

Production of Endogenous Nitric Oxide in Chronic Obstructive Pulmonary Disease and Patients with Cor Pulmonale

Correlates with Echo-Doppler Assessment

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Exhaled nitric oxide (NO) production in stable chronic obstructive pulmonary disease (COPD) has been loosely related to the severity of illness, being significantly reduced in the most severe cases. Pulmonary hypertension is associated with lower NO output from the lung. In this study expired NO was measured in patients with severe stable COPD with or without cor pulmonale (CP). Echocardiographic estimates of right heart function, lung function, diffusion capacity, respiratory muscle strength, and arterial blood gases were obtained in 34 consecutive patients with stable COPD (mean age, 68 ± 7 yr). Expired NO was measured by chemiluminescence to obtain fractional exhaled concentrations at peak (FENOp) and at plateau (FENOpI) points of the single-breath curve and resting NO output (\dot{V}_{NO}). All measurements of expired NO output, FENOp, FENOpI and \dot{V}_{NO} showed a negative correlation with both systolic pulmonary artery pressure (Pspa) ($r = -0.51, -0.63, \text{ and } -0.63$, respectively, $p < 0.01$ for all) and right ventricle wall dimension ($r = -0.41, -0.59, \text{ and } -0.43$, respectively, $p < 0.05$ for all), but not with any measurement of lung function. When the patients were divided according to the Pspa using a cutoff limit of 35 mm Hg, those subjects with CP showed lower FENOp (13.2 ± 4.0 versus 36.7 ± 30.8 ppb, $p < 0.05$), FENOpI (5.7 ± 1.9 versus 8.9 ± 4.7 ppb, $p < 0.05$), and \dot{V}_{NO} (69.2 ± 5.6 versus 107.6 ± 14.6 nL/min, $p = 0.02$) than did those with a normal resting Pspa. NO production from the airways was significantly lower and inversely related to development of CP in patients with severe COPD. Impaired endothelial release may account for the reduced levels of expired NO.

Chronic obstructive pulmonary disease (COPD) is a condition characterized by progressive airflow obstruction and chronic inflammation. The development of cor pulmonale (CP) and right-sided heart failure in the later stages of the disease is commonly associated with a poor prognosis (1).

Endogenous nitric oxide (NO) plays a key role in the physiologic regulation of pulmonary vascular tone (2), and it is also considered to be an important mediator in the airways (3). Evidence exists showing that NO release is impaired in the pulmonary vasculature in patients with COPD (4–6).

NO derived from the lungs (7) has been detected in the expired air of both animals and humans (8). Recent studies have suggested both an alveolar and an airway source of NO output (\dot{V}_{NO}) (9). Increased expired fractional exhaled NO (FENO) has been demonstrated in various inflammatory lung disorders such as asthma (10), bronchiectasis (11), and systemic sclerosis (12). However, the development of pulmonary hypertension

appears to be associated with a decrease in \dot{V}_{NO} at rest (13–16) or during exercise (17).

In patients with COPD, contrasting findings have been reported. Robbins and colleagues (18) demonstrated similar levels of expired NO in a small sample of patients with COPD and in healthy subjects, and they concluded that COPD is different from other inflammatory disorders of the airways. More recently, in patients with stable COPD, FENO has been shown to be loosely related to the severity of illness, being significantly lower in the most severe cases (Stage III of the American Thoracic Society [ATS] standard) as compared with mild or moderate cases (19). Conversely, Maziak and colleagues (20) found an increase in exhaled NO levels in patients with unstable COPD and an inverse relationship between FEV₁ and FENO values. Similarly, Kanazawa and colleagues (21) found FENO and sputum nitrite/nitrate levels in COPD in between those of asthmatics and healthy subjects and positively correlated to neutrophil count in induced sputum.

The hypothesis underlying this study was that the development of CP in patients with stable COPD is associated with decreased \dot{V}_{NO} from the lung.

