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A comparison between high dose nitric oxide delivered from pressurized cylinders and nitric oxide produced by an electric generator from air. A safety pilot study.

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Author's Contribution

SG, RDF, BSF, ALM, FI, WMZ, LB: study design.

SG, CCAM, ALM: data collection.

SG, ALM, RWC: analysis of data.

SG, RDF, CCAM, BY, LB: manuscript preparation.

BSF, ALM, RWC, FI, WMZ: Review of manuscript

SG, CCAM, RDF, BSF ALM, BY, RWC, FI, WMZ and LB agreed to be accountable for all aspects of the work and take responsibility for the integrity of the work as whole.

Summary Conflict of Interest Statement:

SG, RDF, BSF, FI: No conflict of interest to declare.

WMZ, BY licensed patents on electric generation of NO to Third Pole.

RWC receives salary support from UNITAID as the co-principal investigator for work developing tuberculosis diagnostic technologies.

LB receives salary support from K23 HL128882/NHLBI NIH as principal investigator for his work on hemolysis and nitric oxide. LB receives technologies and devices from iNO Therapeutics LLC, Praxair Inc., Masimo Corp. LB receives grants from “Fast Grants for COVID-19 research” at Mercatus Center of George Mason University and from iNO Therapeutics LLC.

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ABSTRACT

Background: High dose (≥ 80 parts-per-million[ppm]) inhaled nitric oxide (NO) has antimicrobial effects. We designed a trial to test the preventive effects of high dose NO on coronavirus disease (COVID-19) in healthcare providers working with COVID-19 patients. The study was interrupted prematurely due to the introduction of COVID-19 vaccines for healthcare professionals. We thereby present data on safety and feasibility of breathing 160 ppm NO using two different NO sources, namely pressurized nitrogen/NO cylinders (iNO) and electric NO generators (eNO).

Methods: Nitric oxide gas was inhaled at 160 ppm in air for 15 minutes twice a day, before and after each work shift, over 14 days to healthcare providers (NCT04312243). During NO administration vital signs were continuously monitored. Safety was assessed by measuring transcutaneous methemoglobinemia (SpMet) and the inhaled nitrogen dioxide (NO₂) concentration.

Results: 12 healthy healthcare professionals received a collective total of 185 administrations of high dose NO (160 ppm) for 15 minutes twice daily. 171 doses were delivered by iNO and 14 doses by eNO. During NO administration SpMet increased similarly in both groups ($p=.82$). Methemoglobin decreased in all subjects at five minutes after discontinuing NO administration. Inhaled NO₂ concentrations remained between 0.70 [0.63-0.79] and 0.75 [0.67-0.83] ppm in the iNO group and between 0.74 [0.68-0.78] and 0.88 [0.70-0.93] ppm in eNO group. During NO administration peripheral oxygen saturation and heart rate did not change. No adverse events occurred.

Conclusion: This pilot study testing high dose inhaled NO (160 ppm) for 15 minutes twice a day using eNO seem feasible and similarly safe when compared with iNO.

Key words: nitrogen dioxide, electric NO generator, nitric oxide, methemoglobin, spontaneous breathing, pulmonary vasodilator

INTRODUCTION

Nitric Oxide (NO) gas is approved by the United States Food and Drug Administration for the treatment of hypoxia associated with pulmonary hypertension in the newborn.(1,2) In clinical practice, NO gas is widely used to reduce pulmonary artery pressure and to improve oxygenation in adult patients with acute respiratory distress syndrome (ARDS).(3–5) An elevated dose of NO gas at 160 parts per million (ppm) and higher has previously been proposed to produced antibacterial and antiviral activity in experimental studies(6,7) as well as pediatric and adult patients.(8–10) Recent in-vitro studies demonstrate that NO donors can inhibit replication of the SARS-CoV-2 virus (11) and clinical trials are investigating the clinical benefits of inhaled high dose NO in patients with coronavirus disease (COVID-19).

Nitric oxide gas is commonly administered with delivery systems that use pressurized NO in nitrogen (NO/N₂) cylinders. Pressurized inhaled nitric oxide cylinders (denoted iNO) are widely available and have been used in more than a half-million patients worldwide.(12) Despite being safe and reliable, the use of pressurized cylinders as the source of NO requires an extensive supply chain and trained personnel to deliver and manage the (NO/N₂) cylinders. Further, cylinder NO therapy can be expensive.(13)

Electrical NO generators (eNO) have been proposed as an alternative source. These devices ionize air (nitrogen and oxygen) with a pulsed, high voltage electrical discharge leading to the generation of NO, nitrogen dioxide (NO₂) and metal microparticles (released by the electrodes during electrical discharge)(14,15). A scavenger containing calcium hydroxide can reduce nitrogen dioxide (NO₂) levels below

the safety threshold (less than 3 ppm for NO₂)(16) while a high-efficiency 0.22 micron particulate air (HEPA) filter removes metal particles generated by electric discharge.(17) These eNO devices can provide inhaled NO therapy without the need for bulky and expensive cylinders potentially making NO therapy widely available both inside and outside the hospital.

