Exhaled Nitric Oxide and Exercise in Stable COPD Patients*

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Study objective: **To evaluate exhaled nitric oxide (eNO) during exercise in patients with stable COPD.**
Setting: **Outpatient evaluation in a rehabilitation center.**
Patients: **Eleven consecutive male patients with stable COPD (age, 65 ± 6 years; FEV_1, 56 ± 10% predicted). Eight healthy (six men; age, 51 ± 16 years) nonsmoking, nonatopic volunteers served as control subjects.**
Methods: **In each subject, a symptom-limited cycle ergometry test was performed by monitoring eNO with the tidal-breath method to assess eNO concentration (FENO) and output ($\dot{V}$NO) at rest, peak exercise, and recovery time.**
Results: **Resting FENO (9.8 ± 5.1 and 14.1 ± 6.3 parts per billion, respectively) and $\dot{V}$NO (4.2 ± 2.0 and 5.9 ± 3.4 nmol/min, respectively) were lower, although not significantly, in COPD patients than in control subjects. In both groups, FENO significantly decreased whereas $\dot{V}$NO significantly increased during exercise. Both variables returned to baseline during the recovery time. Peak exercise $\dot{V}$NO, but not FENO, was significantly lower in COPD patients than in control subjects (7.9 ± 5.4 and 12.7 ± 6.0 nmol/min, respectively, $p < 0.05$). The rise in $\dot{V}$NO was weakly correlated to oxygen consumption ($\dot{V}O_2$) both in control subjects ($r = 0.31$, $p = 0.002$) and in COPD patients ($r = 0.22$, $p = 0.03$). FENO showed an inverse correlation to $\dot{V}O_2$ in both groups ($r = -0.53$, $p = 0.000$; $r = -0.31$, $p = 0.003$ in control subjects and COPD patients, respectively).**
Conclusions: **In patients with mild and moderate COPD, eNO during exercise parallels that observed in normal control subjects. $\dot{V}$NO, but not FENO, is significantly reduced at peak exercise in COPD patients as compared with control subjects. The long-term effects of exercise training on eNO has to be evaluated by further studies.** ***(CHEST 2000; 117:702–707)***

Key words: chemiluminescence analyzer; chronic respiratory diseases; respiration

Abbreviations: eNO = exhaled nitric oxide; FENO = fractional exhaled nitric oxide concentration; NO = nitric oxide; ppb = parts per billion; $\dot{V}$E = minute ventilation; $\dot{V}$NO = eNO output; $\dot{V}O_2$ = oxygen consumption

Nitric oxide (NO) deriving from the lungs[1] has been recently detected in the expiratory air of both animals and humans.[2] Although the origin of exhaled NO (eNO) still remains unclear, it has been shown to increase in inflammatory diseases[3,4] as well as to decrease in conditions leading to chronic pulmonary hypertension.[5,6]

COPD is a condition characterized by progressive airflow obstruction[7] and a presumably chronic inflammation.[8] In COPD, eNO has been shown to be related, although weakly, to the degree of illness severity, being lower in the most severe patients.[9] It may be considered as a useful marker to monitor clinical instability in these patients.[10]

eNO during mild physical exercise has been studied in healthy subjects.[11] eNO concentration (FENO) decreased during physical exercise. However, taking the increased minute ventilation ($\dot{V}$E) into account, eNO markedly increased during exercise.[11] In a study of eNO during steady-state exercise in subjects with different levels of training, Maroun et al[12] found that only the athletes had a significant linear increase in eNO output ($\dot{V}$NO) with increasing oxygen consumption ($\dot{V}O_2$). These results suggest that physical conditioning increases $\dot{V}$NO during exercise.[12]

To the best of our knowledge, there is no information on eNO in COPD patients during exercise. It has been suggested that chronic inactivity and muscle deconditioning are important factors in the loss of muscle mass and strength and related reduction in exercise capacity in COPD patients.[13] Therefore, we

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wondered whether exercise would result in lower eNO in COPD patients than in sedentary healthy subjects. The aim of the present study was therefore to assess eNO during exercise in patients with mild to moderate COPD.

Materials and Methods

Patients gave their informed consent to participate into the study, which was approved by the Ethical Committee of Salvatore Maugeri Foundation IRCCS and was conducted according to the Declaration of Helsinki.

