EFFECTS OF INHALED NITRIC OXIDE AT REST AND DURING EXERCISE IN IDIOPATHIC PULMONARY FIBROSIS

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Supported by grants FIS 00980994, SEPAR and SOCAP.

Running head: Inhaled NO in IPF

Word count (body of the manuscript): 3355
ABSTRACT

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 signalling 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 7 patients with IPF (6 men/1 woman; 57±11 years; forced vital capacity, 60±13% predicted; CO diffusing capacity, 52±10% predicted). Levels of endothelin, 6-keto-prostaglandin-F1α (PGF1α), thromboxane-B2 and nitrates were measured at rest and during submaximal exercise. Pulmonary hemodynamics and gas exchange, including ventilation-perfusion (V_A/Q) relationships, were assessed breathing ambient air and 40 ppm NO, both at rest and during submaximal exercise.

The concentration of thromboxane-B_2 increased during exercise (p=0.046), whereas levels of other mediators did not change. The change in PGF1α correlated with that of mean pulmonary artery pressure (mPAP) (r=0.94; p<0.005). Inhaled NO reduced mPAP 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 V_A/Q distributions in any of the study conditions. Alveolar-to-capillary oxygen diffusion limitation, which accounted for the decrease of PaO_2 during exercise, was not modified by NO administration.

We conclude that in IPF some endothelium-derived signalling 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.

Word count (abstract): 243
52 **Key words:** pulmonary hemodynamics; gas exchange; ventilation-perfusion relationships; pulmonary circulation; vasodilator agents.
INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive disease characterized by inflammation and fibrosis of the lung parenchyma. Abnormal gas exchange and pulmonary hypertension 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). Pulmonary hypertension (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 anti-proliferative 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 HIV infection. To what extent specific PAH therapy would also be beneficial in IPF-associated PH remains unknown. Prostanoids, phosphodiesterase-5 inhibitors (PDE-5i) and endothelin receptor antagonists
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(ERA) 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 ERA (22) or PDE-5i (2) 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 we have 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 ($V_A/Q$) imbalance resulting from the inhibition of hypoxic pulmonary vasoconstriction (HPV) (4; 6; 7). Patients with IPF also show HPV, which contributes to $V_A/Q$ matching and is related the degree of vascular impairment (5).

Inhaled NO is a selective pulmonary vasodilator commonly used to test pulmonary vasorreactivity 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 signalling 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 years were studied. In 4 of them (57%) a diagnosis of usual interstitial pneumonia was established by surgical biopsy, in the remaining 3 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 ($DL_{CO}$), 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 ($W_{\text{max}}$) that each patient could tolerate on a cyclo-ergometer (E. Jaeger, Germany) was determined by an incremental exercise test. The day of the study, a triple-lumen Swan-Ganz catheter (Edwards Laboratories, USA) was placed into the pulmonary artery under pressure-wave monitoring (M1166A; Hewlett-Packard, Germany), 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, USA). Measurements of endothelium-derived vasoactive agents (ET-1, thromboxane-$B_2$ (TMXB$_2$) and 6-keto prostaglandin-$F_{1\alpha}$ (PGF1$\alpha$)) in arterial blood were performed in 6 patients, at rest and during
submaximal exercise. The plasma concentrations of ET-1 and PGF$_{1\alpha}$ were measured by radioimmunoassay, and the concentration of TMXB$_2$ 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 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 formulae. Ventilation ($V_E$) and respiratory rate were recorded using a calibrated Wright spirometer (MK8; BOC-Medical, UK). Oxygen uptake and carbon dioxide production were calculated from mixed expired oxygen and carbon dioxide concentrations (Medical Graphics, USA). Arterial and mixed venous PO$_2$, PCO$_2$ and pH were analyzed in duplicate, using standard electrodes (IL 1302; Instrumentation Laboratories, Italy).

