Effects of inhaled nitric oxide at rest and during exercise in idiopathic pulmonary fibrosis

Isabel Blanco, Jesús Ribas, Antoni Xaubet, Federico P. Gómez, Josep Roca, Robert Rodriguez-Roisin, and Joan A. Barberà

1Department of Pulmonary Medicine, Institut Clínic del Tórax, Hospital Clínic-Institut d’Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, and 2Hospital Universitari de Bellvitge-IDIBELL, Barcelona; and 3Centro de Investigación Biomédica en Red de Enfermedades Respiratorias, Badalona, Spain

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Blanco I, Ribas J, Xaubet A, Gómez FP, Roca J, Rodriguez-Roisin R, Barberà JA. Effects of inhaled nitric oxide at rest and during exercise in idiopathic pulmonary fibrosis. J Appl Physiol 110: 638–645, 2011. First published December 23, 2010; doi:10.1152/japplphysiol.01104.2010.—Patients with idiopathic pulmonary fibrosis (IPF) usually develop hypoxemia and pulmonary hypertension when exercising. To what extent endothelium-derived vasodilating agents modify these changes is unknown. The study was aimed to investigate in patients with IPF whether exercise induces changes in plasma levels of endothelium-derived signaling mediators, and to assess the acute effects of inhaled nitric oxide (NO) on pulmonary hemodynamics and gas exchange, at rest and during exercise. We evaluated seven patients with IPF (6 men/1 woman; 57 ± 11 yr; forced vital capacity, 60 ± 13% predicted; carbon monoxide diffusing capacity, 52 ± 10% predicted). Levels of endothelin, 6-keto-prostaglandin-F1α, thromboxane B2, and nitrates were measured at rest and during submaximal exercise. Pulmonary hemodynamics and gas exchange, including ventilation-perfusion relationships, were assessed breathing ambient air and 40 ppm NO, both at rest and during submaximal exercise. The concentration of thromboxane B2 increased during exercise (P = 0.046), whereas levels of other mediators did not change. The change in 6-keto-prostaglandin-F1α correlated with that of mean pulmonary arterial pressure (r = 0.94; P < 0.005). Inhaled NO reduced mean pulmonary arterial pressure at rest (−4.6 ± 2.1 mmHg) and during exercise (−11.7 ± 7.1 mmHg) (P = 0.001 and P = 0.004, respectively), without altering arterial oxygenation or ventilation-perfusion distributions in any of the study conditions. Alveolar-to-capillary oxygen diffusion limitation, which accounted for the decrease of arterial PO2 during exercise, was not modified by NO administration. We conclude that, in IPF, some endothelium-derived signaling molecules may modulate the development of pulmonary hypertension during exercise, and that the administration of inhaled NO reduces pulmonary vascular resistance without disturbing gas exchange.

pulmonary hemodynamics; gas exchange; ventilation-perfusion relationships; pulmonary circulation; vasodilator agents

IDIOPATHIC PULMONARY FIBROSIS (IPF) is a chronic progressive disease characterized by inflammation and fibrosis of the lung parenchyma. Abnormal gas exchange and pulmonary hypertension (PH) are common consequences of the disease. Gas exchange may be normal or mildly impaired at rest, but worsens markedly during exercise due to oxygen diffusion limitation (5, 35). PH may be present in up to 46% of patients with severe disease (30, 31, 35). It results from vessel remodeling and vasculature loss in fibrotic areas (11).

Gas exchange and hemodynamic abnormalities in IPF are, at least in part, related to the imbalance of endothelium-derived vasoactive agents that are normally present in the pulmonary circulation. Endothelin-1 (ET-1), a potent vasoconstrictor and mitogenic agent, is prominently expressed in lung tissue in IPF (14). Plasma levels of ET-1 are increased in IPF, and its concentration correlates with disease progression and the presence of PH (32). Furthermore, the expression of endothelial nitric oxide (NO) synthase in pulmonary arteries is decreased in both the idiopathic and the associated forms of PH (including IPF) (15), suggesting a role for a reduced synthesis and release of NO, a potent vasodilator and antiproliferative agent, in the development of PH.