Patients

Eleven consecutive male patients with stable COPD were studied. Diagnosis of COPD was made according to the American Thoracic Society guidelines.[7] All the patients were well known in our institutions to which they were referred in the outpatient clinic for periodic medical visits and lung function testing. All patients were ex-smokers, and none had any history of atopy. At the time they were recruited for this study, all the patients were in stable condition, as assessed by stability in blood gas values and pH (> 7.35), and were free from exacerbation in the preceding 4 weeks. Patients with other organ failure, cancer, or inability to cooperate were excluded from the study. All patients were receiving their regular treatment with inhaled bronchodilators (anticholinergic drugs and rescue short-term β_2-agonists) and neither systemic nor inhaled steroids. No change in medical therapy was made the week before the study. Eight healthy, sedentary, nonsmoking, nonatopic volunteers served as control subjects. Demographic, anthropometric, and functional characteristics of patients and control subjects are shown in Table 1.

Measurements

Lung Function: Static and dynamic lung volumes were measured by means of a constant-volume body plethysmograph (Medical Graphic Corp; St. Paul, MN) with the patient in the seated posture according to standard procedure. The predicted values according to Quanjer[14] were used.

Table 1—*Anthropometric and Functional Characteristics of the Patients and Volunteers Studied

Characteristic	COPD Patients	Control Subjects	p Value
N	11	8	
Sex, male/female	11/0	6/2	
Age, yr	65 (6)	51 (16)	NS
Height, cm	168 (4)	171 (9)	NS
Weight, kg	77 (9)	72 (9)	NS
BMI	27 (3)	24 (1)	NS
BSA, m^2	1.87 (0.11)	1.84 (0.16)	NS
FEV_1, % predicted	56 (10)	109 (22)	< 0.01
FVC, % predicted	77 (10)	110 (25)	< 0.01
FEV_1/FVC, %	55 (12)	78 (6)	< 0.05
TLC, % predicted	108 (14)	98 (6)	NS
RV, % predicted	143 (41)	80 (18)	< 0.01

*Results are mean (SD) unless otherwise indicated.
BMI = body mass index; BSA = body surface area; TLC = total lung capacity; RV = residual volume; NS = not significant.

Exercise Test: Symptom-limited incremental exercise test was performed on an electrically braked cycle ergometer (Ergometrics 800S; Sensormedics; Yorba Linda, CA) using the standard 1-min incremental cycle exercise protocol. Functional and metabolic data were determined at rest and during exercise by means of a computerized system (model 2900Z; Sensormedics). Breathing pattern and $\dot{V}E$, $\dot{V}O_2$, and CO_2 production were continuously monitored as average values of 20-s intervals. ECG activity was monitored continuously, and systemic arterial BP was recorded every minute using a sphygmomanometer. After stabilization and a 2-min period of unloaded pedaling at 60 cycles/min, the load was increased by 10 W each minute. The patients were strongly encouraged to cycle to the point of intolerable breathlessness, discomfort, or exhaustion; until maximal heart rate was achieved or an abnormal ECG was noted; or to whenever the patient wanted to stop (symptom-limited exercise test).
NO Measurement: Patients and healthy subjects were asked to abstain from food ≥ 4 h and from alcohol ≥ 24 h before the experiment. In patients, the study was conducted ≥ 12 h after the last drug administration. During exercise, tidal eNO and CO_2 were obtained simultaneously over the last 20 s of each workload through a Teflon catheter connected to a side port of a special facial mask (7934 two-way NRBV-T-shape; Hans Rudolph Inc; Kansas City, MO) with a separate nose compartment excluding the mixing effect caused by nasal NO. eNO measurements were performed at rest and at each workload by means of a high-resolution (0.3 parts per billion [ppb]) chemiluminescence analyzer (LR 2000 series; Logan Research; Kent, UK) adapted for on-line recording of NO concentration. This feature obviates the need of collection into a reservoir with its variable loss of reactive NO: the sampling rate was 250 mL/min. Exhaled CO_2 was simultaneously assessed by single-beam infra-red absorption (resolution, 0.1%; response time, 0.2 s). Mouth pressure and airflow were also assessed. Ambient air was monitored for NO concentration immediately before the study; if NO concentration in the air was > 30 ppb, patient testing was delayed.

FENO was assessed by the tidal breathing method and recorded online as previously described.[15] The mean values of measurements obtained during the last 20 s of recording of each workload were considered. $\dot{V}NO$ from the airways of each subject at each workload was calculated as follows:

$$\dot{V}NO = \dot{V}E \times FENO / 24.04$$

where 24.04 corresponds to the volume of 1 mol of dry air at ambient temperature in standard conditions.[12]

Statistic Analysis

Results of measurements at rest, half peak, and peak exercise as well as at the third minute of recovery time were analyzed. All data are shown as mean ± SD. Within-subject reproducibility of eNO measurements was analyzed by analysis of variance for repeated measures with Huynh-Feldt correction. Between- and within-group differences were evaluated by analysis of variance; *post hoc* test with Bonferroni correction was then used when required. Spearman analysis was used to evaluate the correlation between eNO and exercise variables. A p value < 0.05 was considered to be statistically significant.

