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Nasal, pulmonary and autoinhaled nitric oxide at rest and during moderate exercise

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Abstract *Objective:* To investigate nasal nitric oxide (NO) excretion, pulmonary NO excretion, and autoinhalation of nasally released NO at rest compared with that during moderate exercise in smokers and non-smokers.
Design: Prospective observational study.
Setting: University laboratory.
Participants: Fourteen healthy adult volunteers.
Interventions: Breathing of NO-purified air supplied via a tube system at rest and during a bicycle-ergometer workload of 60 Watt over a time of 10 min.
Measurement and results: We examined nasal and pulmonary NO excretion in smoking ($n = 7$) and non-smoking ($n = 7$) adult human volunteers. At rest, we measured constant nasal NO excretion rates of 311 ± 89 nl/min for non-smokers and 261 ± 142 nl/min for smokers (mean ± SD, n.s.). During 60 W exercise, nasal NO release remained unchanged, while pulmonary NO excretion doubled compared with the rates at rest (non-smokers: 40 ± 21 nl/min versus 23 ± 14 nl/min, $p < 0.05$; smokers: 41 ± 8 nl/min versus 22 ± 8 nl/min, $p < 0.05$). The differences between smokers and non-smokers in nasal or pulmonary NO excretion were not significant. To determine the autoinhaled amount of nasally released NO, we also measured the NO concentration within the nasopharynx of five volunteers during nasal breathing. The average inhaled NO concentration was 17.8 ± 3.1 ppb at rest and this decreased to 9.3 ± 1.8 ppb during exercise of 60 W, while minute ventilation approximately doubled from 9 ± 2 to 21 ± 3 l/min.
Conclusion: Our results demonstrate that moderate exercise increased exclusively pulmonary NO excretion. Nasal NO release, which is 10 times higher at rest, was not changed. The decrease in autoinhaled NO concentration during exercise results from dilution of the continuous nasal release by the increased respiratory gas flow. The individual NO release allows no conclusion about smoking habits.

Key words Nitric oxide · Excretion · Autoinhalation · Exercise · Smoking

Introduction

Nitric oxide (NO) is a gaseous free radical, which acts as an efficient vasodilator [1, 2] with marked local activity. This characteristic can be utilized in the treatment of patients with acute respiratory distress syndrome (ARDS), where the exogenous administration of NO at concentrations between 1 and 50 parts per million (ppm) increases arterial oxygenation due to selective vasodilation in ventilated areas of the lung [3]. In addi-

tion, NO has bronchodilating [4, 5], antiviral and antibacterial [6] properties.

NO is also produced endogenously in the respiratory tract and can be detected in the expired gas of humans and various animal species [7, 8]. An increase of the exhaled NO amount during moderate exercise has been demonstrated in several studies [9, 10]. Furthermore, smoking habits have been reported to influence the range of expired NO [11, 12, 13]. It was assumed by the majority of researchers that the measured NO originated from the lower respiratory tract. Later it was demonstrated that NO is also released into the nasal cavity [14, 15], where it was measured in concentrations between 200 and 600 parts per billion (ppb). By comparison, amongst intubated and ventilated patients, in whom the upper and lower airways are necessarily separated from each other, the concentration of NO in exhaled gas is less than 3 ppb [14, 15, 16]. These results suggest that the exhaled NO is normally composed of a greater portion originating from the nose, while the lesser portion is excreted from the lower airways and alveoli. NO released in the upper airways is considered to take part in local host defence [17]. In addition, nasal NO is inhaled into the lung, where it is partly absorbed. This process has been termed NO autoinhalation [15] and it is hypothesized that this improves arterial oxygen tension.

In many of the studies conducted to date, exhaled gas contained NO in higher concentrations than those measured in intubated patients, indicating an admixture of nasally produced NO. Thus it is unclear to what extent differences in the NO excretion due to exercise or smoking habits result from the upper or lower respiratory tract. Only two studies [18, 19] were concerned with separate measurement of the pulmonary NO portion at rest and during exercise, which can be detected directly in exhaled gas, if nasal NO is removed by continuous aspiration via both nostrils [18] or by blocking the nasal passage using balloon catheters inflated within the oropharynx [19]. Both studies demonstrated a considerable increase in pulmonary NO excretion, even during moderate exercise of 60 W and 75 W, which became more emphasized at a level of 150 W [19].

