

Interrelation between Oxygen Tension and Nitric Oxide in the Respiratory System

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To understand the relationship between oxygen tension and nitric oxide (NO) function, one animal and two human studies were designed. In the animal study, the effect of NO in inducing the relaxation of aortic specimens was significantly lower by 68% under 480 mm Hg of oxygen tension than under 28 mm Hg, indicating that oxygen tension has an important role in determining the biological effects of NO. In a clinical analysis with nonsmokers ($n = 23$), the alveolar-to-arterial difference for oxygen ($A-aDO_2$) was reciprocally correlated with exhaled NO concentrations ($r = 0.53$). Because NO concentration in the lower respiratory zone depends partly on the amount of inspirable NO originating in the upper airway, a well-ventilated area, requiring much perfusion, could receive greater amounts of NO than a poorly ventilated one. Thus, the reciprocal relation of $A-aDO_2$ with the concentration of exhaled NO is not necessarily incompatible with the effect of hypoxic pulmonary vasoconstriction in ventilation-to-perfusion (V'_A/Q') imbalance. In our third experiment, with nonsmokers ($n = 21$), pure oxygen inhalation during mechanical ventilation significantly decreased the concentration of exhaled NO and enhanced $A-aDO_2$, indicating a relationship between NO and oxygen similar to that observed in the animal experiment. These findings led us to conclude that a positive relation between exhaled NO and blood oxygenation efficiency exists in the respiratory system, and further, that oxygen might affect this relationship. Thus, the relative balance of NO and oxygen concentrations may be another factor for consideration in respiratory function.

It has become apparent that nitric oxide (NO) is involved in a wide variety of biological processes such as vasodilation, neurotransmission, blood clotting, and host defense mechanisms. Based on these findings, inhalation of NO as a pulmonary vasodilator and bronchodilator in newborns with severe persistent pulmonary hypertension, in patients with acute respiratory distress syndrome, and during cardiac surgery and organ transplantation has been attempted (1–7). The results have apparently been somewhat successful. However, there is much that is still unclear about the biological functions and therapeutic potency of NO in the respiratory system.

Recent studies have shown that 5–150 parts per billion (ppb) of NO is continuously exhaled in the breathing air of human subjects (8–13). Pulmonary epithelial cells, vascular endothelial cells, nonadrenergic nerves, mast cells, macrophages, neutrophils, and fibroblasts in the lung are considered to be the source of exhaled NO (1). This NO is considered to play certain roles in the maintenance of sterility and/or reflect inflammation of the airway (14). However, taking into account the clinical evidence in NO inhalation therapy, it can be as-

sumed that physiologically exhaled NO might also reflect the generation of endogenous NO, which is related with ventilation-to-perfusion (V'_A/Q') mismatching, by affecting vascular and airway smooth muscular tone in the respiratory system.

We recently found that the lifetime and functions of NO greatly increased under lower oxygen tensions, by examining mitochondria, neutrophils, and other eukaryotic cells *in vitro* (15–20). Because oxygen concentrations in most tissues and cells are much lower (< 30 mm Hg) than in air atmospheric conditions (19, 21), the enhancement of NO by lower oxygen tension is considered to be a key factor in understanding its biological function and significance (15–20). In addition, the importance of hypoxia with NO leads us to presume another possible case in which hyperoxia may inhibit NO. In the respiratory system, such possible effects of oxygen may be more important, because it is always exposed to higher concentrations of oxygen than any other organ or tissue, and is sometimes exposed to nearly pure oxygen during therapeutic processes. Thus, when evaluating the function of exhaled NO, possible inhibition by highly concentrated oxygen should also be taken into consideration.

The goal of the present study was to confirm the adverse effect of hyperoxia on the function of NO *in vivo*, and to investigate the relation between exhaled NO and pulmonary gas exchange efficiency, and then to determine whether oxygen, especially in very high concentrations, affects the status and function of endogenous NO as it does *in vivo*.

METHODS

Study Design

One animal study and two clinical human studies were designed. First, the effect of higher oxygen concentrations on the NO-induced relaxation of smooth muscle was studied *in vitro* using rat aortic specimens. Second, the relation between exhaled NO and arterial blood gases was studied in healthy human subjects. Third, the effect of inspiration of higher oxygen concentrations on exhaled NO was studied in human subjects to determine whether oxygen induces a similar effect on NO as it does *in vitro*. All protocols in each study were approved by the Research Committee of the National Hospital Tokyo Disaster Medical Center.