METHODS

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

Patients

Thirty-four consecutive inpatients with COPD were studied. Diagnosis of COPD was made as Stages II and III according to the ATS standards (22). All patients were ex-smokers (mean pack-years, 27 ± 6), and none had any history of atopy. At the time of inclusion into the study, all of the patients were in stable condition, as assessed by the stability of blood gas values and pH (> 7.35), and they had been free from acute exacerbation in the preceding 4 wk. Patients with other organ failure, cancer, or inability to cooperate were excluded from the study. All of the patients were receiving their regular treatment with inhaled bronchodilators, but none were receiving systemic or inhaled steroids. Twelve patients had been receiving long-term domiciliary oxygen for at least 12 consecutive months. No change in medical therapy was made in the week prior to the study. The demographic, anthropometric and functional characteristics of the patients are shown in Table 1.

Measurements

Lung function. Static and dynamic lung volumes were measured by means of a constant-volume body plethysmograph (CAD/NET System; Med Graphics Corp., St. Paul, MN) according to standard procedure. Normalized lung carbon monoxide diffusion capacity (D_{LCO}) was assessed by means of a pulmonary diagnostic system (PF/DX; Med Graphics) with patients in the sitting position. The predicted values according to Quanjer (23) were used.

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TABLE 1
DEMOGRAPHIC, ANTHROPOMETRIC, AND LUNG FUNCTION
CHARACTERISTICS OF PATIENTS IN THE STUDY*

	Group 1 (n = 17)	Group 2 (n = 17)	p Value
Sex, M/F	15/2	14/3	NS
Age, yr	69 (8)	67 (7)	NS
BSA, m ²	1.6 (0.13)	1.8 (0.17)	0.032
Smoking, pack-years	29 (6)	26 (5)	NS
FEV ₁ , % pred	31 (12)	46 (14)	0.003
VC, % pred	65 (17)	84 (22)	0.013
RV, % pred	159 (51)	149 (33)	NS
TLC, % pred	107 (29)	109 (17)	NS
VE, L	8.7 (1.5)	9.4 (1.3)	NS
DL _{CO} , % pred	54 (32)	78 (26)	0.033
MIP, % pred	41 (14)	49 (21)	NS
MEP, % pred	63 (15)	68 (24)	NS
pH	7.40 (0.03)	7.41 (0.03)	NS
Pa _{CO₂} , mm Hg [†]	47 (7)	40 (4)	0.002
Pa _{O₂} , mm Hg [†]	60 (8)	73 (12)	0.001

Definition of abbreviations: BSA = body surface area; DL_{CO} = lung diffusion capacity to carbon monoxide; MEP = maximal expiratory pressure; MIP = maximal inspiratory pressure; RV = residual volume; VE = minute ventilation.

* Numbers in parentheses are SD.

[†] In room air.

Respiratory muscle strength was assessed by measuring maximal inspiratory and expiratory pressures (MIP and MEP, respectively) at the mouth using a respiratory module system (RPM; Med Graphics) starting from the level of FRC and TLC, respectively, according to the method of Black and Hyatt (24). The predicted values according to Bruschi and colleagues (25) were used.

Arterial blood gases were assessed during the day in resting conditions. Arterial oxygen pressure (Pa_{CO₂}), carbon dioxide pressure (Pa_{CO₂}), and pH were measured by means of an automated analyzer (ABL 500; Radiometer, Copenhagen, Denmark) on blood samples taken from the radial artery with patients in the sitting position for at least 1 h while breathing room air.

NO measurement. Patients were asked to abstain from food for at least 4 h and from alcohol for at least 24 h before the experiment. Exhaled NO was assessed by means of a high-resolution (0.3 ppb) chemiluminescence analyzer (LR 2000 series; Logan Research, Rochester, Kent, UK) adapted for on-line recording of NO concentration and equipped with Teflon mouthpiece tubing. The sampling rate was 250 ml/min. The analyzer also measured exhaled CO₂ (resolution, 0.1%; response time, 0.2 s) by single-beam infrared absorption. An internal restrictor in the breathing circuit allowed expiration against a resistance in order to keep the soft palate closed and to prevent contamination of the exhaled air with nasal NO; a single-breath VC maneuver at constant flow (200 ml/s) was performed as previously recommended (26). Ambient air was monitored for NO concentration before starting FENO measurement, and measurements were taken only on days when ambient NO was less than 35 ppb (27).