Results concerning the nasal portion during exercise are conflicting. Regarding the difference between pulmonary and separately measured total NO content in exhaled gas as the nasal contribution, an increase in absolute nasal NO release during exercise was derived indirectly [19]. On the other hand, a decrease by 34 % in nasal NO excretion during heavy exercise of 245 W was detected in gas that was isolated by continuous aspiration via both nostrils [20]. The uncertainty about the nasal NO excretion also prevents reliable prediction of the autoinhaled NO concentration and its possible influence on arterial oxygenation.

The above-mentioned studies were restricted to the measurement of either pulmonary or nasal NO. It was the aim of this study, therefore, to measure the fractions of NO originating from the lower and the upper respiratory tract simultaneously at rest and during moderate exercise. In order to account for possible differences due to smoking habits, we investigated both non-smokers and smokers. We further examined the flow dependency of nasal NO release, and determined the amount of NO autoinhaled into the lung during nasal breathing, again at rest and during exercise.

Methods and subjects

Measurement of nitric oxide

The measurement of NO concentrations was accomplished using chemiluminescence analysis, in which NO is converted to NO_2 in the presence of an excess of ozone. This chemical reaction is associated with the production of infrared radiation, whose intensity is a measure of the NO concentration [21]. Two types of instruments were used, firstly the CLD 700 AL type analyser (ECO-Physics, Duernten, Switzerland) possessing a lower detection limit of 0.6 ppb. Due to its rise time of 2 s, the instrument is appropriate for the display of NO concentrations which do not vary rapidly. The adjustment of the zero level is accomplished via interruption of the radiation between reaction chamber and photomultiplier; hence, NO free gas is not required for the calibration. In order to scale the display, a test gas with a NO content of 90 ppm (AGA, Bottrop, Germany) was used. Considering the high linearity of the apparatus, this calibration is sufficient for all concentrations below 100 ppm. Accuracy in the 100 ppb range was checked using dilutions of a test gas containing 205 ppb NO (AGA). The second chemiluminescence analyser was a CLD 780 TR type (ECO-Physics). It possesses a rise time of 0.3 s for NO signals between 0.5 ppb and 500 ppb, and a lower detection limit of less than 0.05 ppb if the signal is integrated over a time of 1 min. In addition, it allows continuous display of measured values and is, therefore, appropriate for the time resolution of the NO concentrations during a single breath. The gas used for adjustment of the zero level is produced internally from ambient air, whereby NO is completely converted to NO_2 by reacting with ozone. The scale was calibrated with a test gas containing 205 ppb NO.

While the NO concentration denotes a fractional volume, the NO excretion rate represents the volume released per time unit. It can be determined from the NO concentration and the flow rate according to the following formula:

NO rate [nl/min] = NO concentration [ppb]
* flow rate [l/min],

where the result is expressed in nanolitres per minute. Numerical values are given for BTPS conditions. During the measurements, the barometric pressure was 100.4 ± 7 kPa (mean ± SD).

Study population

Fourteen healthy adults, aged 25–41 years, participated in the study. Seven volunteers were smokers with a daily consumption of 20 ± 10 cigarettes during the last 5 years. All subjects declared that they were free of known airway diseases and provided written informed consent. The study was conducted according to the principles of the Helsinki Declaration of 1983 and approved by the local Ethics Committee.

Study protocol

The study was divided into three parts, which were conducted according to the following protocol:

Simultaneous measurement of nasally aspirated and orally exhaled NO at rest and during moderate exercise in smokers and non-smokers

We examined two groups consisting of seven smokers and seven non-smokers both at rest and during exercise. Both phases lasted for at least 10 min. A second post-exercise rest phase of 10 min followed, to determine whether the final resting values were similar to the baseline values. The bicycle-ergometer workload during exercise was adjusted to 60 W. Heart rate was recorded using pulse oximetry (OSCAR, Datex Instruments, Helsinki, Finland). NO from the lower respiratory tract was measured in mixed orally exhaled gas during continuous removal of nasally released NO. All subjects inhaled synthetic air supplied from a gas cylinder. Air was introduced in excess (70 l/min) into a tube via a reservoir consisting of two 5-l bags. A gas source with minimal positive pressure was maintained by a second tube system connected in sidestream. Spontaneous breathing was accomplished exclusively via the mouth. Inspiratory and expiratory gases were divided by a non-rebreathing valve. Exhaled gas was directed through a heated (37° C) mixing chamber with a volume of 12 l. A pneumotachograph (PT36, Jaeger, Würzburg, Germany) allowed monitoring of the respiratory gas flow. The NO concentrations in orally expired gas were determined with a chemiluminescence analyser of type CLD 700 AL at the mixing chamber.