To evaluate the preventative effects of NO in COVID-19, a clinical trial of healthcare workers was performed. Subjects were randomized to the treatment group (subjects received NO via iNO or eNO according to what available) or to the control group (subjects did not receive any gas). This analysis aimed to evaluate the feasibility and safety of administering 160 ppm to spontaneously breathing healthy volunteers using iNO and eNO in the treatment group.

METHODS

This analysis uses data from the trial of healthcare workers who were enrolled between March 2020 and August 2020 (NCT04312243). This study was reviewed and approved by the Institutional Review Board at Massachusetts General Hospital (Protocol 2020P000831). Written informed consent was obtained from each subject prior to initiation of any study procedures. The trial was terminated early (March 10, 2021) due a lack of enrollment after the approval of COVID-19 vaccines. Data from the enrolled participants who received nitric oxide were assessed for safety and feasibility of administration.

Subject Selection

Enrolled subjects were adult (≥ 18 years) healthcare workers (physicians, nurses or respiratory therapists) working at Massachusetts General Hospital who were scheduled to work with SARS-CoV-2 positive patients at least three times a week (defined as 6 or more shifts in 14 days). Subjects were excluded if they previously had a positive SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) test, were pregnant or had a history of hemoglobinopathies or anemia.

Nitric Oxide Gas Administration

The study subjects received inhaled NO at 160 ppm for 15 minutes twice per day, before and after each work shift, over 14 days. The antiviral and antibacterial NO dose, the duration of the single treatment and the number of treatments is now under investigation. Some authors suggest few breathes or short intermittent pulses of high

NO concentrations might work better than continuous inhalation of standard dose of NO. (7) However, it requires thorough experimental investigation and clinical studies. In our laboratory, we performed a study testing high concentration of NO in a murine model of *Klebsiella* pneumonia studies. (6) We found that short periods of breathing high concentration of NO (12 minutes of NO at 300 ppm every 3 hours) is more efficient in eliminating *Klebsiella* pneumonia than continuous NO breathing at lower concentrations (80 ppm, 160 or 200 ppm for 48 hours). It needs to be determined the effective antiviral and anti-SARS-CoV-2 concentrations, the duration of administration and number of administrations required.

We decided to give 160 ppm as previously reported to be safe in humans. We used 15 minutes to mimic our experimental study in mice. We decided to administer NO before and after the work shift.

To provide high concentrations of NO breathing, a facemask and apparatus that was previously designed and tested was utilized.(18) Briefly, the apparatus is composed of standard respiratory circuit connectors, a 3 L reservoir bag, a scavenger containing powdered calcium hydroxide, a 0.22 micron high-efficiency particulate air filter and a snug-fitting mask (Figure 1). Since high dose inhaled NO reacts with the circulating hemoglobin producing methemoglobin, transcutaneous methemoglobin (Masimo rainbow SET, Irvine, CA 92618) was monitored. Using the same device, peripheral oxygen saturation (SpO₂) and heart rate (HR) were evaluated. HR, SpO₂ and methemoglobinemia (SpMet) data were collected before and at the end of NO administration. To continuously monitor the inspired fraction of oxygen (FiO₂), an oxygen analyzer (MiniOX® 1, Ohio Medical Corporation®, Gurnee, IL 60031) was used.

NO and NO₂ Monitoring

To avoid variation of NO gas concentration during the respiratory cycle, the reservoir gas flow was kept constant at 15 L/min during NO treatments.(18) Levels of NO and NO₂ were monitored through a sampling line connected to the inspiratory limb of the circuit, proximal to the patient. Inhaled NO was measured by chemiluminescence (Sievers 280i Nitric Oxide Analyzer, GE Analytical Instruments, Boulder CO) and Cavity Attenuated Phase Shift (CAPS) was used to monitor NO₂ levels (Aerodyne Research Inc, Billerica, MA). When NO and NO₂ concentrations using high sensitivity methods (chemiluminescence and cavity attenuated phase shift) were not available, we set NO and O₂ flow as shown in Table S2. In subjects breathing from eNO generator, the delivered NO-NO₂ concentrations were always measured. To determine NO absorption and NO₂ production in the airway, exhaled NO and NO₂ concentrations were measured in one healthy subject during eNO administration.

eNO and iNO

Two different NO sources were studied, including a pressurized cylinder containing 850 ppm of NO/N₂ (150 A, Airgas, Radnor Township, PA, content = 4089 L at STP) and an electric NO generator. The electric NO generator (Portable NO generator, ODIC Inc. Littleton, MA) combines a gas pump, an NO generation chamber containing an iridium spark plug, an 18 gram scavenger containing calcium hydroxide and a 0.22 µm HEPA filter.(17) To obtain the desired NO concentration the generator was set with the following sparking parameters: sparking frequency 85 Hz and duty

cycle 65%, with an air flow of 2.5 L/min. The decision to use eNO or iNO was based on subject preference, material or personnel availability. Any subject could receive NO using either NO source (iNO and eNO).

