

Short papers

Exhaled NO during graded changes in inhaled oxygen in man

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Abstract

Background – Nitric oxide (NO) is present in the exhaled air of animals and humans. In isolated animal lungs the amount of exhaled NO is decreased during hypoxia. A study was undertaken to determine whether changes in arterial oxygen tension affect levels of exhaled NO in humans.

Methods – Sixteen healthy subjects were randomised to inhale different gas mixtures of oxygen and nitrogen in a double blind crossover study. Eight gas mixtures of oxygen and nitrogen (fractional inspired oxygen concentration (FiO₂) 0.1 to 1.0) were administered. Exhaled NO was measured with a chemiluminescence detector from end expiratory single breath exhalation.

Results – A dose-dependent change in exhaled NO during graded oxygen breathing was observed ($p=0.0012$). The mean (SE) exhaled NO concentration was 31 (3) ppb at baseline, 39 (4) ppb at an FiO₂ of 1.0, and 26 (3) ppb at an FiO₂ of 0.1.

Conclusions – The NO concentration in exhaled air in healthy humans is dependent on oxygen tension. Hyperoxia increases the level of exhaled NO, which indicates increased NO production. The mechanism behind this phenomenon remains to be elucidated.

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presumably mediated by decreased production of endogenous NO.⁹ Several investigators have shown that hypoxia decreases exhaled NO levels in isolated animal lungs.¹⁰⁻¹² The aim of the present study was to investigate whether oxygen tension affects exhaled NO levels in humans. However, due to ethical considerations any study on this subject in humans is limited to moderate hypoxia. We have therefore included different grades of hypoxia and hyperoxia in the present study to obtain a dose-response relationship between changes in inhaled oxygen and exhaled NO.

Methods

SUBJECTS

Sixteen healthy, non-smoking, drug-free volunteers (nine men) of mean (SD) age 28.6 (3.2) years (range 23-35) were studied. None of the subjects reported airway hyperreactivity or lung disease in their medical history.

The study protocol was approved by the ethics committee of Vienna University School of Medicine and written informed consent was obtained from all subjects.

PILOT STUDY

In four of the subjects we performed experiments on the effect of inhalation of 100% O₂ and 10% O₂ + 90% N₂ on arterial oxygen tension (PaO₂). PaO₂ was measured at baseline and at the end of 10 minutes inhalation of 10% O₂ + 90% N₂ or 100% O₂. The washout period between the two inhalation periods was 10 minutes. PaO₂ was determined from capillary blood samples of the earlobe.¹³ The arterialised blood was drawn into a thin glass capillary tube. Arterial pH, PCO₂ and PO₂ were determined with an automatic blood gas analysis system (AVL 995-Hb, Graz, Austria).

STUDY PROTOCOL

Subjects were randomised to inhale different gas mixtures of O₂ and N₂ in a double blind crossover design. Studies were conducted on a single trial day. The following gas mixtures of O₂ and N₂ were administered: 100% O₂ + 0% N₂, 80% O₂ + 20% N₂, 60% O₂ + 40% N₂, 40% O₂ + 60% N₂, 30% O₂ + 70% N₂, 20% O₂ + 80% N₂, 15% O₂ + 85% N₂, and 10%

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Nitric oxide (NO) can be detected in the exhaled air of animals and humans.¹⁻³ Endogenous NO, which is produced by NO synthase from the amino acid L-arginine, may play an important role in the physiology of the respiratory system and in the pathophysiology of airway disease.^{4,5} The absolute concentration of NO in expired air is dependent on the technique used to measure the exhalate. Exhaled NO is greater during nose breathing than during mouth breathing⁶ and breath holding increases exhaled NO,⁷ which suggests accumulation of NO in the upper and lower respiratory tract.

In the pulmonary circulation hypoxia causes pulmonary vasoconstriction *in vivo*.⁸ This effect is specific to the pulmonary vasculature and is