Admixing of nasally released NO into the respiratory gas was prevented by continuous suction, which was directed out of the nose via a tightly fitting nasal mask (Respironics, Murrysville, Pa., USA) covering both nostrils. This caused a steady gas flow from the oropharynx through the nasal cavity while the velum was kept open. The flow rate for the nasal aspiration was produced with a pump and maintained at 1.4 l/min using a mass flow controller (CAL 60l, ECO-Physics). With a second chemiluminescence analyser of the CLD 700 AL type, the nasal NO was measured in the non-pressurized gas behind the pump. The NO concentration of the synthetic air supplied for inhalation was measured immediately before the investigation of each subject.

Measurement of nasal NO concentrations at different aspiration flow rates

In six subjects, the NO concentration was measured in the gas aspirated from the nose at different flow rates. Using a mass flow controller, aspiration flow rates were set to values between 1 and 6 l/min. During maintenance of the nasal suction, the subjects breathed synthetic air spontaneously via their mouths. The set-up for the NO measurements was the same as described for the first part of the protocol. Each adjusted flow rate was investigated during time periods of 4 min.

Measurement of NO concentrations at rest and during exercise within the nasopharynx

In five subjects two thin catheters (diameter 3.3 mm) were advanced through the nostrils via small and tightly fitting openings of a nasal mask and positioned just proximal to the velum palatinum. Humidified synthetic air was provided from a tube system with a non-rebreathing valve connected to the nasal mask. The subjects breathed exclusively via their nose. In order to maximize NO autoinhalation, they were instructed to perform slow and regular inhalation manoeuvres. A pneumotachograph was placed between the nasal mask and non-rebreathing valve. CO_2 was measured in a sideflow of the nasal mask (Normocap, Datex Instruments). The thin catheters were connected directly with the probe entrance of the chemiluminescence analyser of the type TR 780 AL, which enabled us to resolve the time course of NO concentrations during a single breath. The measurements were carried out at rest and during exercise of 60 W in phases lasting 10 min. For later comparison, we determined the nasal NO excretion during continuous aspiration with the technique described for the first part of the protocol in all subjects.

Data collection and statistical analysis

All measured concentration and flow values were stored continuously using a computerized data acquisition system for later analysis. In the case of the first part of the protocol, the values of respiratory gas flow and mixed orally exhaled NO concentration were time shifted by

Table 1 Results of NO measurements in gas aspirated from the nose and in orally expired gas

		Non-Smokers ($n = 7$)			Smokers ($n = 7$)		
		Rest	Exer. 60 W	Rest	Rest	Exer. 60 W	Rest
NO Concentration [ppb]	nasal	219 ± 63	212 ± 54	211 ± 50	183 ± 99	188 ± 104	175 ± 106
	orally exp.	2.9 ± 1.8	2.3 ± 0.9	2.8 ± 1.3	2.2 ± 0.7	2.1 ± 0.6	2.2 ± 0.4
NO Excretion Rate [nl/min]	nasal	311 ± 89	301 ± 77	299 ± 72	261 ± 142	268 ± 149	249 ± 151
	orally exp.	23 ± 14	40 ± 21*	24 ± 15	22 ± 9	41 ± 8*	25 ± 8
Minute Ventilation [l/min]		10 ± 2	20 ± 4*	10 ± 1	11 ± 3	23 ± 5*	12 ± 2
Heart Rate [1/s]		82 ± 6	106 ± 9*	84 ± 5	76 ± 6	103 ± 4*	78 ± 8

All concentrations are given as the difference from the NO level of inspired synthetic air.
* $p < 0.05$ when compared with both rest phases.

1 min against each other in order to account for the retardation due to the gas transfer through the mixing chamber. An offset of 23 ml/sec was added to the expiratory flow data to compensate for the loss due to nasal suction. Flow and NO values were then averaged separately before multiplication to obtain NO excretion rates in orally expired gas. NO excretion rates in nasally aspirated gas were calculated by multiplication of the averaged nasal NO concentration with the suction flow rate of 1.4 l/min. In the case of the third part of the protocol, values were measured in resolution for single breaths. Therefore, NO excretion rates had to be calculated for each time point before integration. Before multiplication, the values of flow and NO concentration were time shifted by 2.2 s against each other in order to correct for the time delay of the chemiluminescence analyser. The corresponding shift for the CO_2 signal was 1.2 s. NO concentrations, flow rates and NO excretion rates were then averaged exclusively over inspiratory or expiratory phases of the respiratory cycles, respectively.