Effect of Oxygen on NO-induced Relaxation of Smooth Muscle *In Vitro*

The aortas from male Wistar rats, weighing 200–250 g, were obtained. Endothelial cells of the aorta were denuded by the method of Muramatsu and coworkers (22), and then cut into pieces 2–3 mm wide and 10 mm long. The aortic specimens were incubated in Krebs–Henseleit solution saturated with varying concentrations of oxygen at 37° C for 2 h. Specimens were contracted by adding norepinephrine (10^{-7} M) and then relaxed by NO (1.3 nM), in the presence or absence of either methylene blue (MB) or oxyhemoglobin (HbO₂). Their tension was continuously measured at 37° C by an electric transducer equipped with an oxygen electrode.

NO gas was obtained from Teisan Co. Ltd. (Tokyo, Japan). Other chemicals were of analytical grade and were purchased from Nacalai

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Tesque Co. (Kyoto, Japan). The saturated NO solution was prepared as described previously by the bubbling of NO gas through a deoxygenated medium for 30 min at 25°C (16). The concentration of NO in the buffer was confirmed using the oxyhemoglobin method (23).

Exhaled NO and Arterial Blood Gases

Exhaled NO and arterial blood gases were analyzed in 23 healthy nonsmokers (20 males and 3 females, 41 ± 4 yr old) in a special air-conditioned room, in which the concentration of NO was less than 4 ppb. Before measuring, the subjects stayed in the room for at least 1 h. Blood samples were anaerobically obtained by puncturing the radial artery with a heparinized syringe, and then analyzed for arterial blood gases (pH, P_{aO_2} , and P_{aCO_2}) using a blood gas analyzer Model 288 (Chiron, MA). The alveolar-to-arterial difference for oxygen ($A-aDO_2$) was estimated with the following standard equation, assuming $R = 0.85$ (24):

$$A-aDO_2 = F_{I_{O_2}} (BP - 47) - \{P_{aCO_2} [F_{I_{O_2}} + [1 - (F_{I_{O_2}} + F_{I_{CO_2}})]/R] + P_{aO_2}\}$$

where $F_{I_{O_2}}$ is the inspired oxygen fraction, $F_{I_{CO_2}}$ is the inspired carbon dioxide fraction, BP is barometric pressure, and R is respiratory quotient.

Next, exhaled NO was measured using a chemiluminescence NO analyzer CML-500 (Shimadzu, Kyoto, Japan). Each subject was instructed to produce a slow vital capacity maneuver over 30–40 s into unobstructed tubing while wearing nose clips. Air samples through a side arm were continuously collected at a flow rate of 400 ml min^{-1} and analyzed. Exhaled NO was calculated as the plateau concentration at the end of expiration, because it well reflects the NO concentration in the lower respiratory tract that is expected to relate to V'_A/Q' matching (25).

Effect of Oxygen on Exhaled NO

A total of 21 nonsmokers, scheduled for unrelated minor cosmetic surgery, were divided into two groups and studied. Group 1 and 2 subjects were anesthetized under 100% ($n = 12$) and 40% oxygen ($n = 9$), respectively. The detailed experimental procedure was performed as follows. In both groups, anesthesia was induced with sevoflurane followed by orotracheal intubation, and then maintained with 2% sevoflurane in 30% oxygen. All subjects received mechanical ventilation with a tidal volume of 10 ml/kg (7–8 times/min), in which the breathing gas was carefully checked for any NO contamination. Ten minutes after induction of anesthesia, the concentration of inspired oxygen was changed to 100% in group 1 and 40% in group 2, and maintained for an additional 12 min. Next, air samples through the sampling tube attached to the endotracheal tube were collected at a flow rate of 400 ml min^{-1} , and analyzed by a chemiluminescence NO analyzer CML-500. Exhaled NO was then calculated as the plateau concentration at the end of expiration (the first measurement). Simultaneously, arterial blood samples were collected through a catheter inserted into the radial artery. Blood gases were analyzed and $A-aDO_2$ was estimated assuming $R = 1.0$ in group 1 and $R = 0.85$ in group 2. During the next 50 min, the concentrations of inspired oxygen were strictly maintained in both groups. Following that, the NO concentrations in exhaled air and arterial blood gases were analyzed in the same way (the second measurement). All of these measurements were performed before the start of surgery.