Statistical Analysis

Data are reported as mean (standard deviation (SD)) or median (interquartile range [IQR]) for continuous variables and as frequencies and proportions for categorical variables. Normality was assessed using the Shapiro-Wilk test. To evaluate the trend of a continuous variable over time (before and after the treatment), a mixed effect model (R package [lme4] counting each patient as a random effect, R package [emmeans] for post hoc analysis) was used. Statistical significance was determined as a two-tailed $P < .05$. All the analyses were conducted using R Core Team (2021) (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>).

RESULTS

Study Population

We enrolled a total of 24 subjects: 12 in the treatment group and 12 in the control group. None of the enrolled subject developed COVID 19 disease. The control group (subjects not receiving NO) were not included in the presented analysis. NO was administered to 12 subjects, including six males and six females. Overall, the mean (SD) age was 43.3 (12.7) years with a body mass index of 28.9 (5.57) kg/m². Two subjects had a past medical history of systemic hypertension and type II diabetes

mellitus, and one received chronic bronchodilator therapy for asthma. Study population description is presented in table S2.

Nitric Oxide Administrations

Twelve subjects received a total of 185 NO gas administrations. iNO was used to administer 171 doses, and eNO was used for 14 doses. Each subject received, on average, 15.4 NO administrations. All the study subjects received NO using the iNO source. Three subjects received NO using both iNO and eNO.

Air flow was maintained at 15 L/min in the reservoir for all treatments. An NO flow of 4 [0] L/min at 850 ppm in nitrogen was added when using pressurized cylinders, and 2.5 [0] L/min at ~1180 ppm in air when using the electric NO generator. FiO_2 was 0.21 in all administrations. When an NO/N₂ pressurized cylinder was used, 1 L/min of supplemental oxygen was added to maintain the FiO_2 at 0.21. If the minute ventilation is higher than the delivered total flow (air flow + O₂ flow + NO flow), room air will enter the delivery system from an inspiratory valve on the inspiratory limb of the delivery system (Figure 1).

Methemoglobin

During the study gas administration SpMet increased in both groups from 0.90% (0.10) to 1.98% (0.11) with iNO (95% CI [-1.44; -0.70], $P < .001$) and from 0.85% (0.17) to 1.89% (0.16) with eNO (95% CI [-1.64; -0.42], $P < .001$). The increase in SpMet was not statistically different between eNO and iNO administrations (95% CI [-0.29; 0.46], $P = .98$; Figure 2).

Five minutes after stopping NO administration, SpMet decreased to 1.87% (0.11) (95% CI [-0.01; 0.22], P = .09) and 1.81% (0.16) (95% CI [-0.32; 0.48], P= .94) with iNO and eNO, respectively.

NO and NO₂ Delivered Concentrations

Inhaled NO and NO₂ concentrations were continuously monitored over 54 administrations, including 39 with iNO and 14 with eNO (Figure 3). During the study inhaled NO concentration median ranged between 164 [156-169] and 170 [165-175] ppm with iNO and between 153 [151-163] and 178 [158-180] ppm with eNO. NO₂ concentrations varied between 0.7 [0.63-0.79] and 0.75 [0.67-0.83] ppm with iNO and between 0.74 [0.68-0.78] and 0.88 [0.70-0.93] ppm with eNO (Figure 4). No statistically significant difference between the delivered NO₂ concentrations between iNO and eNO (95% CI [-0.04; 0.17], P= .35). As shown, the intra-tidal variations of NO concentration are significantly higher with eNO 14.85 (10.60) ppm compared to iNO 8.53 (2.90) ppm (95% CI [-12.30; -0.35], P < .038). This difference may be explained by the higher total gas flow (air flow + NO flow + oxygen flow) with the iNO source 20 L/min as compared to eNO 17.5 L/min.

Exhaled NO and NO₂

To further capture safety data, exhaled NO and NO₂ were measured in one healthcare worker receiving eNO. The average inspired NO and NO₂ concentrations were 158.5 ppm and 0.68 ppm, respectively. At the end of exhalation (alveolar gas

phase), NO concentration decreased to 11.8 ppm and NO₂ concentration was 0.027 ppm (Figure 5) suggesting minimal NO₂ generation in the airways.

Vital Signs during NO Administration

SpO₂ decreased slightly from 97% [97-98] before NO administration, to 96% [95-97] at the end of NO administration (95% CI [1.52; 2], P < .001) with iNO. When eNO was used, SpO₂ remained unchanged (P = .57).

Heart rate was slightly reduced from 80 [72-87] beats-per minute (bpm) to 78 [70.5-85] bpm (95% CI [2; 3.5], P < .001) with iNO and from 79 [73-82] bpm to 74.5 [70-77] bpm (95% CI [2.5; 8], P < .001) with eNO. (Figure 6) During the administrations none of the study subjects reported any discomfort. None of the subjects developed symptoms such as cough and wheezing suggesting a not significant generation of nitric acid into the airways (from the reaction between NO₂ and bronchial moisture). No adverse events were noted.