In IPF, endothelium-derived mediators may play a role in the development and progression of fibrosis or PH. Targeting these mediators could be useful to limit or reverse disease progression. Current specific therapy of pulmonary arterial hypertension (PAH), which targets the imbalance between endothelium-derived vasoactive mediators, exerts beneficial effects not only in idiopathic PAH, but also in associated forms that involve an inflammatory component of pulmonary vessels, as in connective tissue diseases or human immunodeficiency virus infection. To what extent specific PAH therapy would also be beneficial in IPF-associated PH remains unknown. Prostanoids, phosphodiesterase-5 inhibitors, and endothelin receptor antagonists reduce mean pulmonary arterial pressure (mPAP) and may have an antifibrotic effect (9, 16), suggesting that they could be beneficial in IPF-associated PH. Nevertheless, controlled studies using endothelin receptor antagonists (22) or phosphodiesterase-5 inhibitors (19a) have failed to show improvement in exercise capacity in this condition, although there was a trend in delayed disease progression and improvement in quality of life.

Currently, there is no specific therapy approved for patients with PH associated with chronic respiratory diseases. In previous studies, our laboratory has shown that, in chronic obstructive pulmonary disease (COPD), the administration of drugs that target the pulmonary circulation worsen arterial oxygenation due to increased ventilation-perfusion (V̅/Q̅) imbalance, resulting from the inhibition of hypoxic pulmonary vasoconstriction (HPV) (3, 6, 7). Patients with IPF also show HPV, which contributes to V̅/Q̅ matching and is related to the degree of vascular impairment (5).

Inhaled NO is a selective pulmonary vasodilator commonly used to test pulmonary vasoreactivity in PH. Several reports have documented the potential therapeutic role of inhaled NO...
in patients with end-stage IPF (26), where it has been employed as a bridge to lung transplantation (39). In IPF, the administration of inhaled NO predicts the hemodynamic effects of drugs used to treat PAH (13). However, as noted before in lung diseases where V˙A/Q˙ mismatching is the main mechanism of hypoxemia, inhaled NO may worsen gas exchange, due to the inhibition of HPV (6).

Accordingly, the present study was addressed to investigate in patients with IPF: 1) whether exercise induces changes in plasma levels of endothelium-derived signaling mediators; and 2) the acute effects of inhaled NO on pulmonary hemodynamics and gas exchange, both at rest and during exercise.

Methods.

Patients. Seven patients (6 never smokers/1 ex-smoker; 6 men/1 woman) previously diagnosed of IPF (1, 37), aged 41 to 68 yr, were studied. In four of them (57%), a diagnosis of usual interstitial pneumonia was established by surgical biopsy; in the remaining three, a clinical diagnosis of IPF was established on the basis of a compatible radiographic pattern, without other known causes of interstitial lung disease, and after having excluded other entities by transbronchial lung biopsy or bronchoalveolar lavage.

Lung function testing, including forced spirometry, body plethysmography, and single-breath carbon monoxide diffusing capacity, was performed before the study. Data from all patients entering in the study are shown in Table 1. Active smokers and patients receiving vasodilator drugs were excluded. The study was approved by the internal review board of Hospital Clinic (Barcelona, Spain), and written informed consent was obtained from each participant.

Procedures. Before the study, the highest workload that each patient could tolerate on a cycle-ergometer (J. E. Jaeger) was determined by an incremental exercise test.

The day of the study, a triple-lumen Swan-Ganz catheter (Edwards Laboratories) was placed into the pulmonary artery under pressure-wave monitoring (M1166A; Hewlett-Packard), and a polyethylene catheter was inserted into the radial artery. A peripheral vein catheter was inserted for inert-gas infusion. Intravascular pressures were continuously monitored and registered (7754B; Hewlett-Packard). Measurements of endothelium-derived vasoactive agents [ET-1, thromboxane B2 (TxB2) and 6-keto-prostaglandin F1α (PGF1α)] in arterial blood were performed in six patients, at rest and during submaximal exercise. The plasma concentrations of ET-1 and PGF1α were measured by radioimmunoassay, and the concentration of TxB2 was measured by enzyme immunoassay.