During resting conditions FENO concentration as the peak and the plateau points (FENOp and FENOpl, respectively, in ppb) were obtained from the NO curve as previously described (19). The mean value of three reproducible measurements was considered for analysis. Furthermore, \dot{V}_{NO} of each subject was calculated as: \dot{V}_{NO} (ml/min) = \dot{V}_E * FENOpl, where \dot{V}_E is the flow rate in L/min, and FENO is the fractional exhaled NO concentration (in ppb). Measurements of exhaled NO were performed blind to the patient's clinical and functional status.

Doppler echocardiography. All patients were studied using real-time, phased array, two-dimensional Doppler (2-D) echocardiography (CFM 750 CV 2.5 or 3.25 MHz transducer; GE Vingmed, Milan, Italy) while breathing room air. The examinations were taken on patients in a semirecumbent left lateral position, and images were taken from subxiphoid, parasternal, and apical views. The mean value of three measurements was considered. The measurements were carried out by a single examiner unaware of the purpose of the study.

The acceleration time of the systolic pulmonary artery flow (ATP), the time between the onset to the peak systolic flow, was determined

with a pulsed Doppler recording from the parasternal short axis with the 2-D-guided Doppler sample placed at the level of the pulmonary valve (28).

Tricuspid valve regurgitation was identified by color flow mapping, and the flow velocity curve was obtained by continuous wave Doppler echocardiography; measure of peak pressure gradient (TRP) was estimated by means of a simplified Bernoulli equation.

The diameter of the inferior vena cava (IVC) was measured from the subcostal long-axis view, below the level of the hepatic veins and a few centimeters inferior to its junction with the right atrium, performing two-dimensional and M-mode echocardiographic records during expiration and inspiration phases.

The size diameter of the IVC and its respiratory variations were used to estimate right atrial pressure (PRA) as follows (29, 30). (1) PRA = 20 mm Hg if IVC expiratory diameter is > 2 cm and inspiratory collapse is < 50%. (2) PRA = 10 mm Hg if IVC expiratory diameter is < 2 cm and inspiratory collapse is < 50%. (3) PRA = 5 mm Hg if IVC expiratory diameter is < 2 cm and inspiratory collapse is > 50%.

The estimated PRA was added to TRP value in order to calculate the systolic pulmonary artery pressure (Pspa) (29, 31, 32).

Right ventricle diastolic diameter (RVdd) and right ventricle wall dimension (RVwd) were detected with 2-D echocardiography from the apical and subxiphoid four-chamber views.

Study Protocol

On the morning of study Day 1, echo-Doppler was carried out in all subjects after they had breathed room air for at least 30 min. Lung function, arterial blood gases, and respiratory muscle strength were then assessed the same morning. On the morning of Day 2, FENO was measured in ambient air after the subjects had rested for at least 30 min. Measurement of FENO was performed according to the European Respiratory Society (ERS) recommendations (26). Bronchodilators were discontinued 12 h before the study, and during the same period the subjects abstained from alcohol or caffeine.

Data Analysis

All data are expressed as means \pm SD. Inpatient FENO values were analyzed by ANOVA for repeated measures with Huynh-Feldt correction. As no significant difference within subjects was found, the mean value of three consecutive measurements was used. Spearman's correlation coefficients were calculated among all the considered variables. Patients were then assigned to one of two groups according to the level of Pspa as assessed by echo-Doppler using a cutoff of 35 mm Hg: those with an Pspa \geq 35 mm Hg (Group 1: n = 17; FEV₁, 31 \pm 12% pred; Pspa, 55 \pm 17 mm Hg) and those with a Pspa < 35 mm Hg (Group 2: n = 17; FEV₁, 46 \pm 14% pred; Pspa, 29 \pm 5 mm Hg).

