <sup>**,*</sup>
FVC, % pred.	92.8 ± 3.1	86.5 ± 2.5	78.8 ± 2.5 <sup>**</sup>
FEV <sub>1</sub> , L	2.2 ± 0.2	1.7 ± 0.1 <sup>**</sup>	1.2 ± 0.1 <sup>**,*</sup>
FEV <sub>1</sub> , % pred.	93.8 ± 3.8	73.7 ± 3.2 <sup>**</sup>	62.2 ± 3.3 <sup>**,*</sup>
FEV <sub>1</sub> /FVC, %	82.2 ± 1.9	65.1 ± 1.6 <sup>**</sup>	55.9 ± 1.9 <sup>**,*</sup>
MMEF, % pred.	68.0 ± 4.7	36.2 ± 2.6 <sup>**</sup>	24.9 ± 2.0 <sup>**,*</sup>
IC before 6MWT, L	1.9 ± 0.1	1.9 ± 0.1	1.6 ± 0.1 <sup>**,*</sup>
ΔIC, %	7.9 ± 3.7	1.3 ± 2.5	-0.9 ± 2.2 <sup>*</sup>
Walking distance, m	518.1 ± 14.1	491.7 ± 14.5	392.2 ± 14.3 <sup>**,*</sup>
O <sub>2</sub> saturation, pre-6MWT, %	96.9 ± 0.3	96.1 ± 0.2	95.4 ± 0.3
ΔO <sub>2</sub> saturation, %	-9.2 ± 2.3	-7.1 ± 0.7	-13.2 ± 1.2 <sup>**,*</sup>

Data express as mean ± SEM. \**P* < 0.05, \*\**P* < 0.01 compared to normal subjects, \*\*\**P* < 0.05, \*\*\*\**P* < 0.01 compared to patients with asthma. BMI = body mass index, COPD = chronic obstructive pulmonary disease, FEV<sub>1</sub> = forced expiratory volume in 1 second, FVC = forced vital capacity, IC = inspiratory capacity, MMEF = maximum mid-expiratory flow, 6MWT = 6 minute walk test.

<sup>†</sup>Analyzed by Chi-square test.

among groups. COPD patients walked a significantly lower distance (392.2 ± 14.3 m, *n* = 62, *P* < 0.01) than normal subjects (518.1 ± 14.1 m, *n* = 27) and asthmatics (491.7 ± 14.5 m, *n* = 60).

**Exhaled NO (eNO) Before and After 6MWT**

Patients with asthma (30.4 ± 2.9 ppb, *n* = 60, *P* = 0.003) or COPD (27.1 ± 2.5 ppb, *n* = 62, *P* = 0.009) had higher levels of eNO than normal participants (18.3 ± 1.4 ppb, *n* = 27) at baseline (Figure 1A). A significant decrease in the change of eNO level after 6MWT was found in patients with COPD (-7.2 ± 3.9%, *n* = 62, *P* = 0.016) compared to the asthma group (1.1 ± 2.8%, *n* = 60, Figure 1B).

To analyze the difference in the response of eNO changes after walking between the patients with asthma and COPD, we

categorized asthmatics into FAO and non-FAO. COPD patients were divided into those with exercise-induced DH with (ΔIC < 0%; *n* = 31) or those without (ΔIC ≥ 0%, *n* = 31)<sup>20</sup> (Table 2). The baseline characteristics of FAO and non-FAO asthmatics were not different except for lower lung function including FVC, FEV<sub>1</sub>, MMEF (% predicted), and IC in FAO compared to non-FAO (Table 2). A higher proportion of asthmatics with FAO received combination therapy, whereas more non-FAO asthmatics were on ICSs alone (Table 2). There were no significant differences in terms of clinical characteristics and lung function between COPD patients with or without exercise-induced DH, except that the walking distance was lower in those with DH compared to those without DH.