Measurements of pulmonary vascular pressures were taken at the end of expiration. Two measurements were performed in each study condition, and the mean value is reported as the final result. Cardiac output (CO) was determined by the thermodilution technique (M1012A; Hewlett-Packard, Germany), and expressed as the mean of three measurements. Pulmonary and systemic vascular resistances were calculated using standard formulas. Ventilation (V˙E) and respiratory rate were recorded using a calibrated Wright spirometer (MK8; BOC-Medical). Oxygen uptake and carbon dioxide production were calculated from mixed expired oxygen and carbon dioxide concentrations (Medical Graphics). Arterial and mixed venous PO2, PCO2, and pH were analyzed in duplicate, using standard electrodes (IL 1302; Instrumentation Laboratories).

V˙A/Q˙ distributions were estimated using the multiple inert-gas elimination technique (MIGET) (33). The dispersion of perfusion and ventilation distributions on a logarithmic scale (log SDQ˙ and log SDV˙, respectively) were used as indexes of V˙A/Q˙ mismatch (upper normal limit: log SDQ˙, 0.6; log SDV˙, 0.65) (8). Intrapulmonary shunt was defined as the fraction of CO perfusing lung units with V˙A/Q˙ ratios < 0.005 and dead space as the V˙E to units with V˙A/Q˙ ratios > 100. As an overall descriptor of V˙A/Q˙ inequality, we used the difference among retentions and excretions of inert gases, corrected for acetone (DISP R-E; normal value < 3.0) (8, 12). Furthermore, using the measured concentrations in arterial blood and expired breath, inert-gas gradient indexes, namely retentions minus excretions corrected for acetone, were plotted against solubility for each gas to obtain retention-excretion curves (18). The MIGET can also predict the arterial PO2 (PaO2) value that should result from the measured V˙A/Q˙ distribution, taking into account all of the factors that may influence the gas exchange (fraction of inspired O2, V˙E, CO2), on the explicit assumption that there is no oxygen diffusion limitation. The comparison of this predicted PaO2 value with that actually measured in the arterial blood thus provides information on the presence of oxygen diffusion limitation (2). In this manner, the limitation of alveolar-to-capillary oxygen transfer is evident as a higher predicted than measured PaO2.

Table 1. General characteristics and lung function data

| Age, yr | 57 ± 11 |
| Sex, no. | Male 6, Female 1 |
| Height, cm | 163 ± 10 |
| Weight, kg | 74 ± 15 |
| FEV1, liters | 2.11 ± 0.67 |
| %pred | 70 ± 15 |
| FVC, %pred | 60 ± 13 |
| FEV1/FVC, % | 87 ± 6 |
| TLC, %pred | 63 ± 10 |
| DLCO, %pred | 52 ± 10 |

Values are means ± SD. FEV1, forced expiratory volume in the 1st s; %pred, %predicted; FVC, forced vital capacity; TLC, total lung capacity; DLCO, diffusing capacity of the lung for carbon monoxide; Wpeak, work rate at peak exercise; V˙O2peak, oxygen uptake at peak exercise; V˙Epeak, minute ventilation at peak exercise; MVV, estimated maximum voluntary ventilation; HRpeak, heart rate at peak exercise.

Table 2. Endothelium-derived vasoactive agents at rest and during exercise

<table>
<thead>
<tr>
<th>Endothelin, pg/ml</th>
<th>Rest</th>
<th>Exercise*</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.3 ± 6.4</td>
<td>9.2 ± 5.4</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>6-keto-PGF1α, pg/ml</td>
<td>270 ± 201</td>
<td>291 ± 235</td>
<td>NS</td>
</tr>
<tr>
<td>TxB2, pg/ml</td>
<td>155 ± 73</td>
<td>209 ± 108</td>
<td>0.046</td>
</tr>
<tr>
<td>6-keto-PGF1α/TxB2</td>
<td>2.12 ± 1.77</td>
<td>2.04 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>NO2/NO3, nmol/ml</td>
<td>49.4 ± 8.5</td>
<td>46.5 ± 10.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD. 6-keto-PGF1α, 6-keto-prostaglandin F1α; TxB2, tromboxane B2; NO2, nitrite; NO3, nitrate; NS, not significant. *Measurements were performed while subject breathed ambient air. †Wilcoxon signed-rank test.
Study design. Forty-five minutes after starting the inert-gas infusion, patients were connected to a breathing circuit with inhaled NO-free air for 20 min, and measurements at rest were performed. All measurements were taken under steady-state conditions. Following this, each patient was asked to cycle at a workload equivalent to 60% of the maximal tolerated in the previous incremental test. After the subjects reached steady-state conditions, a second set of measurements were performed during exercise. Afterwards, patients rested for 45 min, and, once it was confirmed that systemic and pulmonary hemodynamics had returned to baseline levels, patients were reconnected to the breathing circuit and breathed a mixture of NO in air at 40 ppm during 20 min (Tecfluid, Spain), as previously described (28).