Reduction in the amount of data was achieved by the calculation of average values over the time periods of the corresponding measurement phases. In order to determine the significance of the results, two types of statistical tests were used. In the investigation of the intra-individual changes during exercise, Wilcoxon's signed rank test for matched pairs with Bonferroni correction was applied. The differences between smokers and non-smokers were examined with the Mann-Whitney U test. Results with p values of less than 0.05 were regarded as significantly different. Data in the text and tables are expressed as means ± standard deviations.

Results

NO excretion rates in nasally aspirated and orally expired gas at rest and during moderate exercise in smokers ($n = 7$) and non-smokers ($n = 7$)

The nasal NO concentrations of individuals reached a stable level within less than 1 min. The height of these plateaus differed amongst the individuals. At rest we obtained 219 ± 63 ppb in non-smokers and 183 ± 99 ppb in smokers as differences from the NO concentration of the inspired air, which was measured to be 0.8 ppb. Since the gas was withdrawn from the nose at a constant flow rate of 1.4 l/min, these concentrations corresponded to nasal NO excretion rates of 311 ± 89 nl/min in non-smokers and 261 ± 142 nl/min in smokers. With values of 2.9 ± 1.8 ppb in non-smokers and 2.2 ± 0.7 ppb in smokers, the simultaneously measured oral expiratory concentrations were approximately two orders in magnitude less than the nasal ones. These concentrations were also given as differences from the NO content of the inspired air and corresponded to pulmonary NO excretion rates of 23 ± 14 nl/min for non-smokers and 22 ± 9 nl/min for smokers.

The results are summarized in Table 1. The nasal and the oral expiratory excretion rates of the single subjects are depicted in Figs. 1 and 2. Comparing exercise with rest, no change in the nasal NO excretion was obtained. In contrast, the oral-expiratory rates increased significantly ($p < 0.05$) during exercise, both for non-smokers and smokers. However, the overall increase was only small for the sum of the nasal and the oral-expiratory rates. The differences between smokers and non-smokers were not statistically significant. This resulted from the considerable individual variations, which appeared within each group and from the small differences between the mean values. Occasionally, a distinct rise of the nasal and the oral-expiratory NO excretion was detected following gastric eructations; an example of this is shown in Fig. 3. Time intervals with such peak concentrations were excluded from further analysis.

Dependence of the nasal NO concentration upon the aspiration flow rate ($n = 6$)

The NO concentrations measured in the gas withdrawn from the nose showed, on average, a dependence upon the aspiration flow rate. It is seen from the data in Table 2 that the concentrations decreased with increasing

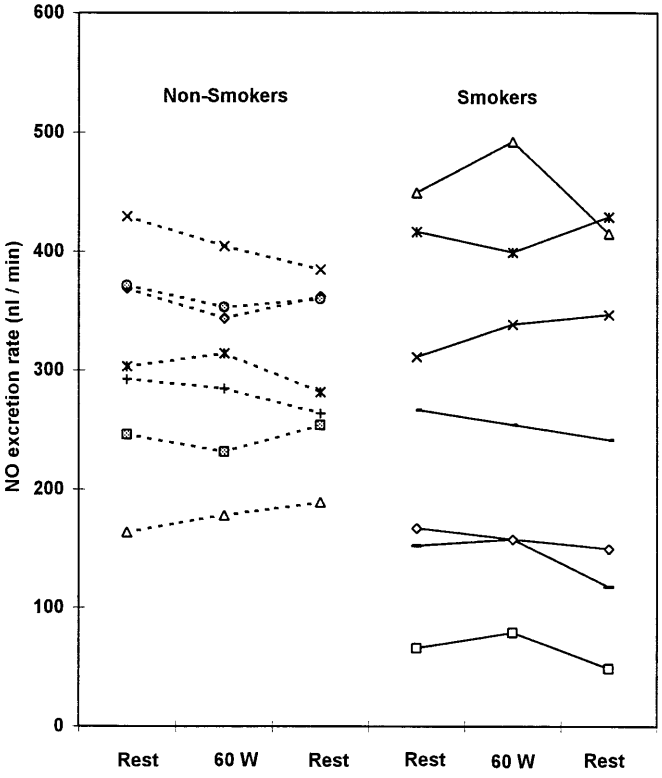


Fig. 1 Nasal NO excretion rates at rest and during exercise of 60 W. Individual values of seven non-smoking and seven smoking healthy adults are shown. Each data point corresponds to a mean value over a time period of 10 min. There was no statistically significant effect of moderate exercise