Results

Demographic, anthropometric, and functional characteristics of patients and control subjects are shown in Table 1. Age and anthropometric charac-

teristics of patients and control subjects were not different. According to the American Thoracic Society standards,[7] airway obstruction of COPD patients could be defined as stages I and II.

Resting and exercise cardiopulmonary variables of subjects in this study are shown in Table 2. Healthy, sedentary control subjects reached significantly higher levels of exercise capacity than COPD patients.

The time courses of mean values of FENO and $\dot{V}NO$ in COPD and control groups during exercise are shown in Figure 1. Resting FENO and $\dot{V}NO$ were lower, although not significantly, in COPD patients than in control subjects. In comparison with resting values, at peak exercise FENO significantly decreased in both groups (from 9.8 ± 5.1 to 5.0 ± 2.7 ppb and from 14.1 ± 6.3 to 6.2 ± 3.5 ppb in COPD patients and control subjects, respectively; $p < 0.0005$ for both) whereas $\dot{V}NO$ significantly increased (from 4.2 ± 2.0 to 7.9 ± 5.4 nmol/min, $p < 0.01$, in COPD patients; from 5.9 ± 3.4 to 12.7 ± 6.0 nmol/min, $p < 0.001$, in control subjects). Peak exercise $\dot{V}NO$ (7.9 ± 5.4 and 12.7 ± 6.0 nmol/min, respectively, $p < 0.05$), but not FENO, was significantly lower in COPD patients than in control subjects. Both FENO and $\dot{V}NO$ returned to baseline values in COPD patients and control subjects during recovery time (Fig 1).

The rise in $\dot{V}NO$ was correlated to $\dot{V}O_2$ both in control subjects ($r = 0.31$, $p = 0.002$) and in COPD patients ($r = 0.22$, $p = 0.03$). FENO showed an inverse correlation to $\dot{V}O_2$ in both groups ($r = -0.53$, $p = 0.000$; $r = -0.31$, $p = 0.003$ in control subjects and COPD patients, respectively).

DISCUSSION

To the best of our knowledge, this is the first study of eNO during exercise in COPD patients, and it shows that in mild to moderate COPD, eNO during exercise parallels that observed in normal control subjects. Peak exercise $\dot{V}NO$ but not FENO is significantly lower than in control subjects. Changes in eNO (as assessed by both output and concentration) were correlated to $\dot{V}O_2$ more in control subjects than in COPD patients.

Inasmuch as it has been shown that β_2-agonists may induce a significant increase in eNO,[16] in our patients, the study was performed ≥ 12 h after the last drug administration. In this study, we used the tidal-breath method to assess eNO. To standardize methods, a European Task Force[17] recently published recommendations on measurements of eNO and proposed that the single-breath method was preferable in adults and the tidal-breath method in children as well as in individuals unable to maintain steady-state exhalation during a slow exhalation maneuver, as in the case of exercise. It could be argued that measurement of NO in the mixed exhaled air by means of the tidal-breath method would not be able to indicate the real source of NO. Nevertheless, Gabbay et al[15] found a consistent relationship between end-exhaled NO concentrations in the lower airways and at the mouth. FENO recorded at rest in different respiratory conditions with the tidal-breath method is reported to be higher than when evaluated at the plateau point of the single-breath exhalation curve.[18] This is also the case of both our patients (9 ± 4 and 4 ± 2 ppb, $p < 0.001$ for FENO and plateau point NO, respectively) and control subjects (14 ± 6 and 7 ± 5 ppb, $p < 0.001$ for FENO and plateau point NO, respectively). Furthermore, we found a significant correlation among resting FENO as assessed by the tidal-breath method and peak ($r = 0.51$, $p < 0.02$) or plateau ($r = 0.55$, $p < 0.01$) values evaluated from the single-breath exhalation curve[17] in our sample study. Although the contribution of nasal NO decreases during exercise relative to rest, it still accounts for approximately 30% of total production,[19,20] and we are not certain that primarily