### Table 3. Hemodynamic response to exercise and inhaled NO

<table>
<thead>
<tr>
<th></th>
<th>Air</th>
<th>NO</th>
<th>Main Effects, P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest Exercise</td>
<td>Rest Exercise</td>
<td>NO Exercise Interaction, NO-Exercise</td>
</tr>
<tr>
<td>mPAP, mmHg</td>
<td>20 ± 7</td>
<td>40 ± 16</td>
<td>16 ± 6</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>5.5 ± 1.0</td>
<td>12 ± 1.5</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>80 ± 12</td>
<td>126 ± 14</td>
<td>84 ± 19</td>
</tr>
<tr>
<td>PCWP, mmHg</td>
<td>7 ± 5</td>
<td>9 ± 5</td>
<td>5 ± 5</td>
</tr>
<tr>
<td>PVR, dyn·s·cm⁻⁵</td>
<td>192 ± 85</td>
<td>204 ± 84</td>
<td>154 ± 63</td>
</tr>
<tr>
<td>RAP, mmHg</td>
<td>3 ± 4</td>
<td>5 ± 5</td>
<td>1 ± 4</td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>104 ± 16</td>
<td>127 ± 20</td>
<td>97 ± 14</td>
</tr>
</tbody>
</table>

Values are means ± SD. NO, nitric oxide; mPAP, mean pulmonary artery pressure; CO, cardiac output; HR, heart rate; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RAP, mean right atrial pressure; SAP, mean systemic arterial pressure. *Significance of the effects of NO and exercise, and their interaction, on a two-way repeated-measures ANOVA.

Fig. 1. Individual effects of inhaled nitric oxide (NO) on mean pulmonary arterial pressure (mPAP) and arterial oxygen tension (PaO₂), at rest and during exercise. Thick bars denote mean values. ns, Non-significant.
Measurements at rest and during submaximal exercise were repeated as before. Inspired concentrations of NO, nitric dioxide, and oxygen were continuously monitored.

**Statistical analysis.** The results are expressed as means ± SD. The Wilcoxon signed-rank test was used to compare plasma concentrations of vasoactive agents at rest and during exercise. The Spearman correlation coefficient was used to explore the relationship between concentrations of vasoactive agents and gas exchange and pulmonary hemodynamics. The effects of exercise and inhaled NO on hemodynamic and gas exchange measurements were assessed on a two-way repeated-measures ANOVA. A P value < 0.05 was considered significant in all cases.

**RESULTS**

Patients suffered from moderate-to-severe restrictive ventilatory impairment and severe reduction of carbon monoxide diffusing capacity (Table 1). The incremental exercise test revealed mild-to-moderate impairment of exercise tolerance (Table 1).

**Plasma levels of vasoactive agents at rest and during exercise.** Plasma concentrations of endothelium-derived agents at rest and during exercise, breathing ambient air, are shown in Table 2. Only TxB2 increased significantly during exercise. Despite a lack of change in mean value, PGF_{1α} increased during exercise in some patients and decreased in others. Interestingly, the change in plasma levels of PGF_{1α} from rest to exercise correlated with the increase in mPAP during exercise (r = 0.94; P < 0.005) (Supplemental Fig. S1; The online version of this article contains supplemental data).