No difference of eNO level before and after 6MWT was found in healthy controls (Figure 2A), and in asthma with non-

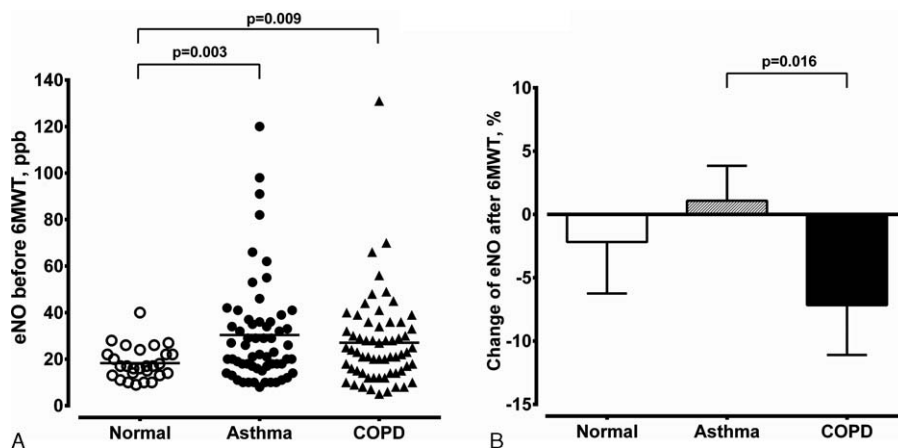


FIGURE 1. (A) Levels of eNO before 6MWT in normal participants (normal, *n* = 27), in patients with asthma (asthma, *n* = 60), and in COPD patients (COPD, *n* = 62). The horizontal line is mean. (B) Change in eNO after 6MWT in normal subjects (normal, *n* = 27), in patients with asthma (asthma, *n* = 60), and in COPD patients (COPD, *n* = 62). Data are expressed by mean ± SEM. COPD = chronic obstructive pulmonary disease, eNO = exhaled nitric oxide, 6MWT = 6 minute walk test.

**TABLE 2.** Clinical Characteristics of Different Groups of Patients With Asthma or COPD

	Asthma			COPD		
	Non-FAO	FAO	<i>P</i>	$\Delta IC \geq 0\%$	$\Delta IC < 0\%$	<i>P</i>
Number	31	29		31	31	
Gender, male*	19	20	0.595	31	31	1.000
Age, years	54.9 ± 3.1	60.9 ± 2.7	0.155	72.1 ± 1.6	70.9 ± 1.8	0.616
BMI, kg/m <sup>2</sup>	25.0 ± 0.7	23.9 ± 0.5	0.225	22.7 ± 0.8	22.5 ± 0.7	0.852
FVC, L	3.0 ± 0.2	2.1 ± 0.1	0.0001	2.1 ± 0.1	2.2 ± 0.2	0.622
FVC, % pred.	97.9 ± 3.0	73.9 ± 2.4	<0.0001	77.7 ± 3.0	80.1 ± 4.2	0.643
FEV <sub>1</sub> , L	2.2 ± 0.1	1.2 ± 0.1	<0.0001	1.1 ± 0.1	1.2 ± 0.1	0.810
FEV <sub>1</sub> , % pred.	91.3 ± 2.9	55.0 ± 3.2	<0.0001	62.0 ± 4.5	62.6 ± 4.8	0.930
FEV <sub>1</sub> /FVC, %	73.6 ± 1.4	56.1 ± 1.9	<0.0001	57.5 ± 2.9	54.5 ± 2.4	0.438
MMEF, % pred.	49.6 ± 3.1	21.8 ± 2.2	<0.0001	27.1 ± 3.2	22.4 ± 2.5	0.245
IC before 6MWT, L	2.2 ± 0.1	1.6 ± 0.1	<0.0001	1.5 ± 0.1	1.7 ± 0.1	0.079
$\Delta IC$ , %	2.1 ± 3.0	0.4 ± 3.9	0.738	11.8 ± 2.6	-13.8 ± 1.7	<0.0001
Walking distance, m	517.9 ± 22.7	463.7 ± 16.3	0.060	423.6 ± 11.6	357.4 ± 23.9	0.015
O <sub>2</sub> saturation, pre-6MWT, %	96.7 ± 0.3	95.5 ± 0.4	0.012	95.5 ± 0.4	95.4 ± 0.3	0.764
$\Delta O_2$ saturation, %	-6.8 ± 0.9	-7.4 ± 1.1	0.648	-13.4 ± 1.6	-12.8 ± 1.7	0.823
Medication over previous year <sup>†</sup>			0.002			0.884
ICS alone, n	15	5		0	0	
ICS with LABA, n	16	19		10	9	
LAMA, n	0	0		6	7	
ICS with LABA and LAMA, n	0	5		15	15	

Data express as mean ± SEM. BMI = body mass index, COPD = chronic obstructive pulmonary disease, FAO = fixed airway obstruction, FEV<sub>1</sub> = forced expiratory volume in 1 second, FVC = forced vital capacity, IC = inspiratory capacity, ICS = inhaled corticosteroid, LABA = long-acting  $\beta_2$  agonist, LAMA = long-acting muscarinic antagonist, MMEF = maximum mid-expiratory flow, 6MWT = 6 minute walk test.