Data Analysis

Comparisons between the two groups were analyzed by variance. The correlation coefficient (r) was calculated by two linear regression analyses. A p value of less than 5% was considered significant. All values are expressed as mean \pm standard error of the mean (SEM).

RESULTS

Effect of Oxygen on NO-induced Relaxation of Smooth Muscle *In Vitro*

To understand the effect of high oxygen concentrations on NO-induced relaxation, endothelium-denuded arterial specimens were contracted by norepinephrine and then relaxed by

adding NO. The amount of contraction by the specimens remained unchanged at oxygen tensions between 17.5 and 500 mm Hg. The effect of NO was expressed as the percentage change of contraction compared with the contraction induced by norepinephrine alone (Figure 1). When NO was added to the medium, the specimens relaxed transiently. The effect of NO significantly decreased with higher oxygen concentrations in the medium. The relaxing effect was decreased by 68% more under 480 mm Hg of oxygen than that under 28 mm Hg. The inset shows the contraction curve induced by norepinephrine and the relaxing effect of NO with these oxygen tensions.

Exhaled NO and Arterial Blood Gases

Analysis of the exhaled air samples revealed that concentrations of the exhaled NO ranged from 16 to 53 ppb. Values of P_{aO_2} , P_{aCO_2} , and pH in the blood samples were 91.2 ± 2.0 mm Hg, 40.8 ± 0.8 mm Hg, and 7.40 ± 0.01 , respectively. Among these examined parameters, P_{aO_2} was positively correlated with the concentrations of exhaled NO ($r = 0.65$), and $A-aDO_2$ was negatively correlated with NO concentration ($r = 0.53$) (Figure 2). However, no significant correlation was found between the concentrations of exhaled NO and pH or P_{aCO_2} .

Effect of Oxygen on Exhaled NO

Minute volumes for ventilation in group 1 and 2 were 4.2 ± 0.2 and $4.7 \pm 0.3 \text{ L min}^{-1}$, respectively. Age, height, and weight were 36 ± 4.3 yr, 165 ± 2.5 cm, and 58 ± 2.1 kg in group 1, and 41 ± 5.6 yr, 163 ± 2.6 cm, and 58 ± 2.8 kg in group 2, respectively. These parameters were not significantly different be-

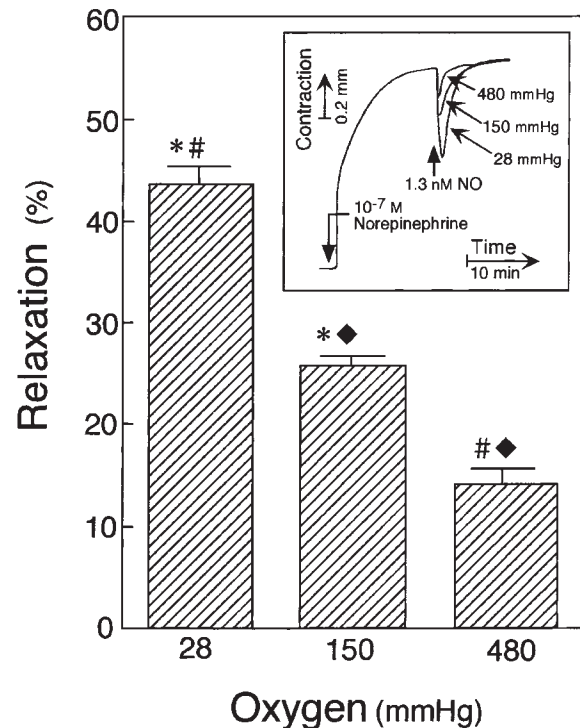


Figure 1. Effect of oxygen tension on NO-induced arterial relaxation. Arterial specimens were prepared by denuding endothelial cells. Contraction and relaxation of the specimens were elicited at 37°C, by adding 1×10^{-7} M norepinephrine and 1.3 nM NO, respectively. Oxygen tensions used were 28, 150, and 480 mm Hg. The effect of NO is expressed as the percentage change of contraction compared with the contraction induced by norepinephrine alone. #, *, ♦: difference is significant ($p < 0.05$). The inset shows the contraction curve when induced by norepinephrine and the relaxing effect of NO.