DISCUSSION

Over 185 consecutive NO administrations, we showed that administering high dose NO (160 ppm) for 15 minutes using an electric NO generator appears to be feasible and as safe compared to NO delivered from pressurized cylinder-based delivery systems.

We were able to reach and maintain a stable NO concentration of 160 ppm with both iNO and eNO throughout the 15-minute administration interval. All NO administrations were well tolerated and without any adverse events. Volunteers were

comfortable as suggested by their significant reductions of heart rate during the administrations.

During the NO administrations, SpMet rose in a similar fashion (with a percentage increase of 55%-64%) with both NO sources, suggesting a similar biological effect. Five minutes after the end of the administration, SpMet decreased in both groups reflecting a robust reduction of methemoglobin by the subjects' methemoglobin reductase.

Monitoring NO₂ concentration is imperative when administering NO at high levels. Inhaled NO₂ concentration, despite being slightly higher with eNO (but not statistically significant), was below the Occupational Safety and Health Administration (OSHA) safety levels. Although not measured during these administrations, we reported low levels of ozone produced by the eNO generator in previous studies. (19)

The administration of inhaled high dose NO led to encouraging results in a patient with cystic fibrosis and chronic lung infection (10) and improved oxygenation and reduced hospital length of stay in 69 infants admitted with acute bronchiolitis.(9) During the COVID-19 pandemic, six pregnant women with severe or critical COVID-19 pneumonia were treated with high dose iNO (160-200 ppm) for 30 mins twice a day, resulting in improved oxygenation and a reduction in respiratory rate. iNO produced symptomatic relief of shortness of breath and dyspnea in these patients.(20)

The main limitation of the widespread use of inhaled high dose NO is the need for dedicated personnel to manage bulky equipment and cylinders. The electric NO generator we studied weighs 1.5 kg, reducing the need for trained personnel or bulky materials, making it easy to use during a pandemic or in a low resource setting.

The development of novel electric NO generators delivering high dose NO from air that continuously monitor inhaled NO/NO₂ concentration and transcutaneous methemoglobin will facilitate the use of eNO for ambulatory and home use (particularly important for remote or low resource areas).

The main limitation of this study is that the decision to use iNO or eNO was based on availability of materials (tanks and eNO generator) and preference of the subjects, rather than random assignment. Thus, the number of high dose NO administrations using iNO and eNO were unequal. Lastly one should note that the number of administrations using eNO in the presented case series is limited.

Conclusion

This is the first pilot study showing promising preliminary results on feasibility and safety of high dose NO (160 ppm) using an electric NO generator. All volunteers tolerated well the treatments and the increase in SpMet during NO gas delivery is comparable between iNO and eNO.

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Figure Legends

Figure 1: Apparatus used to deliver inhaled high dose nitric oxide. NO: nitric oxide; NO₂: nitrogen dioxide; HEPA: high-efficiency particulate air filter; O₂: oxygen.

Figure 2: Non-invasive peripheral saturation of methemoglobin (SpMet) before initiating NO gas, at the end of NO administration and 5 minutes after cessation. iNO: pressurized NO/N₂ cylinder; eNO: electric NO generator.

Figure 3: Nitric oxide (NO) and nitrogen dioxide (NO₂) concentration during of the 15-minute study gas administrations. Panel A depicts the use of a pressurized cylinder (iNO) as a nitric oxide source. In Panel B the gas source was the electric NO generator (eNO).

Figure 4: Nitric oxide (NO) (Panel A) and nitrogen dioxide (NO₂) (Panel B) intra-tidal concentration variation (minimum on the left and maximum on the right for each NO source) with iNO and eNO. iNO: pressurized NO/N₂ cylinder; eNO: electric NO generator.

Figure 5: Inspiratory and expiratory nitric oxide (NO) (blue line) and nitrogen dioxide (NO₂) (red line) concentrations during three consecutive breaths (Panel A, B, C).