**Pulmonary hemodynamics at baseline and during NO inhalation.** At rest, with the subject breathing ambient air, mPAP was, on average, in the upper limit of normal (23) (20 ± 7 mmHg). Four patients presented abnormal mPAP (>20 mmHg): in two it was marginally increased (21–24 mmHg), and in two it was >25 mmHg. All patients had normal pulmonary capillary wedge pressure (PCWP) (Table 3). During exercise while subjects breathed ambient air, mPAP increased up to 40 ± 16 mmHg (P < 0.002) (Table 3, Fig. 1), with no change in PCWP. The transpulmonary pressure gradient (TPG) (mPAP-PCWP), also increased significantly (Fig. 2). CO doubled during exercise, whereas pulmonary vascular resistance (PVR) remained unaltered.

At rest, inhaled NO significantly decreased mPAP and PVR, whereas PCWP and CO remained essentially unchanged (Table 3, Fig. 2, and Supplemental Fig. S2). During exercise, the decrease in mPAP induced by inhaled NO was significantly greater than at rest (Table 3). CO during exercise remained unaltered during NO inhalation (Table 3 and Supplemental Fig. S2). Compared with baseline conditions, during NO inhalation, the relationship between transpulmonary pressure gradient and CO shifted downwards (Fig. 2), and its slope decreased significantly, from 2.70 ± 1.20 to 1.37 ± 0.71 mmHg-min^{-1}L^{-1} (P = 0.008).

**Pulmonary gas exchange at baseline and during NO inhalation.** At rest, patients showed moderate hypoxemia with arterial PCO₂ within the normal range (Table 4, Fig. 1). All patients exhibited a moderate degree of V̇A/Q̇ inequality, as shown by increased log SDQ̇ and log SDV̇ (Table 4, Fig. 3). The DISP R-E was also moderately increased, and intrapulmonary shunt was negligible (<3% in all cases). At rest, V̇A/Q̇ inequality was the main cause of hypoxemia, as shown by the close agreement between measured PaO₂ and that predicted from observed V̇A/Q̇ inequality (−0.4 ± 8.3 Torr) (33). During exercise, PaO₂ decreased 18 ± 12 Torr, and alveolar-arterial PO₂ difference increased by 26 ± 15 Torr, while arterial PCO₂ remained unchanged (Table 4, Fig. 1). The distribution of blood flow remained unaltered, whereas that of V̇E was more homogeneous, and dead space decreased (Table 4, Fig. 3).

Figure 5 shows the plot of direct measurements of retention minus excretion, corrected for acetone, of each inert gas vs. its solubility. Taken all gases together, there were no significant differences among the four different study conditions (ANOVA). Individual analysis of each gas reveals a decrease in the retention-excretion of gases with intermediate solubility (ethane and cyclopropane) during exercise, consistent with a more homogenous distribution of V̇E. No effect of NO inhalation was noticed.

The difference between predicted PaO₂ from V̇A/Q̇ inequality and that actually measured increased markedly during exercise (20 ± 11 Torr; P = 0.001 compared with value at rest), thus indicating that oxygen diffusion limitation emerged as an important factor contributing to decrease PaO₂ (Fig. 4).

At rest, inhaled NO did not modify arterial oxygenation, and V̇A/Q̇ distributions remained essentially unchanged (Table 4, Fig. 1 and Supplemental Fig. S2). The difference between predicted and measured PaO₂ was 6.0 ± 9.8 Torr, a value slightly greater than that observed breathing ambient air that suggests some degree of alveolar-to-capillary O₂ diffusion limitation; although, as mentioned, no deterioration on arterial oxygenation was observed. During exercise, NO inhalation did not modify arterial blood gases or V̇A/Q̇ distributions (Table 4 and Supplemental Fig. S2). During NO inhalation, the contribution of oxygen diffusion limitation to exercise-induced hypoxemia, as assessed by the difference between predicted and measured PaO₂, was similar to that observed while the subjects breathed ambient air (Fig. 4).

Analysis of individual inert-gas gradients (retention minus excretion) did not show any effect of inhaled NO (P = 0.201) (Fig. 5).
Results of the present study show that, in IPF, exercise induces changes in endothelium-derived vasoactive agents, and that the inhalation of NO improves pulmonary hemodynamics, especially during exercise, without altering gas exchange.