The between-group differences were evaluated by Mann-Whitney analysis for nonparametric variables. A p value of less than 0.05 was considered to be statistically significant.

RESULTS

NO was detectable in the expired breath of all subjects. Variation coefficients (SD/mean %) of intra-patient measurements of FENOp and FENOpl were 5 \pm 3% (range, 2 to 9) and 6 \pm 4% (range, 2 to 12), respectively.

Linear regression analysis showed a good correlation between Pspa and acceleration time of the pulmonary valve (ATP) (r = 0.64, p = 0.001), RVdd (r = 0.73, p < 0.001), and RVwd (r = 0.734, p < 0.001). Pspa also showed a significant negative correlation with FENOp (r = -0.51, p = 0.003), FENOpl (r = -0.63, p < 0.001) (Figure 1A), and \dot{V}_{NO} (r = -0.63, p < 0.001). Linear regressions and correlation coefficients between TRP, RVwd, and the FENOpl are shown in Figures 1B and C. No significant relationships between expired NO (assessed as FENOp, FENOpl, or \dot{V}_{NO}) and spirometry (Figure 2A), arterial blood determinations, (Figure 2B), respiratory muscle strength, gas transfer (Figure 2C), demographics, or previous smoking habits were found.

As shown in Table 1, when the data were analyzed accord-

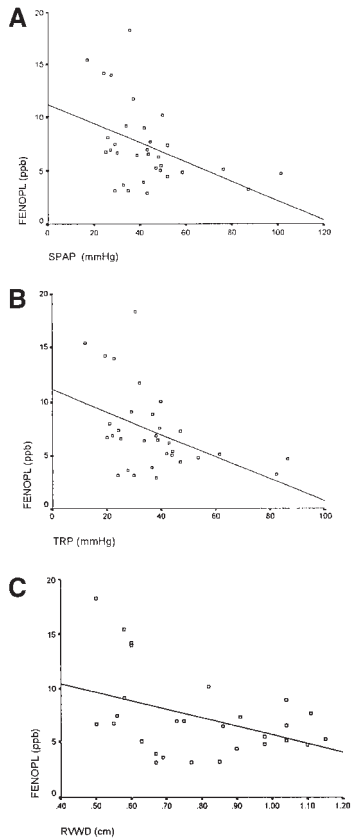


Figure 1. Linear regression between FENOpL = Plateau value of exhaled nitric oxide versus A, sPAP = systolic pulmonary artery pressure ($r = -0.63$, $p < 0.01$). (B) TRP = tricuspid valve regurgitation pressure ($r = -0.45$, $p < 0.01$). (C) RVwd = right ventricle wall dimension ($r = -0.59$, $p < 0.05$).

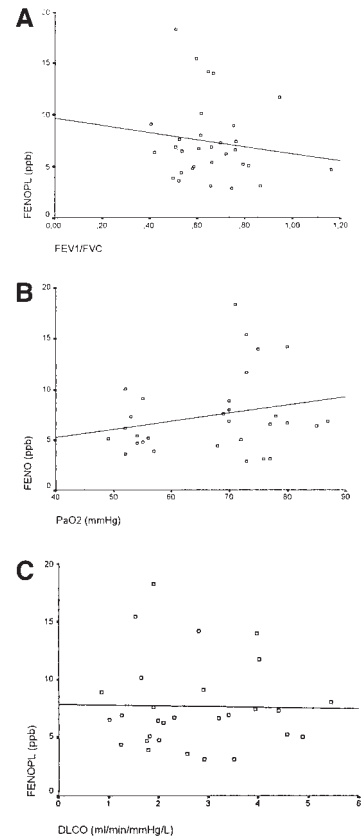


Figure 2. Linear regression between FENOpL = plateau value of exhaled nitric oxide versus A, FEV1/FVC = forced expiratory volume in one second/forced vital capacity ($r = 0.14$, $p = 0.5$). (B) PaO₂ = partial arterial oxygen pressure ($r = 0.24$, $p = 0.2$). (C) DLCO = lung diffusion capacity to carbon monoxide ($r = 0.02$, $p = 0.9$).