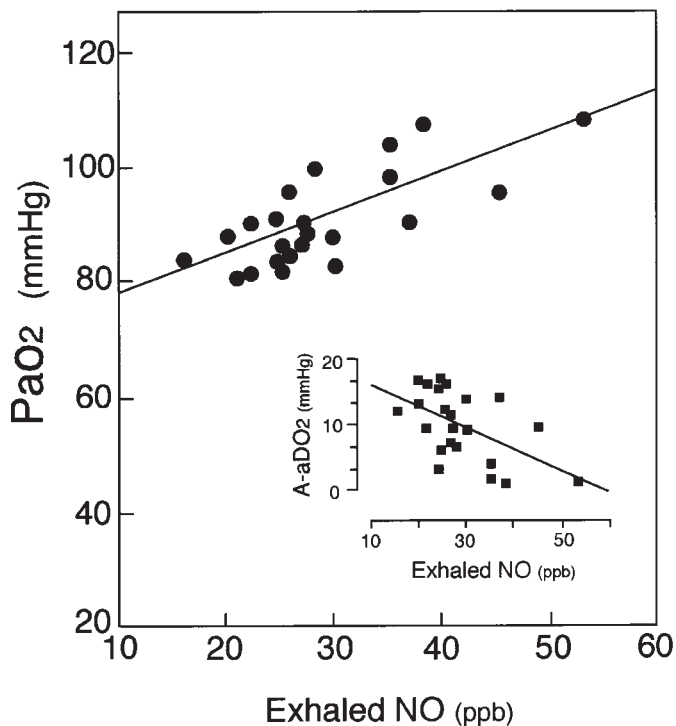


Figure 2. Relationship between P_{aO_2} and exhaled NO. Exhaled air samples were collected from 23 healthy subjects by a slow vital capacity maneuver. P_{aO_2} was significantly correlated with the concentrations of exhaled NO ($r = 0.65$). The inset shows the correlation between $A-aDO_2$ and exhaled NO ($r = 0.53$).

tween group 1 and group 2. In group 1, mean blood pressure, heart rate, and body temperature were 61 ± 1.7 mm Hg, 75 ± 4.3 beat min^{-1} , and $36.3 \pm 0.2^\circ$ C in the first measurement, and 67 ± 2.0 mm Hg, 73 ± 5.7 beat min^{-1} , and $36.1 \pm 0.2^\circ$ C in the second measurement. In group 2, mean blood pressure, heart rate, and body temperature were 61 ± 4.6 mm Hg, 72 ± 4.3 beat min^{-1} , and $36.3 \pm 0.2^\circ$ C in the first measurement, and 62 ± 3.6 mm Hg, 72 ± 3.0 beat min^{-1} , and $36.2 \pm 0.2^\circ$ C in the second measurement. There were no significant differences in each parameter among the four measurements.

The values of arterial blood pH and P_{aCO_2} at the first mea-

surement were 7.46 ± 0.01 and 35.3 ± 0.8 mm Hg in group 1 (100% oxygen inspiration group), and 7.48 ± 0.01 and 32.0 ± 1.0 mm Hg in group 2 (40% oxygen inspiration group), respectively. No significant differences were found between the two groups. In contrast, P_{aO_2} in group 1 (576.2 ± 15.1 mm Hg) was significantly higher than in group 2 (170.0 ± 14.2 mm Hg), reflecting the difference in concentration of inspired oxygen. The concentration of exhaled NO at the first measurement ranged from 4 to 16 ppb as a whole, but there were no significant differences between the two groups. These values are about one-third of those from naturally breathing healthy subjects.

Changes in exhaled NO and $A-aDO_2$ between the first and second measurements in both groups are shown in Figure 3. The concentrations of exhaled NO significantly decreased with concomitant enlargement of $A-aDO_2$ at the second measurement in group 1, while both exhaled NO and $A-aDO_2$ remained unchanged between the first and second measurements in group 2. In the case of pH and P_{aCO_2} , there were no significant changes between the first and second measurements in either group.