\*Analyzed by Fisher exact test.

<sup>†</sup>Calculated by Chi-square test for trend.

FAO (Figure 2B) or FAO (Figure 2C) but the level of eNO after walking was significantly reduced from 29.6 ± 4.4 to 24.8 ± 2.8 ppb in COPD patients with DH (n = 31, *P* = 0.006, Figure 2D), but not observed in COPD patients without DH (Figure 2E).

### Delta eNO Correlated to Delta IC Before and After 6MWT

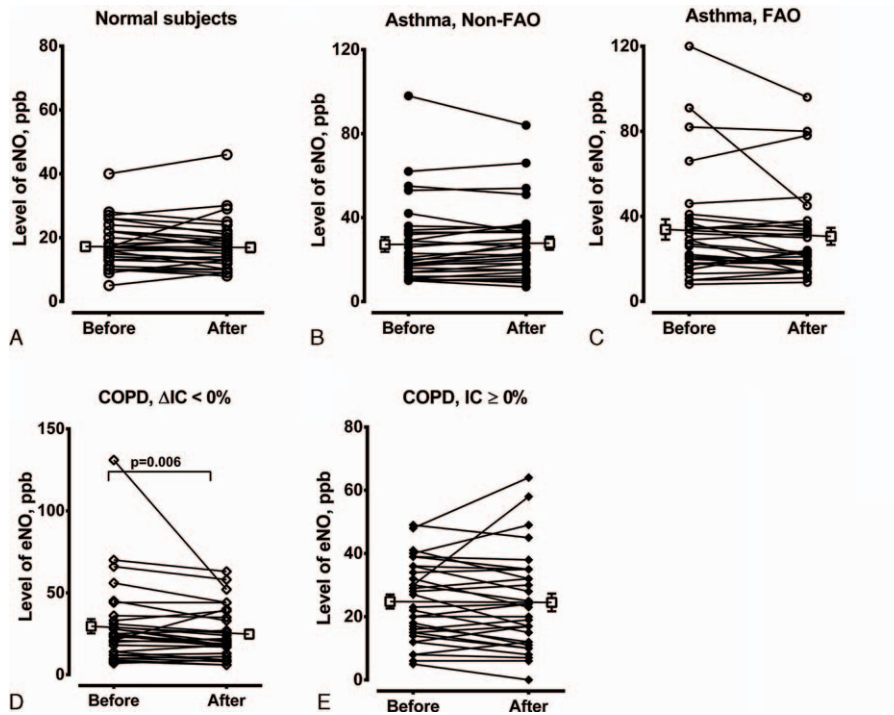
There was no association between delta eNO% and delta IC% after walking (*r* = -0.208, n = 27, *P* = 0.299) in normal subjects. For asthmatic patients, there was a significant negative correlation between delta eNO% and lower delta IC% in the FAO group (*r* = -0.380, n = 29, *P* = 0.042, Figure 3A), whereas a significant positive correlation between delta eNO% and delta IC% in the non-FAO group (*r* = 0.414, n = 31, *P* = 0.021, Figure 3B). There were significantly inverse associations between the delta eNO% and MMEF (% predicted) before exercise (*r* = -0.475, n = 29, *P* = 0.009, Figure 4A) or after exercise (*r* = -0.485, n = 29, *P* = 0.008, Figure 4B) in asthmatics with FAO, but not in the non-FAO group. There was no correlation between the delta eNO% and the % predicted value of FEV<sub>1</sub> or the percent change of FEV<sub>1</sub> before or after 6MWT in asthma either with FAO or without FAO. The baseline levels of eNO before walking were positively correlated to percent-predicted MMEF prior to (*r* = 0.467, *P* = 0.011) and after (*r* = 0.587, *P* = 0.008) 6MWT in the FAO asthma group, but not in the non-FAO group.