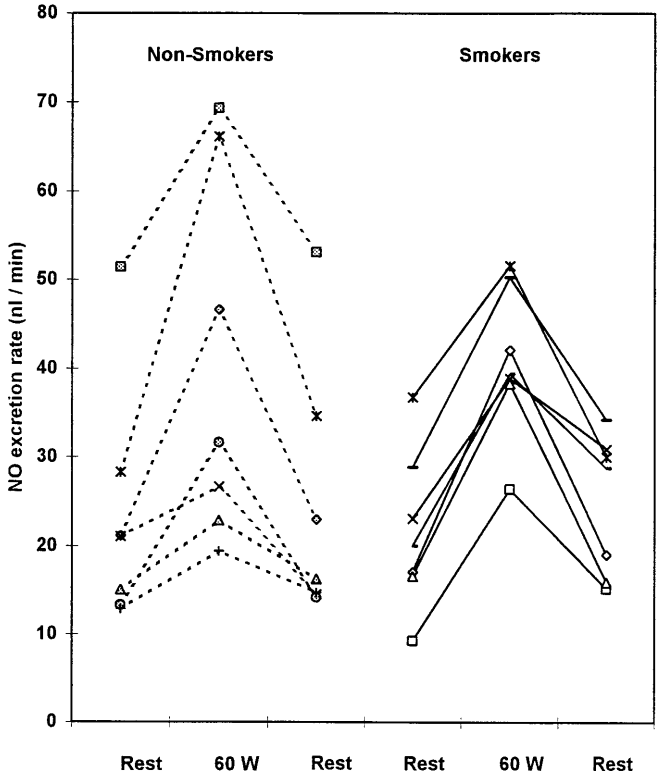


Fig. 2 Effect of moderate exercise on oral expiratory NO excretion rates. Individual values for the same subjects as in Fig. 1, again depicted as mean values over a time period of 10 min. The orally expired NO was determined during removal of nasal NO and was therefore regarded to be pulmonary in origin. A statistically significant increase ($p < 0.05$) caused by moderate exercise of 60 W was demonstrated

flow. Constancy of the corresponding NO rates demonstrates a dilutional effect of the aspiration flow. The adjustment of the aspiration flow rate to a distinct value between 1 and 6 l/min therefore has no influence on the demonstrated amount of NO per time unit. The NO concentration of synthetic air supplied for inhalation was less than 0.9 ppb.

Autoinhaled amount of NO measured within the nasopharynx at rest and during exercise ($n = 5$)

Representative examples for the time course of NO concentrations in the nasopharynx during nasal breathing are shown in the Figs. 4a and 4b. In addition, the curves for respiratory gas flow rate and CO_2 concentration are provided. The NO concentration increased during the inspiratory phase with increasing flow through the nose, and decreased in the expiratory phase to a minimal value. Bicycle exercise to a level of 60 W yielded lower NO concentrations during both inspiration and expiration when compared with at rest. The results are summarized in Table 3. On average, 18 ± 3 ppb of NO were autoinhaled at rest, and 9 ± 2 ppb at an exercise level of 60 W. These values were given as differences from the NO concentration of the inhaled synthetic air, which was measured to be less than 1.2 ppb. The decrease in the autoinhaled NO concentration was accompanied by a doubling of the minute ventilation, indicating a dilutional effect of the respiratory gas flow. The amount of autoinhaled NO per second increased slightly in all five subjects by 16 % from its mean value at rest of 146 ± 38 nl/min to 173 ± 46 nl/min during exercise. In

Table 2 NO in the gas aspirated from the nose at different flow rates

Aspiration Flow Rate [l/min]	NO Concentration [ppb]	NO Excretion Rate [nl/min]
1.0	337 ± 61	337 ± 61
2.0	166 ± 27	331 ± 54
3.0	113 ± 13	338 ± 39
4.0	85 ± 7	341 ± 30
5.0	70 ± 11	348 ± 54
6.0	59 ± 8	354 ± 49