DISCUSSION

The *in vitro* experiment with aortic specimens demonstrated that NO-induced relaxation was suppressed by higher oxygen tension levels. Because the norepinephrine-induced contraction of the specimens was constant up to 500 mm Hg of oxygen, oxygen did not directly affect this process. Considering our previous findings concerning the lifetime of NO, this observed phenomenon is considered to be a result of a shortening of the lifetime of NO induced by oxygen and its derived radicals, which is due to the high reactivity of NO to these molecules (15, 16, 26). The result demonstrates the validity of our supposition that the effect of NO is inversely regulated by the oxygen tension in cases when the range of oxygen tension is much higher. These adverse actions of oxygen could potentially arise in various clinical settings, and, thus, might be important. However, up to this time there has been limited attention paid to this interaction in clinical practice.

The present clinical study indicates the relation of pulmonary gas exchange with exhaled NO. The production of such endogenous NO is not considered to be large and is easily affected by various surrounding factors. As a result, the correlation shown in the present study may have been overlooked

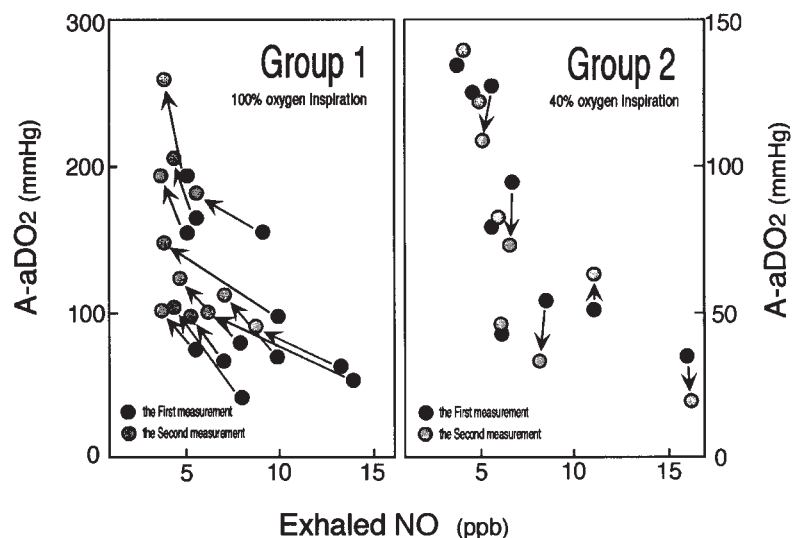


Figure 3. Effect of inspired oxygen concentrations on exhaled NO and $A-aDO_2$. A total of 21 nonsmokers were anesthetized, and then mechanically ventilated during 50 min under 100% oxygen (group 1; $n = 12$) and 40% oxygen (group 2; $n = 9$). A closed circle indicates the value at the beginning of ventilation (the first measurement) and a gray circle indicates the value after 50-min ventilation (the second measurement). Exhaled NO and $A-aDO_2$ were significantly changed only in group 1.

thus far. Previous studies have reported that exhaled NO was found to be increased in airway inflammation associated with patients with asthma and others (9, 11), and possibly reflects the inflammatory status of the airway. Other studies have reported that cigarette smoke contains abundant NO, which could affect NO production in smokers (9, 10, 13). Therefore, human subjects with upper or lower respiratory inflammation and smokers (including passive cigarette smoke inhalers) were strictly excluded from the present study. In addition, to minimize environmental external NO, we performed the measurements in a special room, in which the concentration of atmospheric NO was very low. These careful procedures may have been helpful to determine the exact relationship between exhaled NO and Pa_{O₂}.