Ventilation-perfusion 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 indices 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 ventilation 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 PO$_2$ value that should result from the measured $V_A/Q$ distribution, taking into account all the factors that may influence in the gas exchange ($F_{1O_2}$, $V_E$, CO), on the explicit assumption that there is no
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154 oxygen diffusion limitation. The comparison of this predicted PO$_2$ value with that actually
155 measured in the arterial blood thus provides information on the presence of oxygen diffusion
156 limitation (3). In this manner, the limitation of alveolar-to-capillary oxygen transfer is evident
157 as a higher predicted than measured PaO$_2$(3).

158 Study design

159 Forty-five minutes after starting the inert gas infusion, patients were connected to a breathing
160 circuit with inhaled NO-free air for 20 min and measurements at rest were performed. All
161 measurements were taken under steady-state conditions. Following this, each patient was asked
162 to cycle at a workload equivalent to 60% of the maximal tolerated in the previous incremental
163 test. After having reached steady-state conditions, a second set of measurements were
164 performed during exercise. Afterwards, patients rested for 45 min and once confirmed that
165 systemic and pulmonary hemodynamics had returned to baseline levels, patients were
166 reconnected to the breathing circuit and breathed a mixture of NO in air at 40 ppm during 20
167 min (Tecfluid, Spain) as previously described (28). Measurements at rest and during
168 submaximal exercise were repeated as before. Inspired concentrations of NO, nitric dioxide
169 (NO$_2$) and oxygen were continuously monitored.

170 Statistical analysis

171 The results are expressed as mean±SD. The Wilcoxon signed-rank test was used to compare
172 plasma concentrations of vasoactive agents at rest and during exercise. The Spearman
173 correlation coefficient was used to explore the relationship between concentrations of
174 vasoactive agents and gas exchange and pulmonary hemodynamics. The effects of exercise
175 and inhaled-NO on hemodynamic and gas exchange measurements were assessed on a two-
176 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 $\text{DL}_{\text{CO}}$ (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 TMXB$_2$ increased significantly during exercise. Despite a lack of change in mean value, PGF$_{1\alpha}$ increased during exercise in some patients and decreased in others. Interestingly, the change in plasma levels of PGF$_{1\alpha}$ from rest to exercise, correlated with the increase in mPAP during exercise ($r=0.94$; $p<0.005$) (Supplementary Figure 1).

Pulmonary hemodynamics at baseline and during NO inhalation

At rest, breathing ambient air, mPAP was on average in the upper limit of normal ($23 \pm 7$ mmHg). Four patients presented abnormal mPAP ($>20$ mmHg); in 2 it was marginally increased ($21-24$ mmHg), and in 2 it was $>25$ mmHg. All patients had normal pulmonary capillary wedge pressure (PCWP) (Table 3). During exercise breathing ambient air, mPAP increased up to $40 \pm 16$ mmHg ($p<0.002$) (Table 3, Figure 1), with no change in PCWP. The transpulmonary pressure gradient (TPG) (mPAP-PCWP), also increased significantly (Figure 2). Cardiac output doubled during exercise, whereas pulmonary vascular resistance (PVR) remained unaltered.

At rest, inhaled NO, decreased significantly mPAP and PVR, whereas PCWP and CO remained essentially unchanged (Table 3, Figure 2 and Supplementary Figure 2). During
exercise, the decrease in mPAP induced by inhaled NO was significantly greater than at rest (Table 3). Cardiac output during exercise, remained unaltered during NO inhalation (Table 3, and Supplementary Figure 2). Compared with baseline conditions, during NO inhalation, the relationship between TPG and CO shifted downwards (Figure 2) and its slope decreased significantly, from 2.70±1.20 to 1.37±0.71 mmHg·min·L⁻¹ (p=0.008).

**Pulmonary gas exchange at baseline and during NO inhalation**

At rest, patients showed moderate hypoxemia with PaCO₂ within the normal range (Table 4, Figure 1). All patients exhibited a moderate degree of V_A/Q inequality, as shown by increased Log SDQ and Log SDV (Table 4, Figure 3). The DISP R-E was also moderately increased and intrapulmonary shunt was negligible (<3% in all cases). At rest, V_A/Q inequality was the main cause of hypoxemia as shown by the close agreement between measured PaO₂ and that predicted from observed V_A/Q inequality (-0.4±8.3 mmHg) (33). During exercise, PaO₂ decreased 18±12 mmHg and AaPO₂ increased by 26±15 mmHg, while PaCO₂ remained unchanged (Table 4, Figure 1). The distribution of blood flow remained unaltered, whereas that of ventilation was more homogeneous and dead space decreased (Table 4, Figure 3).