Table 2—*Cardiorespiratory Variables During Exercise

	Baseline		Peak		Recovery	
Variable	COPD Patients	Control Subjects	COPD Patients	Control Subjects	COPD Patients	Control Subjects
$\dot{V}E$, L/min	10.6 (1.9)	9.2 (2.6)	37.7 (14.0)	52.7 (14.4)†	16.7 (9.2)	16
RR, bpm	17.6 (2.8)	16.4 (3.1)	28.6 (4.4)	26.5 (3.6)	19.1 (3.1)	19.4 (4.2)
HR, bpm	86 (14)	80 (9)	124.3 (20)	159 (17)‡	98 (15)	104 (17)
W maximum			83 (21)	126 (30)†		
$\dot{V}O_2$, mL/min/kg	3.72 (0.6)	4.1 (1.1)	15.3 (5.8)	25.9 (5.1)†	4.77 (2.4)	5.6 (1.8)

*Data are presented as mean (SD); RR = respiratory rate; HR = heart rate.
†$p < 0.001$ vs COPD patients.
‡$p < 0.05$ vs COPD patients.

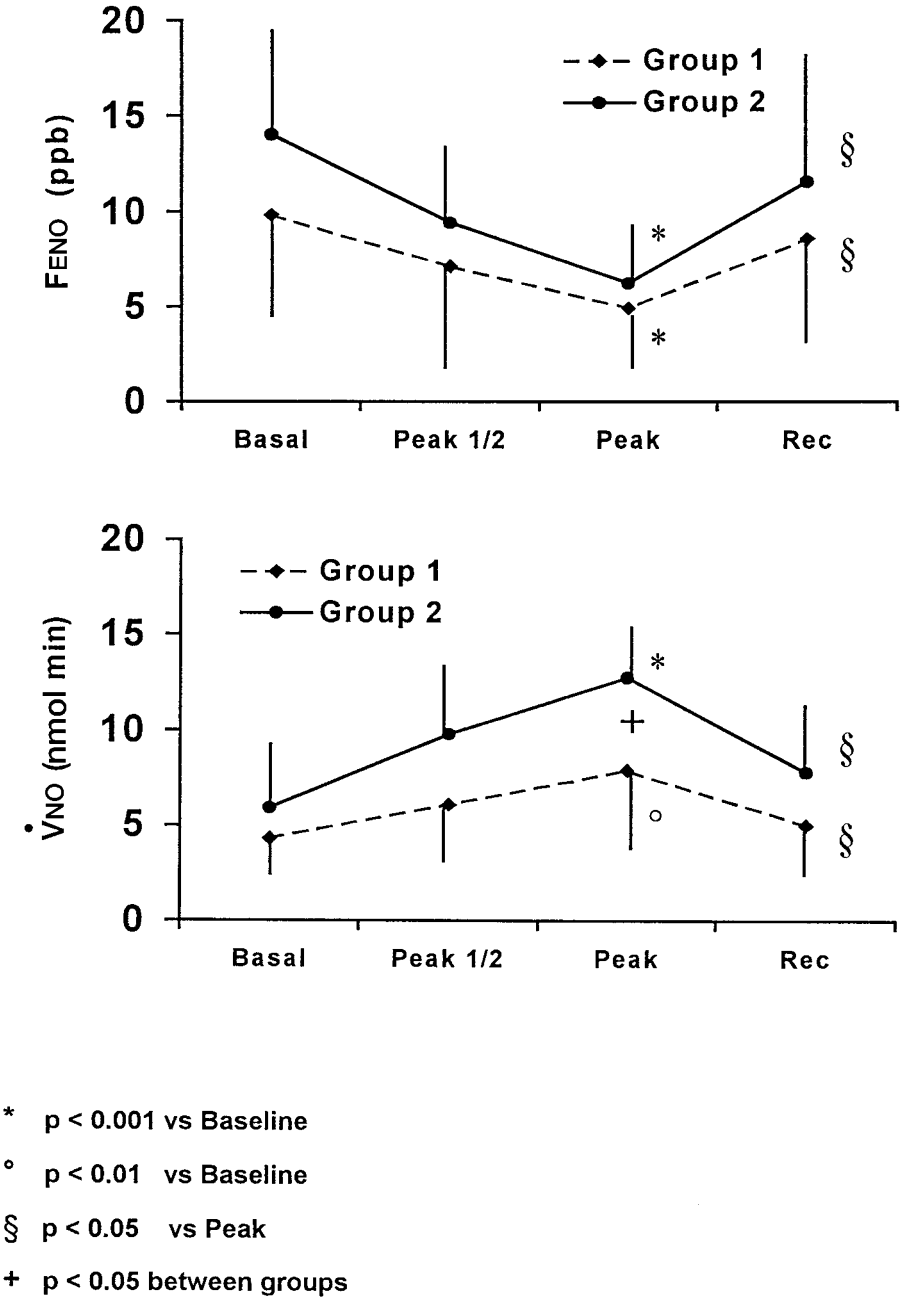

FIGURE 1. Time course of FENO (*top*) and V̇NO (*bottom*) in COPD (group 1) and control subjects (group 2) during exercise test evaluated at rest (Basal), half peak (Peak 1/2), peak exercise (Peak), and recovery (Rec).