The correlation between exhaled NO and Pa_{O₂} might seem to be incompatible with the effect of hypoxic pulmonary vasoconstriction (HPV), because the potent vasodilating effect of NO might even inhibit the HPV that is essential for the correction of V'_A/Q' imbalance and worsen the pulmonary gas exchange. However, the favorable contribution of endogenous NO against pulmonary oxygen uptake and vascular resistance has also been reported by other researchers (27, 28). Theoretically, the NO concentrations involved in respiratory gas exchange are not equal throughout the respiratory zone, as it would be dependent on the amount of inspirable NO generated in the upper airway in addition to intrinsic NO generated in the lower respiratory zone (27–29). Thus, a well-ventilated zone, which requires much perfusion, could receive much more NO than a poorly ventilated one, which is not necessarily incompatible with the active process of HPV. On the other hand, it was reported that inhalation of nebulized N_G-nitro-L-arginine methyl ester (L-NAME) did not affect the gas exchange in patients with asthma, indicating the limited role of endogenous NO in V'_A/Q' control (30). However, L-NAME inhalation did not modify pulmonary artery pressure, but conversely decreased pulmonary vascular resistance in these patients. Thus, when considering an interpretation, it should be noted that delivery of L-NAME may be inadequate or that the NO dependency in patients with asthma is somewhat different from healthy subjects. Recently, Terraz and coworkers demonstrated that treatment of the small pulmonary artery with L-NAME inhibits HPV, indicating the requirement of some amount of endogenous NO for normal HPV function (31). Their evidence indicates that L-NAME treatment might have potentially biphasic effects on HPV, inhibition in addition to enhancement, depending on conditions, which would make the interpretation of the effect of L-NAME inhalation more complicated. Thus, the ineffectiveness of L-NAME inhalation on the efficiency of pulmonary gas exchange in this study would not seem to absolutely indicate a decreased involvement of endogenous NO in pulmonary gas exchange.

Consistent with previous observations, the concentration of exhaled NO in our study decreased in mechanically ventilated subjects (12). Because NO is partly synthesized in the nasopharynx (14), the reduction may be due to a lack of nasopharynx NO fraction by orotracheal intubation. In addition, the inhalation of anesthetic gas and/or an increase in airway pressure caused by mechanical ventilation might also affect measurable endogenous NO (32). Thus, the physiological environment of endogenous NO in a mechanically ventilated subject does not seem to be exactly identical with that in a naturally breathing subject. However, mechanical ventilation has a great advantage in that the same respiratory conditions can be maintained against different concentrations of inspired oxygen. During measurement, our subjected patients were only anesthetized, without any other stimulation such as surgery.

Blood pressure, heart rate, body temperature, and the partial pressure of carbon dioxide in arterial blood were maintained at the same level, indicating that stable hemodynamic and metabolic conditions were maintained throughout the measurement period, though cardiac output and other specific parameters were not examined. Mechanical ventilation with oxygen may induce minor atelectasis (33). However, it has been demonstrated that such atelectasis develops within a few minutes after starting mechanical ventilation and the amount does not change as long as the ventilation conditions do not change (33–35). Thus, under the present study conditions, it seems likely that the difference in oxygen concentration was a main causative factor of the effect on endogenous NO, though several other factors must be taken into consideration with such a conclusion, as discussed above. In this process, excess oxygen and its derived radicals might function against NO in a manner similar to the case of aortic specimens. A potent *in vivo* antioxidant system, however, was able to attenuate the effect of such an amount of oxygen and related radicals. Thus, it is conceivable that inhalation of only the highest concentrations of oxygen for a longer period, which could seriously enhance oxidative stress, induced the reduction of NO concentration, whereas 40% oxygen inhalation, which was unable to greatly enhance oxidative stress, did not. This might be the first evidence suggesting the adverse effects of high concentrations of oxygen on NO function in humans.

The results of the present study indicate the relation between exhaled NO and the oxygenation of blood in the respiratory system, and also suggest that oxygen tension might play an important role in determining the biological functions of NO. Hence, controlling the relative balance of NO and oxygen concentrations might be of great importance for maintaining respiratory functions, and should be considered when treating various respiratory diseases.