Figure 5 shows the plot of direct measurements of retention minus excretion, corrected for acetone, of each inert gas versus its solubility. Taken all gases together there were no significant differences among the 4 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 ventilation. No effect of NO inhalation was noticed.

The difference between predicted PaO₂ from V_A/Q inequality and that actually measured, increased markedly during exercise (20±11 mmHg; p=0.001 compared with value at rest), thus indicating that oxygen diffusion limitation emerged as an important factor contributing to decrease PaO₂ (Figure 4).
At rest, inhaled NO did not modify arterial oxygenation and $V_A/Q$ distributions remained essentially unchanged (Table 4, Figures 1 and Supplementary Figure 2). The difference between predicted and measured PaO$_2$ was 6.0±9.8 mmHg, a value slightly greater than that observed breathing ambient air that suggests some degree of alveolar-to-capillary O$_2$ diffusion limitation; although, as mentioned, no deterioration on arterial oxygenation was observed. During exercise, NO inhalation did not modify arterial blood gases or $V_A/Q$ distributions (Table 4 and Supplementary Figure 2). During NO inhalation, the contribution of oxygen diffusion limitation to exercise-induced hypoxemia, as assessed by the difference between predicted and measured PaO$_2$, was similar to that observed while breathing ambient air (Figure 4).

Analysis of individual inert gas gradients (retention minus excretion) did not show any effect of inhaled NO (p=0.201) (Figure 5).

**DISCUSSION**

Results of the current 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 the release of thromboxane and prostacyclin has been proposed as a possible mechanism implicated in the development of PH (36; 40). In our study, during exercise, TMXB$_2$ increased, whereas the concentration of the prostacyclin metabolite PGF$_{1\alpha}$ and the ratio between PGF$_{1\alpha}$ and TMXB$_2$ remained stable. Interestingly, the change in PGF$_{1\alpha}$ correlated with that of mPAP (Supplementary Figure 1). Taken together we hypothesize that exercise induced the endothelial production of eicosanoids with opposite actions on...
pulmonary vascular tone, being the increase in 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$_{1\alpha}$ during exercise and the increase of PAP, suggests that PGF$_{1\alpha}$ could be a potential non-invasive 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-signalling 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 $V_{A}/Q$ imbalance due to HPV inhibition (4; 6; 7; 21), we carefully evaluated the effects of inhaled NO on $V_{A}/Q$ 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 (SAP), 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 (Supplementary Figure 2). Whereas breathing ambient air mPAP increased 3 mmHg per litre of CO, during NO inhalation such an increase was of only 1.4 mmHg per litre ($p=0.008$) (Supplementary Figure 2). 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 an 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 $V_{A}/Q$ 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 is 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 in a greater extent pulmonary vessels than small airways. Accordingly, inhaled NO appears to target lung vessels in well-ventilated areas, thus not increasing blood flow in poorly ventilated alveolar units with low $V_{A}/Q$ ratios. What makes this finding clinically relevant is that, despite HPV contributes 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 $\text{PaO}_2$ 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 (Figure 4), consistent with the lack of changes in CO, heart rate and, presumably, in red cell transit time. Furthermore, the larger increase in ventilation than in cardiac output during exercise shifted
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VA/Q distributions to units with higher VA/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 VA/Q distribution (13; 27). This selective effect of intravenous prostanoids 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, in 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 breathing ambient air were performed first and breathing NO secondly, although we left 45 minutes 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 6 out of 7 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 breathing ambient air only, such that the effects of inhaled NO on those measurements could not be established. Finally, our
study was not addressed 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 signalling pathway as a therapeutic target (2), 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 signalling molecules may contribute to modulate pulmonary vascular tone during exercise; and that inhaled NO reduces pulmonary vascular resistance, 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.
Acknowledgments

The authors would like to thank, F. Burgos, C. Gistau and M. Simó for their invaluable support and collaboration in the studies; and to J. Ruiz Manzano for the collaboration in recruiting the patients.
References


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FIGURE LEGENDS

Figure 1
Individual effects of inhaled nitric oxide (NO) on mean pulmonary artery pressure (mPAP) and arterial oxygen tension (PaO₂), at rest and during exercise. Thick bars denote mean values.