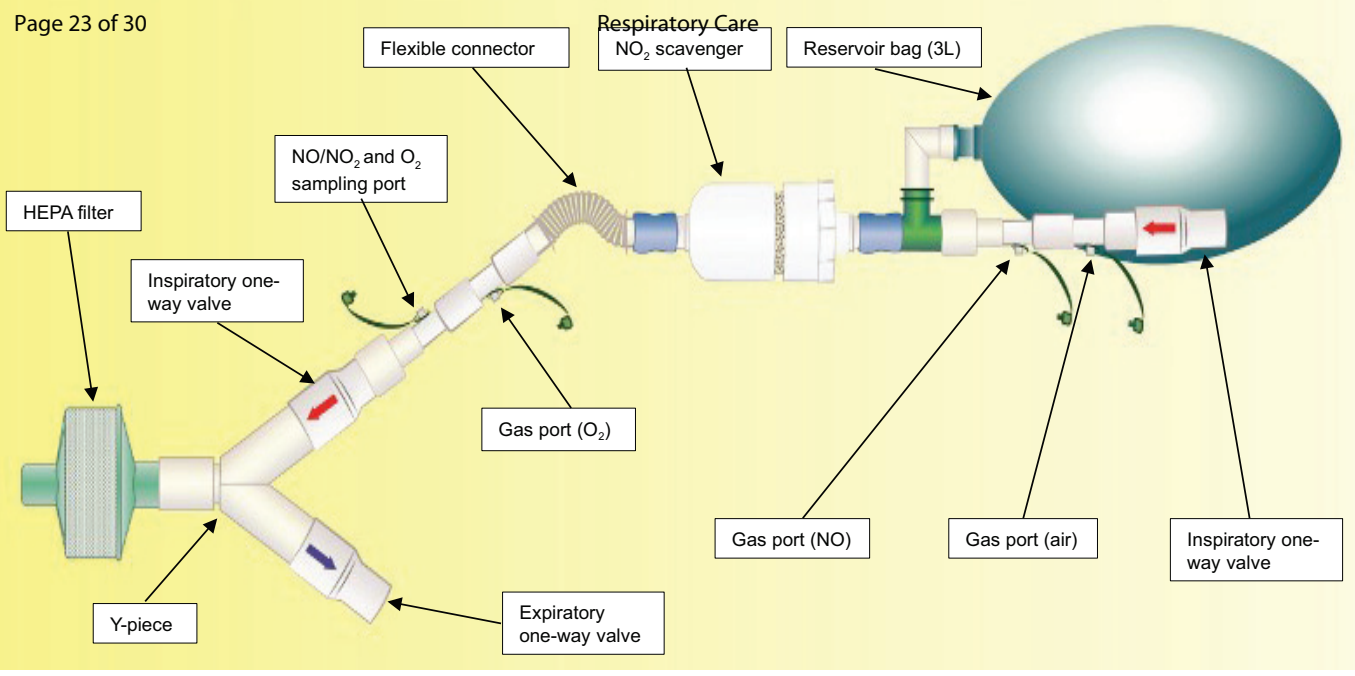
Figure 6: Peripheral oxygen saturation (SpO_2) (Panel A) and heart rate (HR) (Panel B) before NO gas administration and at the end of NO administration. iNO: pressurized NO/ N_2 cylinder; eNO: electric NO generator.

Quick Look*Current Knowledge*

The clinical application of high dose nitric oxide to treat bacterial and viral infections is under investigation through several clinical trials. The standard nitric oxide (NO) source is a pressurized cylinder containing nitric oxide balanced in nitrogen. Over the last several years, electric NO generators capable of generating NO from air using a pulsed electrical discharge have been developed.

What this Paper Contributes to our Knowledge

High dose NO was successfully administered to healthy volunteers using both pressurized cylinders and electric NO generators as an NO source. The delivery of high dose NO in this pilot study appeared to be feasible and safe using both sources. Methemoglobin increased in the same fashion in both groups. The delivered nitrogen dioxide levels were always below the prescribed safety levels.