Pulmonary vascular tone is modulated by balanced actions of endothelium-derived vasodilators (NO, prostacyclin) and vasoconstrictors (ET-1, thromboxane). An imbalance between

**Table 4. Gas-exchange response to exercise and inhaled NO**

<table>
<thead>
<tr>
<th></th>
<th>Air Rest</th>
<th>Air Exercise</th>
<th>NO Rest</th>
<th>NO Exercise</th>
<th>NO Exercise Interaction NO-exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{PaO}_2 ), Torr</td>
<td>77 ± 11</td>
<td>59 ± 16</td>
<td>75 ± 11</td>
<td>58 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>( \text{PaCO}_2 ), Torr</td>
<td>39 ± 2</td>
<td>37 ± 5</td>
<td>37 ± 3</td>
<td>37 ± 4</td>
<td>0.03 NS</td>
</tr>
<tr>
<td>A-a( \text{PO}_2 ), Torr</td>
<td>19 ± 11</td>
<td>45 ± 22</td>
<td>21 ± 13</td>
<td>44 ± 21</td>
<td>NS</td>
</tr>
<tr>
<td>( \text{Ve} ), l/min</td>
<td>7.9 ± 2.7</td>
<td>33.9 ± 10.3</td>
<td>8.1 ± 2.4</td>
<td>35.1 ± 11.6</td>
<td>NS</td>
</tr>
<tr>
<td>( \text{Vd/Vt} ), %</td>
<td>49.2 ± 11.3</td>
<td>43.5 ± 9</td>
<td>47.4 ± 12.5</td>
<td>43.0 ± 9.6</td>
<td>NS</td>
</tr>
<tr>
<td>\text{Log SDQ}˙</td>
<td>0.84 ± 0.46</td>
<td>0.88 ± 0.50</td>
<td>0.78 ± 0.45</td>
<td>0.81 ± 0.51</td>
<td>NS</td>
</tr>
<tr>
<td>Low ( \text{Vd}/\text{Q} ), %CO</td>
<td>3.8 ± 5.7</td>
<td>2.9 ± 4.0</td>
<td>3.3 ± 4.0</td>
<td>3.3 ± 4.7</td>
<td>NS</td>
</tr>
<tr>
<td>Mean Q</td>
<td>0.72 ± 1.0</td>
<td>1.93 ± 0.51</td>
<td>0.76 ± 0.16</td>
<td>1.79 ± 0.62</td>
<td>0.0001 0.002</td>
</tr>
<tr>
<td>Shunt, %CO</td>
<td>1.2 ± 1.0</td>
<td>1.6 ± 1.8</td>
<td>1.1 ± 0.8</td>
<td>1.4 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>\text{Log SDV}</td>
<td>0.72 ± 0.23</td>
<td>0.53 ± 0.13</td>
<td>0.65 ± 0.45</td>
<td>0.48 ± 0.24</td>
<td>0.02 NS</td>
</tr>
<tr>
<td>Mean V</td>
<td>1.23 ± 0.21</td>
<td>3.00 ± 0.82</td>
<td>1.19 ± 0.23</td>
<td>2.85 ± 0.85</td>
<td>NS</td>
</tr>
<tr>
<td>Dead space, %( \text{Ve} )</td>
<td>31.6 ± 11.2</td>
<td>28.8 ± 7.9</td>
<td>41.7 ± 9.7</td>
<td>27.5 ± 15.4</td>
<td>NS</td>
</tr>
<tr>
<td>DISP R-E</td>
<td>6.4 ± 3.2</td>
<td>5.6 ± 3.4</td>
<td>5.2 ± 2.7</td>
<td>4.9 ± 2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Pred-Meas ( \text{PaO}_2 ), Torr</td>
<td>−0.4 ± 8.3</td>
<td>19.6 ± 11.1</td>
<td>6.0 ± 9.8</td>
<td>21.3 ± 12.8</td>
<td>0.001 0.006 NS</td>
</tr>
</tbody>
</table>