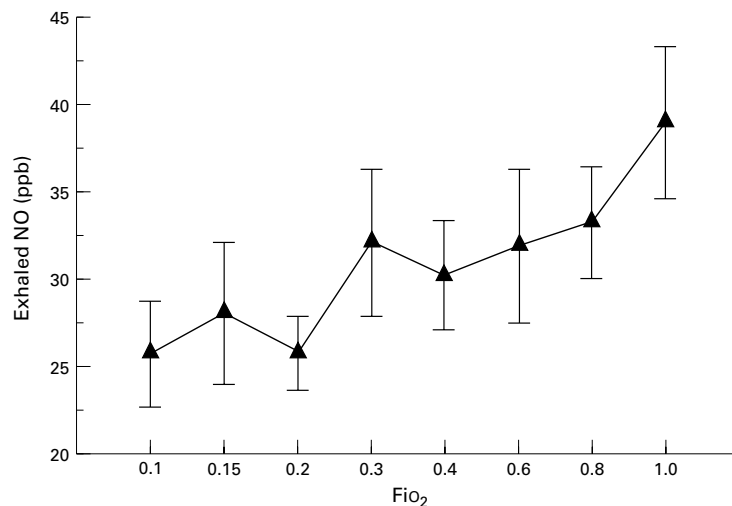


Figure 1 The effect of different mixtures of O₂ and N₂ (Fio₂) on exhaled NO in 16 healthy volunteers. The error bars represent the SE.

O₂ + 90% N₂. Balanced randomisation was used to ensure that groups of two of the 16 subjects received one of the above treatments first. All gases were delivered through a partially expanded reservoir bag at atmospheric pressure under nasal occlusion.

All subjects were asked to refrain from alcohol and caffeine for at least 12 hours before each trial day. After a 20 minute resting period in the sitting position, baseline measurements of exhaled NO and systemic haemodynamics were performed. Thereafter, a 10-minute breathing period of the first gas mixture was started. Measurements were obtained during the last three minutes of each breathing period. Subjects subsequently crossed over to the next treatment where measurements were performed in an identical fashion. The washout period between consecutive inhalation periods was 10 minutes. After four inhalation periods measurements were again performed under resting conditions before crossing over to the other gas mixtures under study. Recordings during breathing of ambient air were repeated at the end of the study. The readouts during breathing of ambient air were taken to calculate short term variability of measurements.

STUDY METHODS

Exhaled NO was measured with a chemiluminescence detector (Nitrogen oxides analyser, Model 8840, Monitor Labs Inc, USA). Calibration of the instrument was done with certified gases (1000 and 300 ppb NO in N₂, AGA, Vienna, Austria) using precision flow meters. A baseline signal was obtained with pure N₂. With 100% O₂ we did not obtain a signal, which ensured that oxygen itself did not influence the NO readings. One l/min of the exhaled air was allowed to enter the inlet port. Subjects were instructed to fully inflate their lungs, hold their breath for 10 seconds, and exhale for 10 seconds into a Teflon tube. Auto-inhalation of NO from the nasopharynx was avoided by a noseclip. Three consecutive read-

ings were made at each measurement point under nasal occlusion. The end expiratory values from the strip recorder readings were used for analysis to ensure that inspired NO from the ambient air did not distort the results. Previous studies have shown that inhalation of up to 38 ppb NO does not influence the level of exhaled NO after breath holding for 15 seconds.³ The detection limit of this method is 2 ppb.¹⁴

Systolic and diastolic blood pressures and pulse rate were measured by an automated oscillometric device (Siemens Sirecust 888R, Siemens, Erlangen, Germany).

STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistica software package (Release 4.5, Stat-Soft Inc, Tulsa, Oklahoma, USA). To quantify the short term variability of the exhaled NO measurements, intraclass correlation coefficients (κ) were calculated from the three recordings under ambient air breathing. κ was calculated according to the method of Kramer and Feinstein¹⁵ based on a repeated measure ANOVA model. κ can then be calculated from the variance among subjects (v_s), the variance among methods (v_m), and the residual error variance (v_e):

$$\kappa = (v_s - v_e) / (v_s + v_e + 2 \times v_m)$$

The higher the intraclass correlation coefficients the better the reproducibility of the method. A κ of 1 reflects perfect reproducibility. The intraclass correlation coefficient is a widely accepted measure of reliability and is considered more appropriate than older methods such as χ^2 , percentage agreement, product moment correlation or Yule's Y.^{15,16}

The effects of graded changes in Fio₂ on exhaled NO and systemic haemodynamics were assessed by repeated measure ANOVA. Post hoc comparisons were carried out using paired *t* tests. The level of significance was set at $p = 0.05$. Data are presented as means (SE).

Results

The baseline value of Pao₂ in the pilot experiment was 92 (4) mm Hg (12.3 (0.5) kPa). A mixture of 10% O₂ + 90% N₂ caused moderate hypoxia with a Pao₂ of 45 (2) mm Hg (6.0 (0.3) kPa). In contrast, 100% O₂ raised the Pao₂ to 491 (24) mm Hg (65.5 (3.2) kPa).