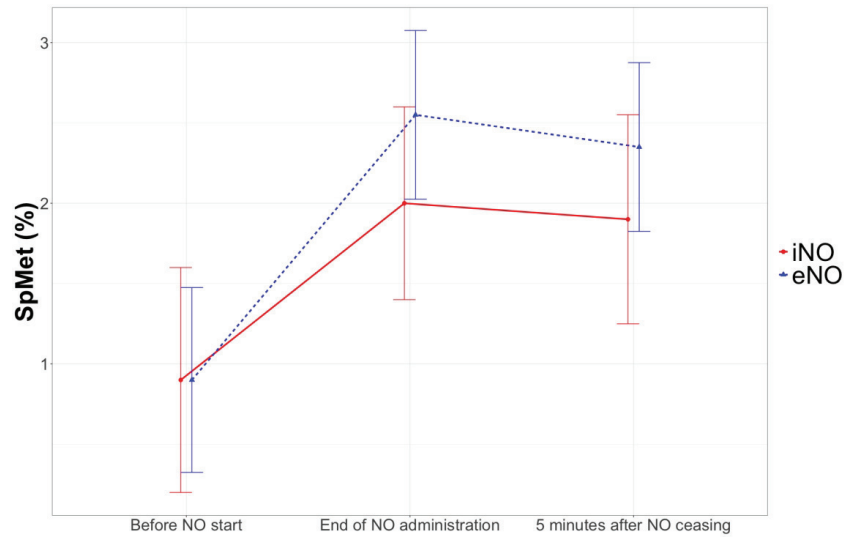
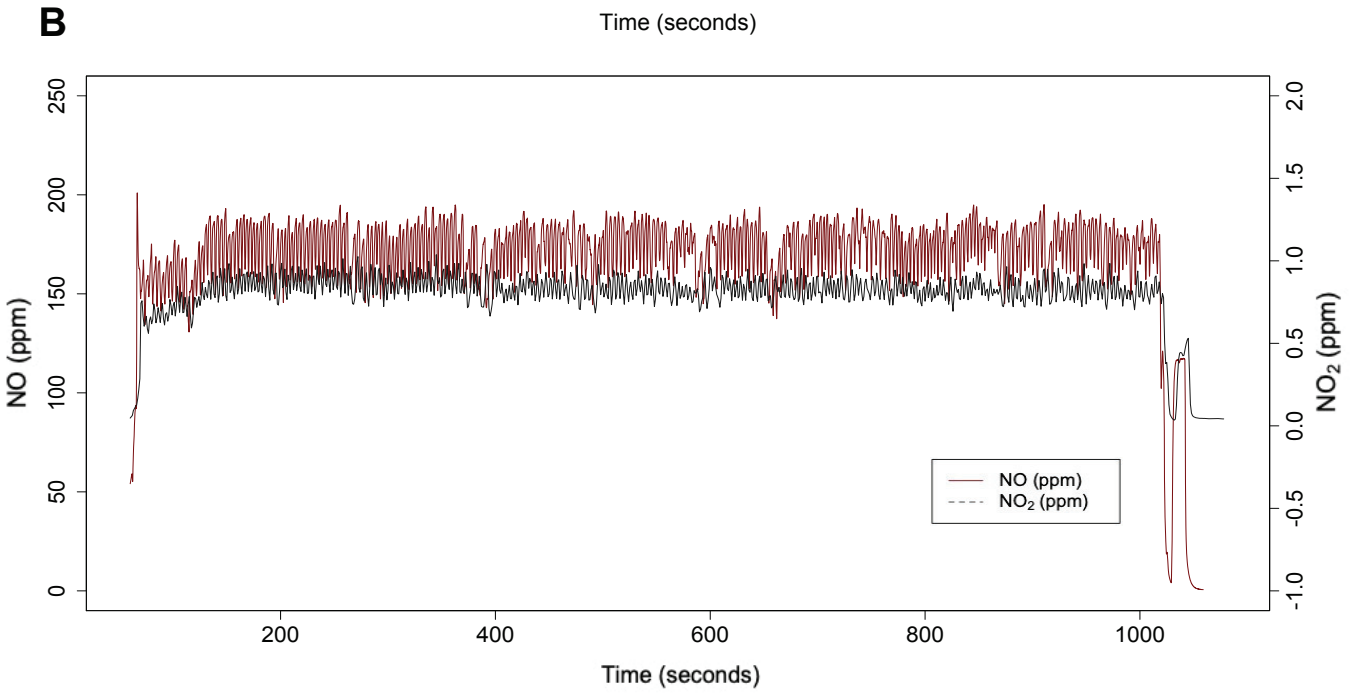
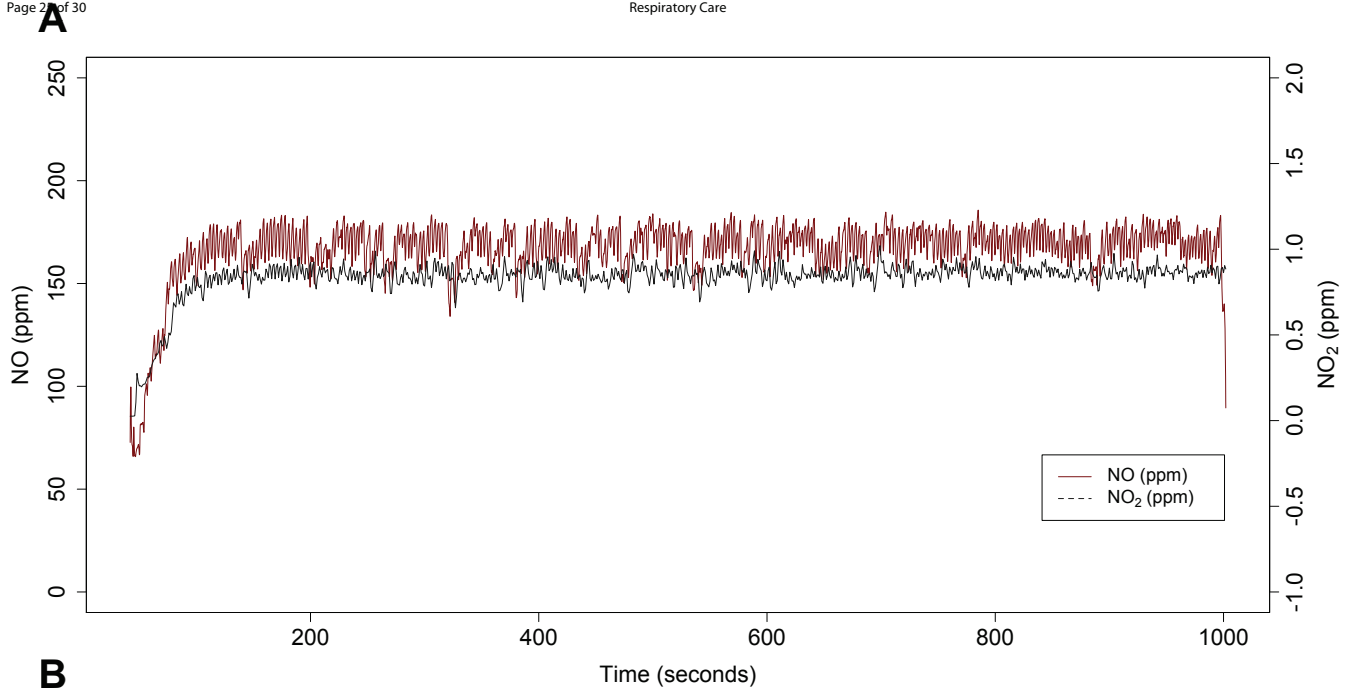


Figure 2: Non-invasive peripheral saturation of methemoglobin (SpMet) before initiating NO gas, at the end of NO administration and 5 minutes after cessation. iNO: pressurized NO/N₂ cylinder; eNO: electric NO generator.