References

- Barnes, P. J., and M. G. Belvisi. 1993. Nitric oxide and lung disease. *Thorax* 48:1034–1043.
- Lunn, R. J. 1995. Inhaled nitric oxide therapy. *Mayo Clin. Proc.* 70:247–255.
- Popke-Zaba, J., T. W. Higenbottam, A. T. Dinh-Xuan, D. Stone, and J. Wallwork. 1991. Inhaled nitric oxide as a cause of selective pulmonary hypertension. *Lancet* 228:1173–1174.
- Rossaint, R., K. J. Falke, F. Lopez, K. Slama, U. Pison, and W. M. Zapol. 1993. Inhaled nitric oxide for the adult respiratory distress syndrome. *N. Engl. J. Med.* 328:399–405.
- Frostell, C. G., M. D. Fratacci, J. C. Wain, R. Jones, and W. M. Zapol. 1991. Inhaled nitric oxide: a selective vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation* 83:2038–2047.
- Rich, G. F., G. D. Murphy, C. M. Roos, and R. A. Johns. 1993. Inhaled nitric oxide: selective pulmonary vasodilation in cardiac surgical patients. *Anesthesiology* 78:1028–1035.
- Snell, G. I., R. F. Salamonson, P. Bergin, D. S. Esmore, S. Khan, and T. J. Williams. 1995. Inhaled nitric oxide used as a bridge to heart-lung transplantation in a patient with end-stage pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* 151:1263–1266.
- Tsujino, I., K. Miyamoto, M. Nishimura, H. Shinano, H. Makita, S. Saito, T. Nakano, and Y. Kawakami. 1996. Production of nitric oxide in intrathoracic airways of normal humans. *Am. J. Respir. Crit. Care Med.* 154:1370–1374.
- Persson, M. G., O. Zetterstrom, V. Agrenius, E. Ihre, and L. E. Gustafsson. 1994. Single-breath nitric oxide measurements in asthmatic patients and smokers. *Lancet* 343:146–147.
- Schilling, J., P. Holzer, M. Guggenbach, D. Gyurech, K. Marathia, and S. Geroulanos. 1994. Reduced endogenous nitric oxide in the exhaled air of smokers and hypertensives. *Eur. Respir. J.* 7:467–471.
- Kharitonov, S. A., D. Yates, R. A. Robbins, R. Logan-Sinclair, E. A. Shinebourne, and P. J. Barnes. 1994. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 343:133–135.
- Gerlach, H., R. Rossaint, D. Pappert, M. Knorr, and K. J. Falke. 1994.