Figure 2
Relationship between mean values of pulmonary vascular pressure gradient (mean pulmonary artery pressure minus pulmonary capillary wedge pressure) and cardiac output, at rest and during exercise, while breathing ambient air (triangles) and 40 ppm NO (circles). The pressure-flow relationship shifted downwards and had a lower slope during NO inhalation (p=0.008).

Figure 3
Ventilation-perfusion distributions at rest (upper panels) and during exercise (lower panels), before and during nitric oxide inhalation, in one of the study patients. The amount of ventilation (○) and blood flow (●) in alveolar units with different ventilation-perfusion (VA/Q) ratios in each study condition is shown.

Figure 4
Relationship between PaO₂ predicted from observed VA/Q inequality and that actually measured, at rest (left panel), breathing ambient air (closed circles) and inhaled NO (open circles); and during exercise (right panel), breathing ambient air (closed triangles) and inhaled NO (open triangles). During exercise, predicted PaO₂ was systematically greater than that actually measured, denoting alveolar-to-capillary O₂ diffusion impairment.
during NO inhalation practically overlapped those breathing ambient air, indicating that NO
did not modify O₂ diffusion limitation during exercise.

**Figure 5**

Plot of retention minus excretion corrected for acetone (R-E*) versus solubility (log scale) for
the 6 inert gases in the four study conditions. Values are expressed as mean±SD.

A repeated measures ANOVA did not show differences in R-E* on the four study conditions.
Individual analysis of each gas reveals that R-E* of gases with intermediate solubility
(cyclopropane and halothane) decreased during exercise, consistent with a reduction in V_A/Q
inequality as a result of a more homogeneous distribution of ventilation. No effects of inhaled
nitric oxide were noticed.
## TABLE 1
GENERAL CHARACTERISTICS AND LUNG FUNCTION DATA

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<table>
<thead>
<tr>
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<tr>
<td>Age, yr</td>
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<td>Gender, No.</td>
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<td>Height, cm</td>
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<td>Weight, kg</td>
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<tr>
<td>% pred</td>
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<td>FVC, % pred</td>
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<td>TLC, % pred</td>
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<tr>
<td>DLCO, % pred</td>
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Incremental exercise test

|                        |        |       |
| W peak                 |        |       |
| Watts                  | 104 ± 28|   |
| % pred                 | 84 ± 29|       |
| VO\textsubscript{2} peak |        |       |
| mL·min\textsuperscript{-1} | 1301 ± 328| |
| % pred                 | 75 ± 24|       |
| mL·Kg·min\textsuperscript{-1} | 17 ± 2 |       |
| V\textsubscript{E} peak |        |       |
| L·min\textsuperscript{-1} | 58 ± 9 |       |
| V\textsubscript{E} peak/MVV, % | 78 ± 40 |       |
| HR peak                |        |       |
| min\textsuperscript{-1} | 135 ± 18|   |
| % pred                 | 84 ± 13|       |

Data are expressed as means±SD.

Definition of abbreviations: FEV\textsubscript{1}= forced expiratory volume in the first second; FVC= forced vital capacity; TLC= total lung capacity; DLCO= diffusing capacity of the lung for carbon monoxide; W peak= work-rate at peak exercise; VO\textsubscript{2} peak= oxygen uptake at peak exercise; V\textsubscript{E}= minute ventilation; MVV= estimated maximum voluntary ventilation; HR peak= heart rate at peak exercise.
### TABLE 2
ENDOTHELium-DERIVED VASOACTIVE AGENTS
AT REST AND DURING EXERCISE