Exhaled NO levels at baseline, after the fourth breathing period, and at the end of the study were 31 (3) ppb, 30 (3) ppb, and 28 (2) ppb, respectively (NS). The intraclass correlation coefficient as calculated from these results was 0.63.

The effect of inhalation of the gases with different Fio₂ on exhaled NO is shown in fig 1. There was a positive correlation between changes in Fio₂ and exhaled NO levels ($p = 0.0012$, ANOVA), with 26 (3) ppb at an Fio₂ of 0.1 and 39 (4) ppb at an Fio₂ of 1.0. An Fio₂ of 0.1, however, did not significantly reduce exhaled NO compared with baseline, as evidenced from post-hoc comparisons.

Systolic blood pressure was 127 (12) mm Hg, diastolic blood pressure was 79 (6) mm Hg, and pulse rate was 79 (11) beats/min at baseline. Systemic haemodynamics showed only minor changes during inhalation periods (data not shown). A significant increase in pulse rate to 87 (7) beats/min was observed only during inhalation of a gas mixture with an Fio_2 of 0.1 ($p=0.023$ compared with baseline).

Discussion

The results of this study show that NO in the exhaled air is dependent upon oxygen tension. Hyperoxia during 100% O_2 breathing resulted in an approximately 25% increase in exhaled NO levels compared with baseline. In contrast, hypoxia only slightly reduced exhaled NO. The latter might be caused by at least two phenomena. On the one hand, the reproducibility of our test system may limit the sensitivity to detect small changes in exhaled NO concentration and, on the other, a study in humans is limited to moderate hypoxia and we cannot exclude the possibility that a more pronounced hypoxia would have resulted with lower concentrations of NO in the exhaled air.

It has already been observed in buffer perfused rabbit lungs that hypoxia decreases exhaled NO.¹¹ In the isolated pig lung hypoxia produced an increase in pulmonary vascular resistance and a fall in exhaled levels of NO.^{10,12} The mechanism by which hypoxia and hyperoxia influence exhaled levels of NO remains unclear. An inhibition of NO synthase by pulmonary hypoxia is unlikely because the vasoconstrictor response to hypoxia is intensified by NO synthase inhibition.¹⁷ It has been speculated that hypoxia limits the availability of oxygen for NO synthesis,¹² which is in keeping with the finding that hypoxia inhibits NO synthase through oxygen depletion.¹⁸ However, our results support the concept that endogenously generated NO is involved in hypoxic pulmonary vasoconstriction.

For interpretation of our data it must be considered that the exact origin of NO in exhaled air in healthy humans has not yet been determined. High concentrations of NO have been observed in paranasal sinuses¹⁹ and it is assumed that exhaled NO to some degree reflects NO derived from the upper airways.²⁰ The endogenous source of exhaled NO is reflected by the reduction in expired NO following inhalation^{3,21} and intravenous administration of NO synthase inhibitors^{22–25} and the increase after orally²⁶ and intravenously²⁴ administered L-arginine. The comparatively high levels of NO measured with our system in exhaled air may be caused by the fact that, on the one hand, autoinhalation of NO, but not the nasal contribution of NO, were avoided by the use of a noseclip and, on the other, breath holding increases NO levels.

In conclusion, we have shown that the NO concentration in exhaled air in healthy humans is dependent on oxygen tension. Hyperoxia increases the level of exhaled NO, which indicates increased pulmonary NO production. The mechanism behind this phenomenon remains to be elucidated.