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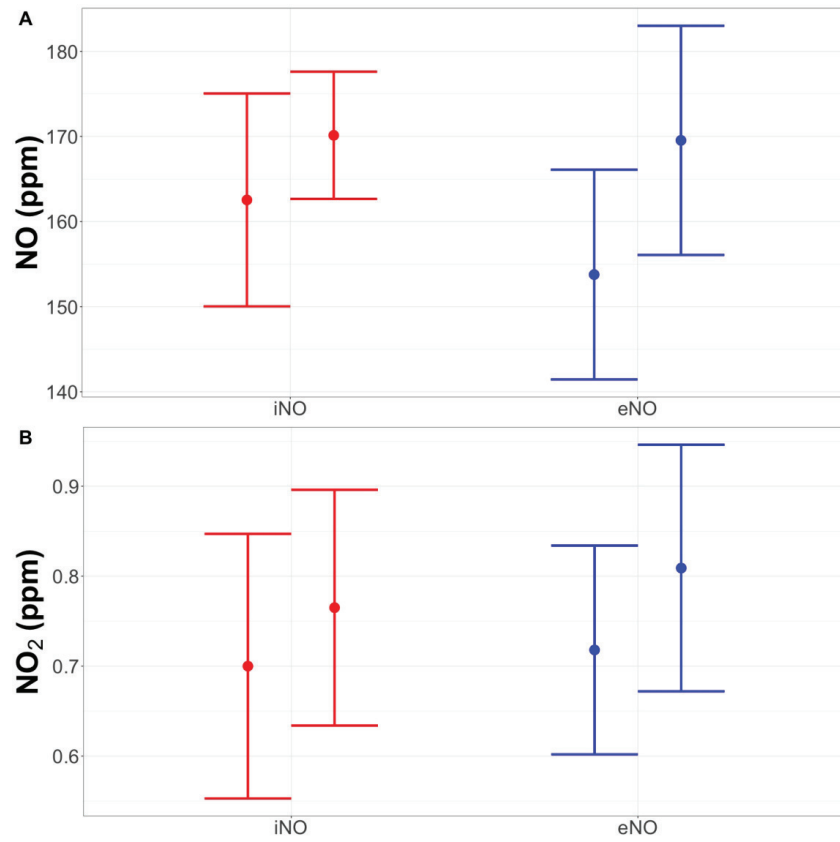
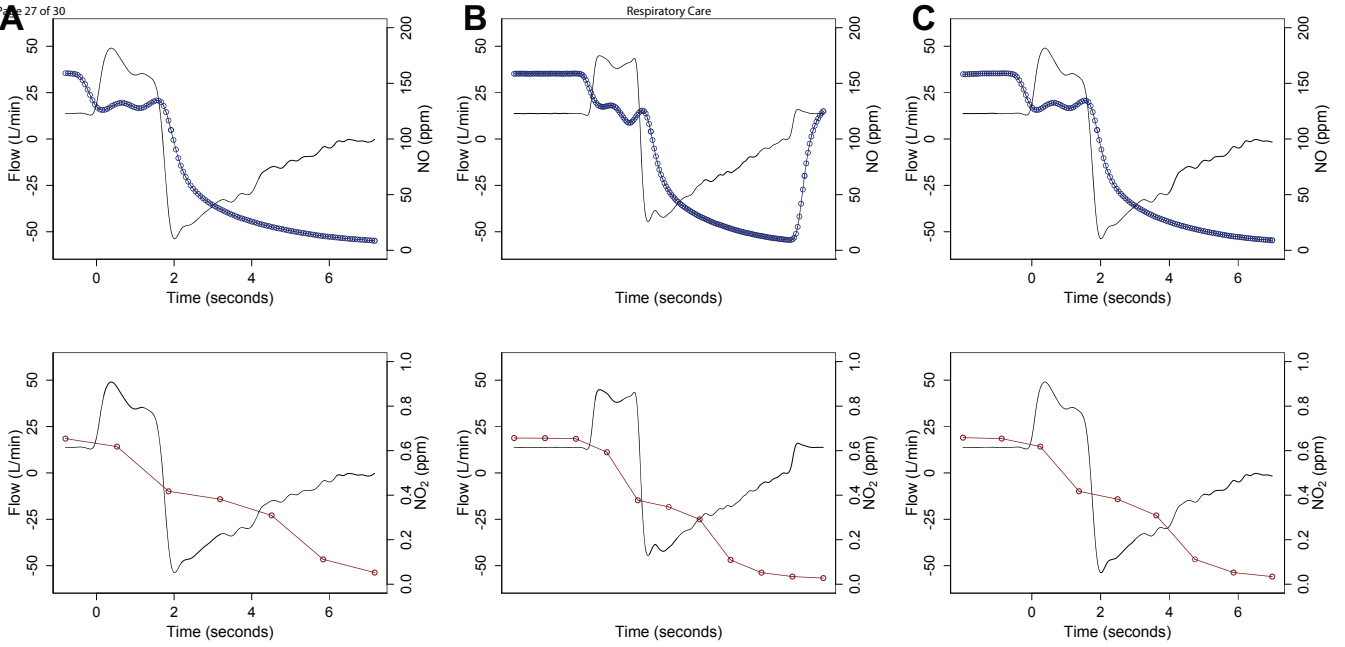