<table>
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<th>Rest</th>
<th>Exercise*</th>
<th>p value†</th>
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<tr>
<td>Endothelin, pg·mL⁻¹</td>
<td>9.3±6.4</td>
<td>9.2±5.4</td>
<td>ns</td>
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<tr>
<td>6-keto-PG F₁α, pg·mL⁻¹</td>
<td>270±201</td>
<td>291±235</td>
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<tr>
<td>Thromboxane B₂, pg·mL⁻¹</td>
<td>155±73</td>
<td>209±108</td>
<td>0.046</td>
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<tr>
<td>6-keto-PG F₁α / TMXB₂</td>
<td>2.12±1.77</td>
<td>2.04±2.4</td>
<td>ns</td>
</tr>
<tr>
<td>NO₂⁻/NO₃⁻, nmol·mL⁻¹</td>
<td>49.4±8.5</td>
<td>46.5±10.6</td>
<td>ns</td>
</tr>
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</table>

Data are expressed as means±SD.

Definition of abbreviations: 6-keto-PGF₁α = 6-keto-prostaglandin F₁α; TMXB₂ = tromboxane B₂; NO₂⁻ = nitrite; NO₃⁻ = nitrate; ns = not significant

* Measurements performed while breathing ambient air

† Wilcoxon signed-rank test.
### TABLE 3
HEMODYNAMIC RESPONSE TO EXERCISE AND INHALED NITRIC OXIDE

<table>
<thead>
<tr>
<th></th>
<th>Air Rest</th>
<th>Air Exercise</th>
<th>NO Rest</th>
<th>NO Exercise</th>
<th>p value*</th>
<th>Main effects</th>
<th>Interaction NO-exercise</th>
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<tr>
<td>mPAP, mmHg</td>
<td>20±7</td>
<td>40±16</td>
<td>16±6</td>
<td>28±10</td>
<td>0.002</td>
<td>0.002</td>
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<tr>
<td>CO, L·min⁻¹</td>
<td>5.5±1.0</td>
<td>12±1.5</td>
<td>5.5±0.8</td>
<td>12.0±1.6</td>
<td>ns</td>
<td>&lt;0.0001</td>
<td>ns</td>
</tr>
<tr>
<td>HR, beats·min⁻¹</td>
<td>80±12</td>
<td>126±14</td>
<td>84±19</td>
<td>125±17</td>
<td>ns</td>
<td>&lt;0.0001</td>
<td>ns</td>
</tr>
<tr>
<td>PCWP, mmHg</td>
<td>7±5</td>
<td>9±5</td>
<td>5±5</td>
<td>4±4</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>PVR, dyn·s·cm⁻⁵</td>
<td>192±85</td>
<td>204±84</td>
<td>154±63</td>
<td>130±52</td>
<td>0.005</td>
<td>ns</td>
<td>0.03</td>
</tr>
<tr>
<td>RAP, mmHg</td>
<td>3±4</td>
<td>5±5</td>
<td>1±4</td>
<td>3±3</td>
<td>0.01</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>104±16</td>
<td>127±20</td>
<td>97±14</td>
<td>119±22</td>
<td>0.007</td>
<td>0.001</td>
<td>ns</td>
</tr>
</tbody>
</table>

Data are expressed as means±SD.

*Significance of the effects of NO and exercise, and their interaction, on a two-way repeated-measures ANOVA.

Definition of abbreviations: 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; ns= not significant.
## TABLE 4
GAS-EXCHANGE RESPONSE TO EXERCISE AND INHALED NITRIC OXIDE