ing to Pspa, Group 1 patients had worse FEV₁, VC, and DLCO values than did the subjects in Group 2: 11 of the 17 patients in Group 1 were defined as ATS Stage III COPD as compared with six of 17 in Group 2. Patients in Group 1 were more hypoxic in ambient air. Nine patients were receiving long-term oxygen in Group 1 and three in Group 2.

The differences in parameters evaluated by echo-Doppler are illustrated in Table 2. As expected, in Group 1 the higher level of Pspa was associated with higher values of both RVdd and RVwd.

Both FENOp and FENOpL as well as \dot{V}_{NO} levels were lower in Group 1 patients than in those in Group 2 (Table 3).

DISCUSSION

This study has shown that in patients with COPD, CP is associated with reduced expired NO concentration and output. In these patients NO production from the airways was related to right ventricular dysfunction as assessed by both pulmonary pressure levels and right ventricular dimensions.

In this study a slow VC maneuver was employed to measure FENO. To standardize methods a European Task Force (26) recently published recommendations on measurements of expired NO and proposed that the single-breath method was preferable in adults. Measurements were performed by using standard procedures and at the same expiratory flow rate in order to minimize the possible influence of the expiratory flow rates of different subjects (33). We are also confident that a constant flow exhalation was performed even in the most com-

promised subjects, as verified by the backup visual control on-line system (flow and pressure) and by the reproducibility of the obtained values (mean variation coefficient < 10%, for both FENOp and FENOpL).

In patients with COPD, FENO levels have been positively associated with increased airway obstruction (19, 20), higher neutrophil counts (in clinically unstable patients) (21), and sputum eosinophils (34). In the present study all subjects were in clinically stable condition as determined by the absence of both respiratory exacerbation and right heart failure, the latter confirmed by the IVC measurements (see Table 2). In a

TABLE 2
ECHO-DOPPLER EVALUATIONS OF PATIENTS IN THE STUDY*

	Group 1 (n = 17)	Group 2 (n = 17)	p Value
ATP, s	0.08 (0.02)	0.11 (0.01)	< 0.001
TRP, mm Hg	48 (15)	24 (6)	< 0.001
IVCe, cm	1.4 (0.7)	1.1 (0.7)	NS
IVCi, cm	0.7 (0.7)	0.3 (0.1)	NS
IVCi/IVCe	0.4 (0.2)	0.4 (0.5)	NS
Pspa, mm Hg	55 (17)	29 (5)	< 0.001
RVdd, cm	3.6 (0.7)	2.8 (0.4)	0.001
RVwd, cm	0.9 (0.1)	0.6 (0.1)	< 0.001

Definition of abbreviations: ATP = acceleration time of the pulmonary value; IVCe = inferior vena cava expiratory diameter; IVCi = inferior vena cava inspiratory diameter; Pspa = systolic pulmonary artery pressure; RVdd = right ventricle diastolic diameter; RVwd = right ventricle wall diastolic thickness; TRP = tricuspid valve regurgitation pressure.

* Numbers in parentheses are SD.

TABLE 3
EXHALED NO LEVELS AND RATE OF PRODUCTION

	Mean	SD	95% Confidence Intervals	
			(+)	(-)
FENOp, ppb				
Group 1	13.2	(4.0)*	11.1	15.3
Group 2	36.8	(30.8)	19.0	54.6
FENOpl, ppb				
Group 1	5.7	(1.9) [†]	4.8	6.8
Group 2	8.9	(4.7)	6.4	11.6
\dot{V}_{NO} , nl/min				
Group 1	69.3	(23.2) [‡]	57.3	81.2
Group 2	107.6	(56.6)	76.3	139.0

Definition of abbreviations: FENOp = peak value of exhaled nitric oxide; FENOpl = plateau value of exhaled nitric oxide; \dot{V}_{NO} = output of exhaled nitric oxide.