Values are means ± SD. \( \text{PaO}_2 \), arterial oxygen partial pressure; \( \text{PaCO}_2 \), arterial carbon dioxide partial pressure; A-a\( \text{Po}_2 \), alveolar-arterial oxygen pressure difference; \( \text{PvO}_2 \), mixed-venous oxygen partial pressure; \( \text{Ve} \), minute ventilation; \( \text{Vd/Vt} \), ratio of dead space to tidal volume; \text{log SDQ}˙, dispersion of blood flow distribution; \text{CO}, cardiac output; low \( \text{Vd}/\text{Q} \), perfusion to alveolar units with \( \text{Vd}/\text{Q} \) ratios between 0.005 and 0.1; mean Q, mean \( \text{Vd}/\text{Q} \) ratio of blood flow distribution; shunt, perfusion to alveolar units with \( \text{Vd}/\text{Q} \) ratios < 0.005; \text{log SDV}, dispersion of ventilation distribution; mean V, mean \( \text{Vd}/\text{Q} \) ratio of ventilation distribution; dead space, ventilation to units with \( \text{Vd}/\text{Q} \) ratios > 100 calculated from inert gases; DISP R-E, dispersion of retention minus excretion of inert gases corrected for acetone; Pred-Meas \( \text{PaO}_2 \), predicted minus measured \( \text{PaO}_2 \). *Significance of the effects of NO and exercise, and their interaction, on a two-way repeated-measures ANOVA.
During exercise in our patients, ET-1 levels in plasma did not change, whereas the concentration of the prostacyclin metabolite PGF_{1α} and the ratio between PGF_{1α} and TxB_{2} remained stable. Interestingly, the change in PGF_{1α} correlated with that of mPAP (Supplemental Fig. S1). Taken together, we hypothesize that exercise induced the endothelial production of eicosanoids with opposite actions on pulmonary vascular tone, with the increase in pulmonary arterial pressure (PAP) mainly due to fixed obliteration and narrowing of pulmonary vessels (10). Yet the finding of an association between the increase in plasma concentration of PGF_{1α} during exercise and the increase of PAP suggests that PGF_{1α} could be a potential noninvasive biomarker of exercise-induced PH in IPF.

Although ET-1 is overexpressed in lungs of patients with IPF (14, 29) and has been implicated in exercise-induced PH in this condition (38), ET-1 levels in plasma did not change during exercise in our patients.

To get further insight into the suitability of specific therapy for treating PH (19) associated with chronic respiratory disorders, we explored the effects of inhaled NO in patients with IPF, since the NO-signaling pathway is a reasonable therapeutic target in this condition (9, 20, 25). Given the detrimental effects of vasodilators on arterial oxygenation in COPD, where these drugs increase V_{A}/Q_{L} imbalance due to HPV inhibition (3, 6, 7, 21), we carefully evaluated the effects of inhaled NO on V_{A}/Q_{L} distributions and pulmonary hemodynamics, both at rest and during exercise. Inhaled NO resulted in a significant reduction of mPAP, especially during exercise. The hemodynamic response to NO was characterized by a decrease of mPAP, with no change in CO, PCWP, or systemic arterial pressure, consistent with its selectivity for the pulmonary circulation. This contrasts with the hemodynamic profile of systemic vasodilators that also produce a decrease in systemic blood pressure when administered to patients with PH secondary to pulmonary fibrosis (27).

The vasodilating effect of inhaled NO during exercise was greater than at rest, with a downward shift and a slope reduction of the pressure-flow relationship (Supplemental Fig. S2). Whereas breathing ambient air mPAP increased 3 mmHg/l CO, during NO inhalation such an increase was of only 1.4 mmHg/l (P = 0.008) (Supplemental Fig. S2). Such changes could be explained by the combined effects of vasodilation and vessel recruitment induced by NO.

Olschewski et al. (27) and Ghofrani et al. (13) already showed reduction of PAP with inhaled NO in IPF at rest. Our study confirms these findings and extends them, showing that the hemodynamic effect of NO was even greater during exercise. Such a hemodynamic improvement could eventually lead to a better exercise tolerance in IPF, since, in this condition, abnormalities in pulmonary circulation play a major role in limiting exercise tolerance (17).