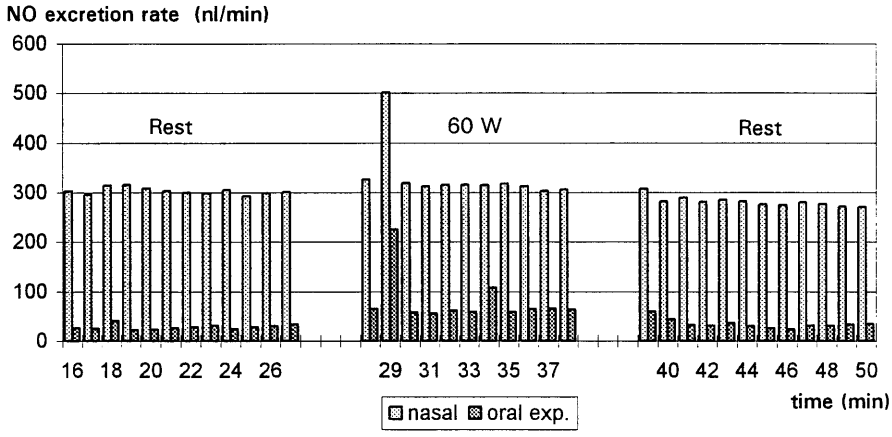
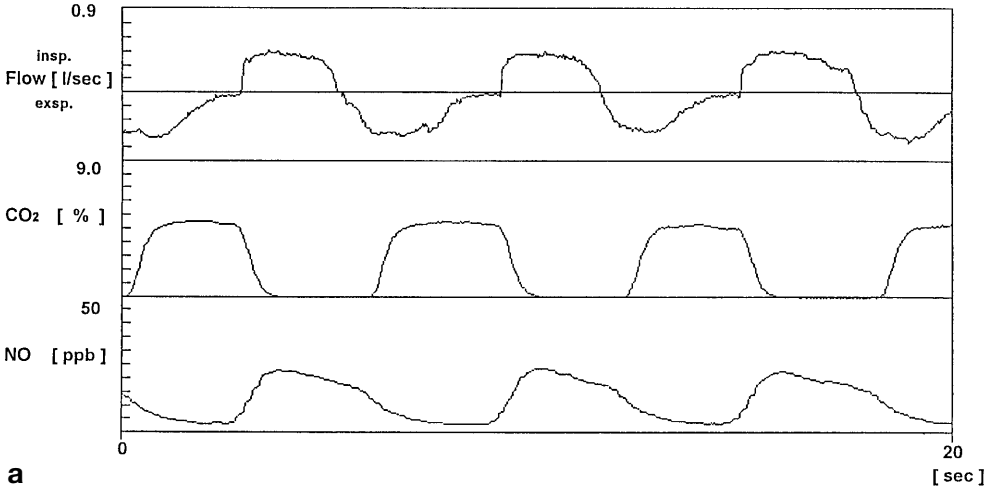


Fig. 3 Nasal and oral expiratory NO excretion rates in a single subject, demonstrating steady state conditions for NO release in upper and lower respiratory tract. A gastric eructation, which occurred in the 29th min, had a considerable influence on both nasally aspirated and orally expired NO