- Autoinhalation of nitric oxide after endogenous synthesis in nasopharynx. *Lancet* 343:518–519.
13. Kharitonov, S. A., R. A. Robbins, D. Yates, V. Keatings, and P. J. Barnes. 1995. Acute and chronic effects of cigarette smoking on exhaled nitric oxide. *Am. J. Respir. Crit. Care Med.* 152:609–612.
 14. Lundberg, J. O. N., T. F. Szallasi, E. Weitzberg, J. Rinder, J. Lidholm, A. Anggard, T. Hokfelt, J. M. Lundberg, and K. Alving. 1995. High nitric oxide production in human paranasal sinuses. *Nature Med.* 11:370–373.
 15. Takehara, Y., T. Kanno, T. Yoshioka, M. Inoue, and K. Utsumi. 1995. Oxygen-dependent regulation of mitochondrial energy metabolism by nitric oxide. *Arch. Biochem. Biophys.* 323:27–32.
 16. Takehara, Y., H. Nakahara, Y. Inai, M. Yabuki, K. Hamazaki, T. Yoshioka, M. Inoue, A. A. Horton, and K. Utsumi. 1996. Oxygen-dependent reversible inhibition of mitochondrial respiration by nitric oxide. *Cell Struct. Funct.* 21:251–258.
 17. Iha, S., K. Orita, T. Kanno, T. Utsumi, E. F. Sato, M. Inoue, and K. Utsumi. 1996. Oxygen-dependent inhibition of neutrophil respiratory burst by nitric oxide. *Free Rad. Res.* 25:489–498.
 18. Inai, Y., Y. Takehara, M. Yabuki, E. F. Sato, J. Akiyama, T. Yasuda, M. Inoue, A. A. Horton, and K. Utsumi. 1996. Oxygen-dependent-regulation of Ehrlich ascites tumor cell respiration by nitric oxide. *Cell Struct. Funct.* 21:151–157.
 19. Nishikawa, M., E. F. Sato, K. Utsumi, and M. Inoue. 1996. Oxygen-dependent regulation of energy metabolism in ascites tumor cells by nitric oxide. *Cancer Res.* 56:4535–4540.
 20. Yabuki, M., Y. Inai, T. Yoshioka, K. Hamazaki, T. Yasuda, M. Inoue, and K. Utsumi. 1997. Oxygen-dependent fragmentation of cellular DNA by nitric oxide. *Free Rad. Res.* 26:245–255.
 21. Kessler, M., D. W. Lubers, B. A. Krumme, K. Scholeben, and H. Bunte. 1977. Oxygen tension in different tissues. *Bibl. Anat.* 16:146–149.
 22. Muramatsu, I., S. Kigoshi, and M. Oshita. 1990. Two distinct alpha-1-adrenoreceptor subtype involved in noradrenaline contraction of rabbit thoracic aorta. *Br. J. Pharmacol.* 101:662–666.
 23. Murphy, M. E., and E. Noack. 1994. Nitric oxide assay using hemoglobin method. *Methods Enzymol.* 233:240–250.
 24. Wu, E. Y., K. W. Barazanji, and R. L. Johnson Jr. 1997. Sources of error in A-aDO₂ calculated from blood store in plastic and glass syringes. *J. Appl. Physiol.* 82:196–202.
 25. Kharitonov, S. A., K. F. Chung, D. Evans, B. J. O'Connor, and P. J. Barnes. 1996. Increased exhaled nitric oxide in asthma is mainly derived from the lower respiratory tract. *Am. J. Respir. Crit. Care Med.* 153:1773–1780.
 26. Ignarro, L. J., R. E. Byms, G. M. Buga, K. S. Wood, and G. Chaudhuri. 1988. Pharmacological evidence that endothelium-derived relaxing factor is nitric oxide: use of pyrogallol and superoxide dismutase to study endothelium-dependent and nitric oxide-elicited vascular smooth muscle relaxation. *J. Pharmacol. Exp. Ther.* 244:181–189.
 27. Lundberg, L. O. N., J. M. Lundberg, G. Settergren, K. Alving, and E. Weitzberg. 1995. Nitric oxide, produced in the upper airways, may act in an 'aerocrine' fashion to enhance pulmonary oxygen uptake in humans. *Acta Physiol. Scand.* 155:467–468.
 28. Settergren, G., M. Angdin, R. Astudillo, S. Gelinder, J. Liska, J. O. N. Lundberg, and E. Weitzberg. 1998. Decreased pulmonary vascular resistance during nasal breathing: modulation by endogenous nitric oxide from the paranasal sinuses. *Acta Physiol. Scand.* 163:235–239.
 29. Kimberly, B., B. Nejadnik, G. D. Giraud, and W. E. Holden. 1996. Nasal contribution to exhaled nitric oxide at rest and during breathholding in humans. *Am. J. Respir. Crit. Care Med.* 153:829–836.
 30. Gómez, F. P., J. A. Barvera, J. Roca, R. Iglesia, J. Ribas, P. J. Barnes, and R. Rodriguez-Roisin. 1998. Effect of nitric oxide synthesis inhibition with nebulized L-NAME on ventilation-perfusion distributions in bronchial asthma. *Eur. Respir. J.* 12:865–871.
 31. Terraz, S., F. Baechtold, D. Renard, A. Barsi, A. Rosselet, A. Gnaegi, L. Liaudet, R. Lazor, J. A. Haefliger, N. Schaad, C. Perret, P. Kucera, M. Markert, and F. Feihl. 1999. Hypoxic contraction of small pulmonary arteries from normal and endotoxemic rats: fundamental role of NO. *Am. J. Physiol.* 276:H1207–1214.
 32. Nakamura, K., K. Terasako, H. Toda, I. Miyawaki, M. Kakuyama, M. Nishiwada, Y. Hatano, and K. Mori. 1994. Mechanisms of inhibition of endothelium-dependent relaxation by halothane, isoflurane, and sevoflurane. *Can. J. Anaesthesiol.* 41:340–346.
 33. Brismar, B., G. Hedestierna, H. Lundquist, A. Strandberg, L. Svensson, and L. Tokics. 1985. Pulmonary densities during anesthesia with muscular relaxation—a proposal of atelectasis. *Anesthesiology* 62:422–428.
 34. Rothen, H. U., B. Sporre, G. Engberg, G. Wegenius, M. Hogman, and G. Hendenstierna. 1995. Influence of gas composition on recurrence of atelectasis after a reexpansion maneuver during general anesthesia. *Anesthesiology* 82:832–842.
 35. Reber, A., G. Engberg, B. Sporre, L. Kviele, H. U. Rothen, G. Wegenius, U. Nylund, and G. Hendenstierna. 1996. Volumetric analysis of aeration in the lung during general anaesthesia. *Br. J. Anaesthesiol.* 76:760–766.