In the present series, we did not observe changes in arterial oxygenation or V_{A}/Q_{L} distributions when administering inhaled NO, neither at rest nor during exercise. This is at variance with what we have observed in COPD, where inhaled NO worsens gas exchange due to the inhibition of HPV (6), despite HPV being also present in IPF (5). The absence of gas exchange deterioration could be explained by the lack of access of inhaled NO to units with greater structural derangement, and hence not counteracting HPV in these units (13), or to the fact that, in IPF, fibrosis affects, to a greater extent, pulmonary vessels than small airways. Accordingly, inhaled NO appears to target lung vessels in well-ventilated areas, thus not increas-
ing blood flow in poorly ventilated alveolar units with low \( V_{A}/Q \) ratios. What makes this finding clinically relevant is that, despite HPV contributing to \( V_{A}/Q \) matching in both COPD and IPF, the effect of NO on gas exchange behaves differently. While in COPD inhaled NO worsens arterial oxygenation (6), in IPF it does not (13, 27).

To our knowledge, this is the first study assessing the effects of inhaled NO during exercise in IPF, when patients are more clinically symptomatic. During exercise, in IPF, alveolar-to-capillary oxygen diffusion limitation becomes an important factor accounting for the \( P_{aO_2} \) decrease (5). In this regard, \( V_{A}/Q \) distribution assessment with the MIGET clearly showed that NO had no effect on exercise-induced oxygen diffusion limitation (Fig. 4), consistent with the lack of changes in CO, heart rate, and, presumably, in red cell transit time. Furthermore, the larger increase in \( V_{E} \) than in \( CO \) during exercise shifted \( V_{A}/Q \) distributions to units with higher \( V_{A}/Q \) ratio, hence minimizing the potential impact of NO on poorly ventilated lung units.

Previous studies have shown that, whereas inhaled NO does not influence gas exchange, the administration of intravenous prostacyclin or iloprost increases intrapulmonary shunt without altering the dispersion of \( V_{A}/Q \) distribution (13, 27). This selective effect of intravenous prostanooids on intrapulmonary shunt could be explained by vasodilation restricted to unventilated lung units or to the increase in CO, changes that are not observed with inhaled NO. We consider the effect on CO more likely, since it has been known for many years that, in conditions of diffuse lung damage, the increase in CO causes a concomitant increase in shunt (24, 34). Accordingly, IPF drugs, with eventual inotropic effect, could deteriorate gas exchange due to their effect on intrapulmonary shunt and, eventually, on alveolar-to-capillary oxygen diffusion.

Our study has limitations. First, it was performed in a small number of patients. Nevertheless, due to the consistency of the findings, we decided not to include more patients to avoid unnecessary invasive studies. Second, the order of the measurements was not randomized, and in all cases measurements of breathing ambient air were performed first and breathing NO second, although we left 45 min after completing the first set of measurements and checked the return to baseline conditions. Third, the majority of patients did not present significant PH at rest, although six out of seven showed a marked increase of mPAP during exercise with values exceeding 40 mmHg. To what extent our findings can be extrapolated to patients with more severe PH remains unsettled. Nevertheless, as shown by Agusti et al. (5), IPF patients with lesser vascular impairment have more preserved HPV and hence could be more susceptible to a detrimental effect of NO on gas exchange. Fourth, plasma concentrations of endothelium-derived vasoactive agents were measured while the subject was breathing ambient air only, such that the effects of inhaled NO on those measurements could not be established. Finally, our study was not conducted to assess a potential treatment of IPF-associated PH. It mainly assessed the impact of inhaled NO on \( V_{A}/Q \) distributions. Given the potential interest of NO signaling pathway as a therapeutic target (19a), adequately designed clinical trials should address this question in IPF patients with established PH.

In conclusion, results of the present study, conducted in a selected population of patients with IPF, suggest that endothelium-derived signaling molecules may contribute to modulate pulmonary vascular tone during exercise, and that inhaled NO reduces PVR, both at rest and during exercise, without altering gas exchange.

Our findings underscore the relevance of evaluating targeted PAH therapy in specific conditions because, despite \( V_{A}/Q \), mismatch underlies arterial hypoxemia in the majority of chronic respiratory disorders; their side effects on gas exchange may not be identical. Indeed, whereas in COPD PH therapy impairs gas exchange, in IPF it may not have such an undesirable effect.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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