<table>
<thead>
<tr>
<th></th>
<th>Air</th>
<th>NO</th>
<th>Main effects</th>
<th>Interaction NO-exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
<td>NO</td>
<td>Exercise</td>
</tr>
<tr>
<td><strong>PaO₂, mmHg</strong></td>
<td>77±11</td>
<td>59±16</td>
<td>ns</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>PaCO₂, mmHg</strong></td>
<td>39±2</td>
<td>37±5</td>
<td>0.03</td>
<td>ns</td>
</tr>
<tr>
<td><strong>AaPO₂, mmHg</strong></td>
<td>19±11</td>
<td>45±22</td>
<td>ns</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>PvO₂, mmHg</strong></td>
<td>37±2</td>
<td>25±2</td>
<td>ns</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Vₑ, L·min⁻¹</strong></td>
<td>7.9±2.7</td>
<td>33.9±10.3</td>
<td>ns</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Vₑ/D/V₉, %</strong></td>
<td>49.2±11.3</td>
<td>43.5±9</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Log SDQ</strong></td>
<td>0.84±0.46</td>
<td>0.88±0.50</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Low Vₑ/Q, %CO</strong></td>
<td>3.8±5.7</td>
<td>2.9±4.0</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Mean Q</strong></td>
<td>0.72±0.17</td>
<td>1.93±0.51</td>
<td>ns</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Shunt, %CO</strong></td>
<td>1.2±1.0</td>
<td>1.6±1.8</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Log SDV</strong></td>
<td>0.72±0.23</td>
<td>0.53±0.13</td>
<td>ns</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Mean V</strong></td>
<td>1.23±0.21</td>
<td>3.00±0.82</td>
<td>ns</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Dead space, % Vₑ</strong></td>
<td>31.6±11.2</td>
<td>28.8±7.9</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td><strong>DISP R-E</strong></td>
<td>6.4±3.2</td>
<td>5.6±3.4</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Pred-Meas PaO₂, mmHg</strong></td>
<td>-0.4±8.3</td>
<td>19.6±11.1</td>
<td>0.001</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Data are expressed as means±SD.

*Significance of the effects of NO and exercise, and their interaction, on a two-way repeated-measures ANOVA.

Definition of abbreviations: PaO₂= arterial oxygen partial pressure; PaCO₂= arterial carbon dioxide partial pressure; AaPO₂= alveolar-arterial oxygen pressure difference; PvO₂= mixed venous oxygen partial pressure; Vₑ= minute ventilation; Vₑ/D/V₉= ratio of dead space to tidal volume; Log SDQ= dispersion of blood flow distribution; CO= cardiac output; Low Vₑ/Q= perfusion to alveolar units with Vₑ/Q ratios between 0.005 and 0.1; Mean Q= mean Vₑ/Q ratio of blood flow distribution; shunt= perfusion to alveolar units with Vₑ/Q ratios < 0.005; Log SDV= dispersion of ventilation distribution; Mean V= mean Vₑ/Q ratio of ventilation.
distribution; dead space = ventilation to units with $V_A/Q$ ratios > 100 calculated from inert gases; DISP R-E = dispersion of retention minus excretion of inert gases corrected for acetone; Pred-MeasPaO$_2$ = predicted minus measured PaO$_2$. 
Figure 3

AMBIENT AIR - REST

Ventilation (○) and Blood flow (●), L/min

 Ventilation-Perfusion ratio

AMBIENT AIR - EXERCISE

Ventilation (○) and Blood flow (●), L/min

 Ventilation-Perfusion ratio

NITRIC OXIDE - REST

Ventilation (○) and Blood flow (●), L/min

 Ventilation-Perfusion ratio

NITRIC OXIDE - EXERCISE

Ventilation (○) and Blood flow (●), L/min

 Ventilation-Perfusion ratio
Figure 4

**EXERCISE**

- Predicted PaO\(_2\), mmHg
  - 40
  - 60
- Measured PaO\(_2\), mmHg
  - 20
  - 40
  - 60
  - 80
  - 100

**REST**

- Predicted PaO\(_2\), mmHg
  - 40
  - 60
- Measured PaO\(_2\), mmHg
  - 20
  - 40
  - 60
  - 80
  - 100

Scatter plots showing predicted vs. measured PaO\(_2\) under rest and exercise conditions, with different markers for Air and NO.
Figure 5

The figure shows the solubility of various gases as a function of atmospheric pressure. The solubility is presented in milliliters per 100 milliliters per millimeter of mercury (mL/100mL/mmHg). The solubility values for different gases are indicated at various pressure levels, ranging from 0.001 to 1000 mmHg. The gases include Ambient air, rest, Ambient air, exercise, NO, rest, and NO, exercise. The graph also includes error bars to indicate the variability in the data.