There was a significant positive correlation between delta eNO% and delta IC% after walking in COPD patients with DH

( $\Delta IC < 0\%$ , *rs* = 0.404, n = 31, *P* = 0.024, Figure 5B), but not in COPD patients without DH (Figure 5A). We also observed a significant positive correlation between delta eNO% and percent-predicted MMEF before (*rs* = 0.373, n = 31, *P* = 0.039) or after (*rs* = 0.393, n = 31, *P* = 0.029) walking in COPD patients with DH, but not in COPD without DH. However, there was no correlation between the delta eNO% and the % predicted value of FEV<sub>1</sub> at baseline or the percent change of FEV<sub>1</sub> caused by the 6MWT in COPD patients either with DH or without DH.

### DISCUSSION

We demonstrated that asthmatic patients can be separated into 2 groups according to different responses in eNO levels (delta eNO%) after 6MWT as function of IC (delta IC%) changes before and after exercise. Thus, in asthma patients with FAO, the delta eNO% negatively correlated with the delta IC%, whereas there was a significant positive correlation between delta eNO% and delta IC% in asthma patients without FAO. There was an inverse association between the delta eNO% and MMEF (% predicted) before or after 6MWT in asthmatics with FAO, but not in the non-FAO group. However, in COPD patients with DH after exercise, the delta eNO% positively correlated with the delta IC%. The different pattern of eNO changes after 6MWT is possibly related to the underlying inflammatory mechanisms. In asthmatic patients with FAO, there may be residual inflammation in the airways, with a resultant increased eNO level after exercise. In contrast, asthmatics without FAO had less airway inflammation and their eNO level after exercise was related to expiratory airflow

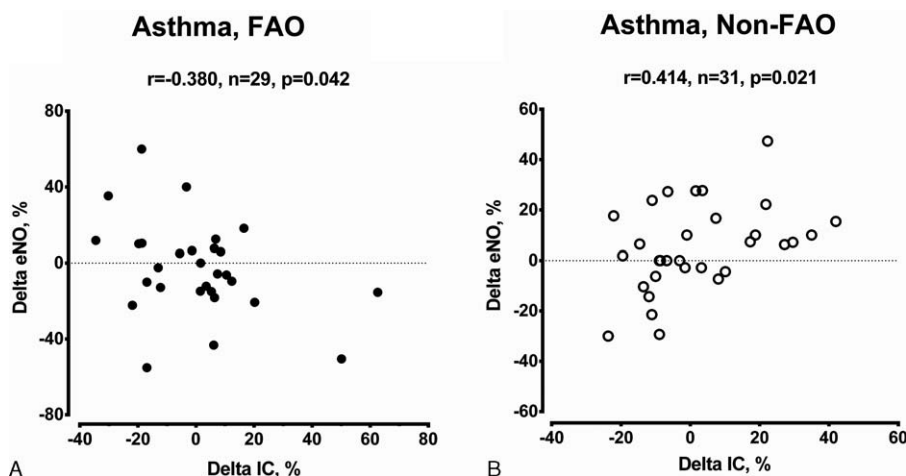


**FIGURE 2.** The level of eNO was measured prior to and after 6MWT in normal subjects (A) (n = 27), asthma patients with non-FAO (B) (n = 31) or FAO (C) (n = 29), and COPD patients with (D) ( $\Delta$ IC < 0%, n = 31) or without (E) ( $\Delta$ IC  $\geq$  0%, n = 31) dynamic hyperinflation on exercise. The P value is indicated. COPD = chronic obstructive pulmonary disease, eNO = exhaled nitric oxide, FAO = fixed airway obstruction, 6MWT = 6 minute walk test.