lower airway NO is being sampled. Furthermore, the facial mask with a separate nose compartment that we used in this study has not been shown to clearly separate sinus from lower respiratory NO.

In this study, resting FENO was lower, although not significantly, in patients with mild and moderate COPD than in control subjects. Although in this study the lack of significant differences in resting eNO might be explained by the small sample size, in a previous study of eNO, assessed by the single-breath method,[17] the most severe COPD patients (stage III of the American Thoracic Society standards), but not stage I and II COPD patients, showed significantly lower concentrations of eNO than control subjects.[9]

The main result of our study is that V̇NO and FENO changed during exercise in COPD patients in a fashion similar to that observed in healthy sedentary

volunteers. The majority of the increase in eNO was related to the increase in $\dot{V}E$; therefore, most of the differences in $\dot{V}NO$ between COPD patients and healthy volunteers can be explained by the fact that these two groups did not reach the same peak $\dot{V}E$. Indeed, it has been reported that FENO from the airways of normal individuals during exercise decreases whereas output increases,[11,21] the latter being more closely related to increased $\dot{V}E$ than to increased blood flow.[20] A mathematical two-compartment model predicts that eNO from the nonexpansible airways and the expansible alveoli significantly contributes to the increased $\dot{V}NO$ with increasing ventilation.[22] Moreover, the reported increase in $\dot{V}NO$ during exercise in healthy individuals has been shown not to reflect an increase of systemic NO production.[23] In our study, the rise of $\dot{V}NO$ in COPD patients was only weakly related to an increase in $\dot{V}E$ ($r = 0.32$, $p = 0.001$), thus suggesting that other factors may be involved. Bauer et al[24] reported that changes in regional or total pulmonary blood flow, but not hyperventilation, may account for the increased stimulus for $\dot{V}NO$ during exercise in humans. Theoretically, we cannot exclude that different mechanisms related to ventilation-perfusion matching and gas exchange[25] could contribute to the behavior of eNO during exercise.

In a study of eNO during steady-state exercise in subjects with different levels of training, Maroun et al[12] found that only the athletes had a significant linear increase in $\dot{V}NO$ with increasing $\dot{V}O_2$ ($r = 0.75$). These results suggested that physical conditioning may induce an increase in $\dot{V}NO$ during exercise.[12] This might be confirmed also by our results. Indeed, although changes in both $\dot{V}NO$ and FENO were better correlated to $\dot{V}O_2$ and $\dot{V}E$ in control subjects than in COPD patients, relationships in both groups were weak.

It has been demonstrated that reduced exercise capacity in COPD shows only a weak relation to lung function impairment.[26] Chronic inactivity and muscle deconditioning are important factors in the loss of muscle mass and strength and related reduction in exercise capacity in COPD patients.[13] Indeed, at peak exercise, COPD patients in our study were able to reach only 66% of the load performed by the sedentary healthy control subjects.

It has been suggested[12] that in trained subjects, increased NO release during exercise by the epithelial cells is likely to improve the perfusion of ventilated lung areas resulting in improvement of ventilation-perfusion distribution and enhancement of pulmonary oxygen exchange. The same authors also suggested that enhanced NO synthesis by the pulmonary endothelial cells and the resultant rise in $\dot{V}NO$ might also serve as an important modulator of bronchomotor tone, NO being likely to reduce airway resistance.[27] These suggested effects, if proved, might confirm the importance of pulmonary rehabilitation programs including exercise training[28] in these deconditioned COPD patients. Although we found that eNO production rapidly returned to baseline values, at least theoretically prolonged exercise might induce long-lasting eNO increases and the related physiologically favorable effects. Whether exercise may induce long-lasting changes in NO synthase activity should be evaluated by further studies.

In conclusion, in patients with mild and moderate COPD, $\dot{V}NO$ during exercise increases to a lesser extent than in normal control subjects. The pathophysiologic mechanisms possibly underlying this result should be further elucidated.

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