- Gustafsson LE, Leone AM, Persson M, Wiklund NP, Moncada S. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea-pigs and humans. *Biochem Biophys Res Commun* 1991;181:852–7.
- Borland C, Cox Y, Higenbottam T. Measurement of exhaled nitric oxide in man. *Thorax* 1993;48:1160–2.
- Kharitonov SA, Yates D, Robbins RA, Logan Sinclair R, Shinebourne E, et al. Increased amount of nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994;343:133–5.
- Barnes PJ, Belvisi MG. Nitric oxide and lung disease. *Thorax* 1993;48:1034–43.
- Barnes PJ, Kharitonov SA. Exhaled nitric oxide: a new lung function test. *Thorax* 1996;51:233–7.
- Kimberley B, Nejadnik B, Giraud GD, Holden WE. Nasal contribution to exhaled nitric oxide at rest and during breathholding in humans. *Am J Respir Crit Care Med* 1996;153:829–36.
- Persson MG, Wiklund P, Gustafsson LE. Endogenous nitric oxide in single exhalations and the change during exercise. *Am Rev Respir Dis* 1993;148:1210–4.
- Marshall BE. Hypoxic pulmonary vasoconstriction. *Acta Anaesthesiol Scand* 1990;34(Suppl 94):37–41.
- Shaul PW, Farrar MA, Zellers TM. Oxygen modulates endothelium-derived relaxing factor production in fetal pulmonary arteries. *Am J Physiol* 1992;262:H355–64.
- Cremona G, Higenbottam T, Takao M, Hall L, Bower EA. Exhaled nitric oxide in isolated pig lungs. *J Appl Physiol* 1995;78:59–63.
- Grimminger F, Spriestersbach R, Weissmann N, Walmrath D, Seeger W. Nitric oxide generation and hypoxic vasoconstriction in buffer-perfused rabbit lungs. *J Appl Physiol* 1995;78:1509–15.
- Nelin LD, Thomas CJ, Dawson CA. Effect of hypoxia on nitric oxide production in neonatal pig lung. *Am J Physiol* 1996;271:H8–14.
- Pitkin AD, Roberts CM, Wedzicha JA. Arterialised earlobe blood gas analysis: an underestimated technique. *Thorax* 1994;49:364–6.
- Jilma B, Kastner J, Mensik C, Hildebrand J, Vondrovec B, Krejcy K, et al. Sex differences in nitric oxide production. *Life Sci* 1996;58:469–76.
- Kramer MS, Feinstein AR. Clinical biostatistics. *Clin Pharmacol Ther* 1981;29:111–23.
- Bartko JJ, Carpenter WT. On the methods and theory of reliability. *J Nerv Men Dis* 1976;163:307–17.
- Persson MG, Gustafsson LE, Wiklund NP, Moncada S, Hedqvist P. Endogenous nitric oxide as a probable modulator of pulmonary circulation and hypoxic pressor response in vivo. *Acta Physiol Scand* 1990;140:449–57.
- Rengasamy A, Johns RA. Characterization of endothelium-derived relaxing factor/nitric oxide synthase from bovine cerebellum and mechanisms of modulation by high and low oxygen tensions. *J Pharmacol Exp Ther* 1991;259:310–6.
- Lundberg JON, Weizberg E, Nordvall SL, Kuylensstierna R, Lundberg JM, Alving K. Primarily nasal origin of exhaled nitric oxide and absence in Kartagener's syndrome. *Eur Respir J* 1994;8:1501–4.
- Schedin U, Frostell C, Persson MG, Jakobsson J, Andersson G, Gustafsson LE. Contribution from upper and lower airways to exhaled endogenous nitric oxide in humans. *Acta Anaesthesiol Scand* 1995;39:327–32.
- Yates DH, Kharitonov SA, Robbins RA, Thomas PS, Barnes PJ. Effect of a nitric oxide synthase inhibitor and a glucocorticosteroid on exhaled nitric oxide. *Am J Respir Crit Care Med* 1995;152:892–6.
- Krejcy K, Schmetterer L, Kastner J, Nieszpauro-Los M, Monitzer B, Schütz W, et al. Role of nitric oxide in hemostatic system activation in humans. *Arterioscler Thromb Vasc Biol* 1995;15:2063–7.
- Schmetterer L, Krejcy K, Kastner J, Wolzt M, Gouya G, Findl O, et al. The effect of systemic nitric oxide-synthase inhibition on ocular fundus pulsations in man. *Exp Eye Res* 1997;64:305–12.
- Schmetterer L, Findl O, Fasching P, Ferber W, Strenn K, Breiteneder H, et al. NO and ocular blood flow in patients with insulin-dependent diabetes mellitus. *Diabetes* 1997;46:653–8.
- Wolzt M, Schmetterer L, Ferber W, Artner E, Mensik C, Eichler HG, et al. Effect of nitric oxide-synthase inhibition on renal hemodynamics in man: reversal by L-arginine. *Am J Physiol* 1997;272:F178–82.
- Kharitonov SA, Lubec G, Lubec B, Hjelm M, Barnes PJ. L-arginine increases exhaled nitric oxide in normal human subjects. *Clin Sci* 1995;88:135–9.