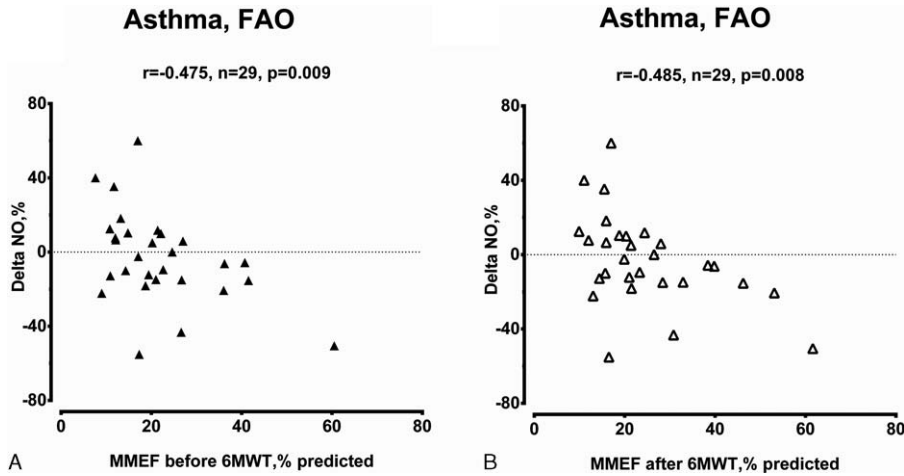
limitation. On the other hand, in COPD patients with DH, air-trapping during exercise may lead to the retention of NO in the distal airspaces.

Assessing airway and lung inflammation is important for investigating the underlying mechanisms of asthma and COPD. In our study, the baseline eNO was higher in asthmatics and COPD subjects than in normal subjects (Figure 1A), thus indicating the presence of persistent airway or lung

inflammation in stable asthmatic and COPD patients. Since airflow limitation is one contributing factor to final eNO measurements,<sup>12,16</sup> 6MWT clearly evoked airflow limitation and DH during exercise.<sup>20,25</sup> Exercise-induced variations in airway and alveolar eNO parameters revealed significantly different patterns of asthma and COPD. The clinical usefulness of 6MWT to discriminate NO gas exchange in patients with asthma and COPD remains to be established, and the



**FIGURE 3.** Correlation between the delta eNO% and the delta IC% in the asthma with FAO group (A) as well in the asthma with non-FAO group (B). The number and significance are indicated. eNO = exhaled nitric oxide, FAO = fixed airway obstruction, IC = inspiratory capacity.

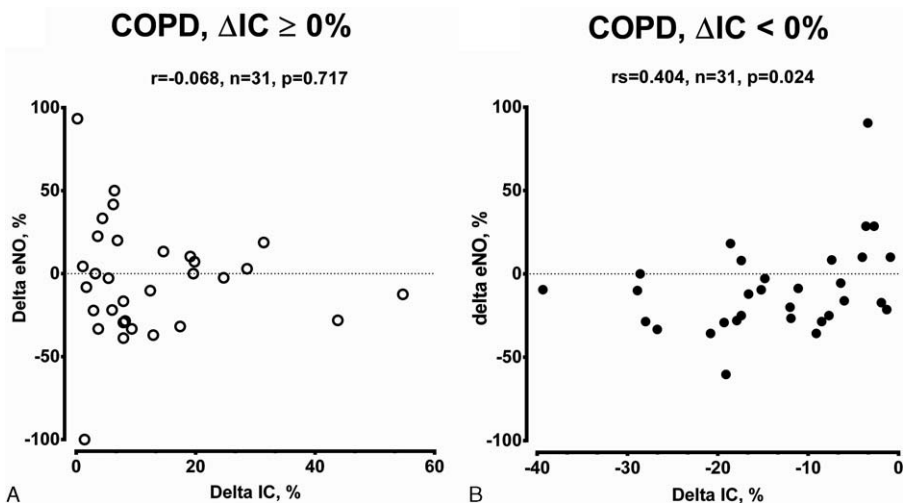


**FIGURE 4.** Correlations between the delta eNO% and percent-predicted MMEF (% predicted) before exercise (A) and after exercise (B) in asthmatics with FAO. The number and significance are indicated. eNO = exhaled nitric oxide, FAO = fixed airway obstruction, MMEF = maximum mid-expiratory flow.

contribution of peripheral smaller airways/alveolar compartments governing the increase or decrease in eNO after walking exercise remains to be determined.