Figure 4: Nitric oxide (NO) (Panel A) and nitrogen dioxide (NO₂) (Panel B) intra-tidal concentration variation (minimum on the left and maximum on the right for each NO source) with iNO and eNO. iNO: pressurized NO/N₂ cylinder; eNO: electric NO generator.

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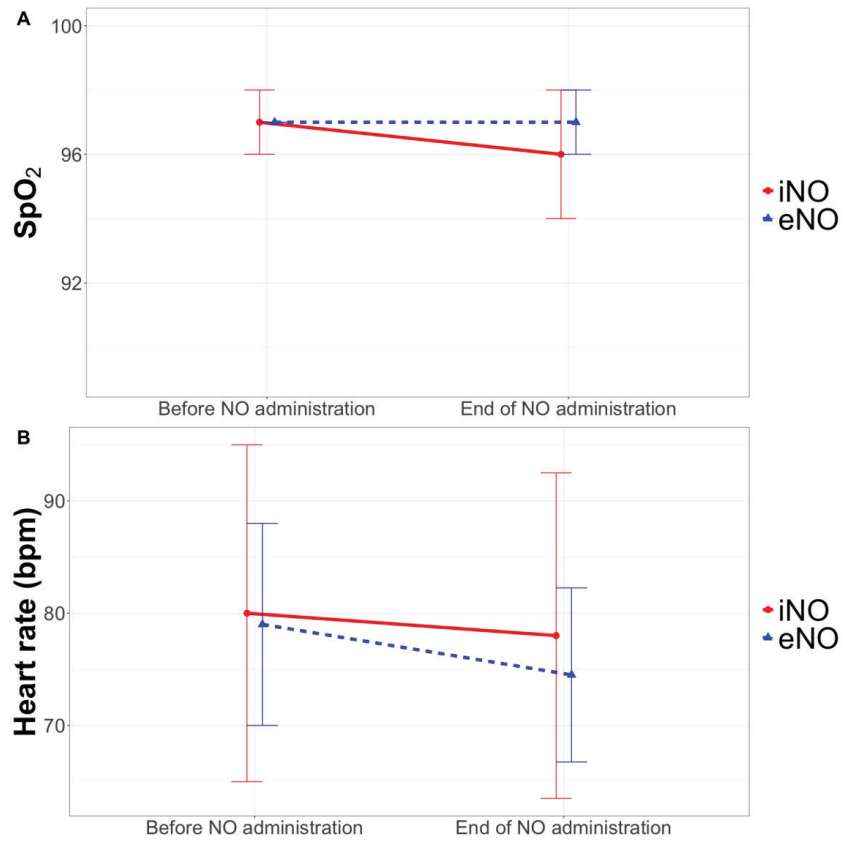


Figure 6: Peripheral oxygen saturation (SpO₂) (Panel A) and heart rate (HR) (Panel B) before NO gas administration and at the end of NO administration. iNO: pressurized NO/N₂ cylinder; eNO: electric NO generator.

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NO = 160 ± 10 ppm			
FiO ₂ (%)	NO flow (L/min)	Air flow (L/min)	O ₂ flow (L/min)
21	4	15	1

Table S1: Table used to set nitric oxide (from a pressurized cylinder containing 850 ppm NO/N₂), air and oxygen flow necessary to reach the desired concentration of 160 ppm. NO: Nitric oxide; O₂: oxygen; N₂: nitrogen.

	<i>iNO</i> (<i>N</i> =9)	<i>eNO + iNO</i> (<i>N</i> =3)
Age (years)	47.1 (12.3)	31.7 (2.52)
Sex		
<i>Female</i>	5 (56%)	1 (33%)
<i>Male</i>	4 (44%)	2 (67%)
BMI (kg/m²)	28.9 (6.4)	28.7 (1.9)
Hypertension	2 (22%)	0 (0%)
Diabetes	2 (22%)	0 (0%)
Asthma	1 (11%)	0 (0%)
Smoking status		
<i>Current Smoker</i>	1 (8%)	0 (0%)
<i>Former Smoker</i>	2 (17%)	0 (0%)
<i>Never Smoked</i>	6 (75%)	3 (100%)

Table S2: Study population. Continuous variables presented as mean (standard deviation), categorical variables presented as frequencies and proportions.