* p = 0.004 versus group 2.

[†] p = 0.016 versus group 2.

[‡] p = 0.016 versus group 2.

previous study (19) performed in outpatients with COPD, we had observed that patients with severe airway obstruction ($FEV_1 < 35\%$ pred) and CP had reduced levels of eNO compared with patients with moderate airway obstruction without CP. The results of the present study performed in other inpatients extend those findings in that NO production from the airways is related to pulmonary pressure levels and right ventricular dimensions.

In that study (19) we had also observed that FENO levels in patients with COPD and severe airway obstruction without CP were similar to those in patients with CP, suggesting that the severity of airway obstruction was an important factor in determining FENO. Nevertheless, although in the present study the CP group exhibited a more severe functional impairment than did patients in that study (19), FENO and \dot{V}_{NO} were not related to the indices of airway obstruction (Figure 2), altered gas transfer, or arterial oxygenation. Also in the previous study (19) no correlation between FEV_1 (% pred) and eNO was found when only patients with COPD were considered. In the present study a weak inverse correlation between Pa_{O_2} and P_{spa} ($r = -0.58$; $p < 0.05$) was found, although there was no difference in the duration of the illness (10 ± 4 and 9 ± 3 yr in Groups 1 and 2, respectively).

Although the exact origin of expired NO is uncertain, both theoretical (35) and experimental (9) work on NO exchange suggest that expired NO may be derived from both an airway and an alveolar source. An alveolar source of expired NO is unlikely as the impaired gas transfer in the subjects with CP (Table 1) would presumably favor a reduced diffusion of NO from the air space to the blood (36) and would therefore cause higher rather than lower FENO levels.

Hypoxia has been associated with reduced FENO in isolated lungs (7), animals (8), and humans (37) in whom expired NO levels were dependent on oxygen concentration when breathing hypoxic mixtures. Conversely, little effect of acute hypoxia on \dot{V}_{NO} was observed in normal subjects breathing 10% oxygen mixtures (38). In the present study, although Group 1 patients had lower mean arterial oxygen tensions, all measurements were performed in room air and no clear association was observed between Pa_{O_2} and FENO or \dot{V}_{NO} .

In isolated human pulmonary arteries, NO-dependent vasorelaxation is impaired in both mild (6) and severe COPD (4). This impairment is related to the degree of intimal thickening and to arterial oxygen tension (4). In isolated human lungs stimulated but not basal release appears to be impaired (39). In rats with chronic hypoxic pulmonary hypertension,

reduction in pulmonary blood flow decreases endothelial nitric oxide synthase (eNOS), mRNA, and protein expression, whereas hypoxia increased eNOS expression (40).

The development of pulmonary hypertension in lung diseases other than COPD has also been associated with reduced FENO levels. In primary pulmonary hypertension this reduction appeared to be related to the amputated peripheral pulmonary vascular bed (15), and the reduced FENO is more apparent during exercise (17). In patients with heart failure and pulmonary hypertension, FENO and \dot{V}_{NO} were negatively correlated with pulmonary vascular resistance and lower mixed venous oxygen tension (41). Similarly, although \dot{V}_{NO} is generally increased in systemic vasculitides (12, 42), a reduction is observed when pulmonary hypertension develops (13, 14). These findings raise the possibility that expired NO may be derived in part from abluminally released endothelial NO (43) and that the reduced expired NO in pulmonary hypertension may reflect either the impaired endothelial release of NO associated with this condition (5) or the increased muscularization of the distal pulmonary arteries typical of pulmonary hypertension, which would take up more NO.

In conclusion, NO output in patients with COPD appears to be inversely related to the development of cor pulmonale. Impaired endothelial release or reduced diffusion of NO from the endothelium into the airway may account for the lower levels of expired NO in these patients.

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