There is evidence to support that eNO emanates from central and/or peripheral airways in asthmatics, and that these levels reflect the presence of airway inflammation, particularly eosinophilic inflammation.<sup>12</sup> The levels of delta eNO after exercise in asthma with FAO were increased as the decrease of IC after walking exercise, which may result from the narrowing airway by submaximal exercise of 6 minute walking. Our results are different from a previous report that eNO decreases during exercise-induced bronchoconstriction in children with asthma.<sup>16</sup> One might have expected that the association between the delta eNO and delta IC is similar in COPD and asthma with FAO. However, opposite results were observed. Asthma with FAO may have increased infiltration of the airways with eosinophils and CD4<sup>+</sup> lymphocytes, which

is accompanied by airway remodeling characterized by vasodilatation, microvascular leakage, and smooth muscle hypertrophy, thus these changes may play a role in the pathogenesis of fixed airflow obstruction.<sup>26,27</sup> The role of the small airways in producing airflow obstruction and AHR may be greater than that of larger airways.<sup>28,29</sup> In asthma with FAO, the basal level of eNO before walking or change of delta eNO after walking is positively correlated with the severity of MMEF, a parameter reflective of small airways obstruction.<sup>30,31</sup> Peripheral airway narrowing is a major determinant of airflow obstruction, because of more inflammation and increased smooth muscle mass, which exhibits increased contractility.<sup>32-34</sup> In addition, increased inflammation as evidenced by infiltration of inflammatory cells and upregulation of cytokines could be considered as the basis for the increased levels of eNO in small airways of asthma with FAO.<sup>11,13</sup> Therefore, high levels of eNO retained in the small airways, where increased levels of eNO correlated



**FIGURE 5.** Percent changes in IC% correlate with exhaled eNO% after walking in COPD patients without dynamic hyperinflation ( $\Delta IC \geq 0\%$ , A) and with dynamic hyperinflation ( $\Delta IC < 0\%$ , B). The number and significance are indicated. COPD = chronic obstructive pulmonary disease, eNO = exhaled nitric oxide, IC = inspiratory capacity.

with the decrease of IC after 6MWT in asthma with FAO. There was no correlation between the delta eNO% and the % predicted value of FEV<sub>1</sub> or the percent change of FEV<sub>1</sub> before or after 6MWT either in asthma with FAO or non-FAO. Thus, the small airways may contribute significantly to the eNO level found in asthma with FAO. In contrast, asthma with non-FAO may have less small airway inflammation and AHR, as well as homogeneous structure with greater reversibility. The change of delta eNO was negatively correlated to decreased IC in asthma with no FAO, which may result from DH after 6MWT, as reported in a previous study.<sup>16</sup>

In COPD, levels of eNO have been shown to be inversely related to FEV<sub>1</sub>, to the carbon monoxide diffusion capacity (DLCO), and to oxygen saturation (SaO<sub>2</sub>), and positively correlated with the residual lung volume/total lung capacity ratio.<sup>35,36</sup> Other studies reported increased<sup>35,36</sup> or reduced or unchanged levels<sup>37,38</sup> in stable COPD patients. Since progression of COPD from GOLD stage 0 to 4 is strongly associated with thickening of the small airway wall due to the remodeling process,<sup>39</sup> eNO production indicates that alveolar concentration of NO in COPD patients may reflect airway remodeling in addition to inflammation. Contrary to asthmatic patients, in COPD patients with exercise induced air-trapping, the percent change of eNO was positively correlated to delta IC after 6MWT. It is hypothesized that the eNO possibly originates from the peripheral airways in COPD patients,<sup>36</sup> and this may explain the decreased eNO in COPD subjects with air-trapping. Since COPD patients presented with heterogeneous etiology, in patients without exercise induced DH possibly represent a different phenotype of COPD. An HRCT study may help to identify different disease phenotypes based on structural changes, such as centrilobular (small airway component) or panlobular (parenchymal component) involvement.

One limitation of this study is the lack of the direct evidence of exercise-induced bronchoconstriction in asthmatics or COPD during the 6MWT. Further physiological observations of flow rate or resistance are necessary. Another limitation of the study is the lack of inflammatory evidence in the bronchi to relate to the levels of eNO.

## CONCLUSION

In conclusion, after a 6MWT, the elevated eNO resulted from persistent inflammation in the small airways in asthma with FAO, while the reduced eNO in COPD patients resulted from air-trapping changes and retained NO in the distal air-space. The eNO levels and DH changes after 6MWT may be helpful in differentiating subgroups of asthma and COPD patients that could be helpful in providing individualized therapy.

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