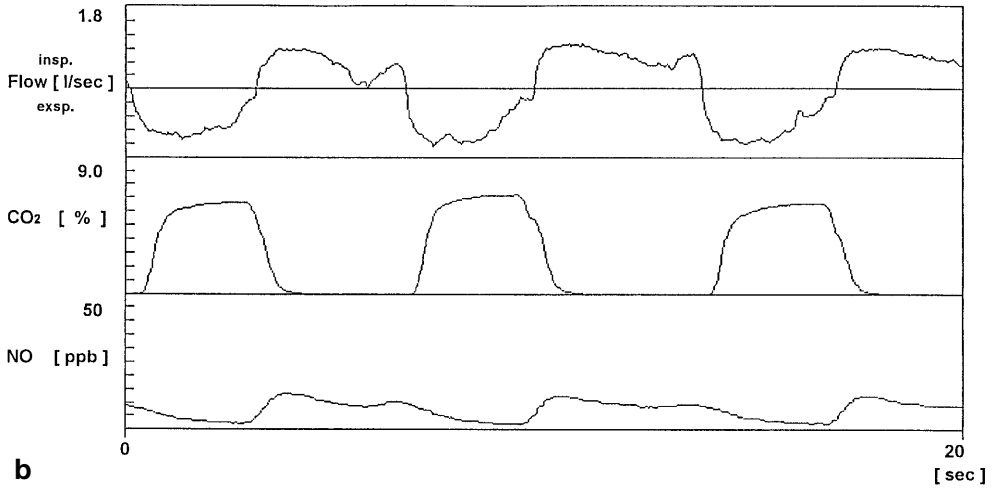


Fig. 4 a NO concentration in the nasopharynx near the velum palatinum with respect to time along with respiratory flow rate and CO_2 curves for individual breaths. The data were collected from a single subject at rest while he breathed exclusively through the nose. **b** NO concentration in the nasopharynx together with respiratory flow rate and CO_2 curve for the same subject during 60 W of exercise. Compared with Fig. 4a, the mean autoinhaled NO concentration is decreased, while the inspiratory gas flow is increased

Table 3 Results of NO measurements within the nasopharynx during nasal breathing

		Rest	Exer. 60 W	Rest
NO Concentration [ppb]	insp.*	17.8 ± 3.1	9.3 ± 1.8	15.1 ± 3.8
	exp.	8.0 ± 3.8	4.9 ± 1.6	7.3 ± 3.1
NO Rate [nl/min]	insp.*	146 ± 38	173 ± 46	147 ± 45
	exp.	68 ± 32	103 ± 40	73 ± 26
Minute Ventilation [l/min]		9 ± 2	21 ± 3	11 ± 3

* data are given as differences from the NO content of the inspired synthetic air.

the same subjects, continuous nasal aspiration during mouth-breathing resulted in a NO release rate of 358 ± 56 nl/min.

Discussion

The data presented suggest an increase of NO excretion in the lower respiratory tract during exercise compared with at rest, while the nasal NO release remains approximately constant. The magnitude of the individual NO excretion appears unrelated to smoking habits in this limited material. Nasal NO excretion was demonstrated to be flow independent at low nasal flow rates. For the first time we measured autoinhaled NO at rest and during exercise. It was found that autoinhaled NO concentrations during nasal breathing result from dilution of a steady nasal NO release by the respiratory gas flow.

The main portion of NO excretion in the respiratory tract originates from the nose. Therefore, the determination of pulmonary NO requires a separation of nasal NO from the respiratory gas, which was accomplished by an aspiration flow directed out of the nose. Successful removal of nasal NO was confirmed by the low oral expiratory concentrations measured during nasal aspiration, which were in the range of values measured in the exhaled air of intubated patients [14, 15, 16, 22]. Since the palate was maintained open during nasal aspiration, nasally released NO was diluted by the respiratory gas drawn continuously through the nasal cavity. Because inspiratory and expiratory NO concentrations during oral breathing were found to be approximately 100 times less than the nasal levels, this leads to an error in the range of only 1 % in the measured nasal values. Nasal NO excretion was found to be independent of flow rates in the range between 1 l/min and 6 l/min.

Even low aspiration flow rates, which are expected to affect the anterior part of the nasal cavity more than the nasopharynx, are sufficient for complete removal of nasally released NO. This is compatible with the finding of Lundberg et al. [17] that nasal NO is mainly produced in the epithelium of the paranasal sinuses, from where it diffuses into the region of the nasal turbinates. As previously reported [14], nasal NO excretion at rest was found to be stable with respect to time. Changes in heart rate and ventilation during exercise had no significant effect on the nasal NO release, which therefore appears to be primarily determined by the local conditions within the nasal cavity and the adjacent paranasal sinuses. This is in accordance with the finding that nasal NO synthesis is mainly controlled by an inducible form of the enzyme NO synthase (i-NOS) [17]. Differences in activation of i-NOS due to cytokines and lipopolysaccharides [23] amongst the subjects could have contributed to the considerable inter-individual variation of nasal NO production.

The oral expiratory NO excretion rates measured during nasal aspiration were regarded as pulmonary in origin. Strictly speaking, they represent an upper limit for the detectable amount of NO in expired gas resulting from lower airways and alveoli. At rest, the release in the lower respiratory tract contributes less than 10 % to the total NO excretion. It is possible that in the alveoli higher concentrations are present and that low levels in the expiratory gas result from uptake by blood and from absorption during passage through the airways. The interpretation of pulmonary NO excretion is complicated by the fact that it is composed of fractions with different origins. Immunohistochemical examinations showed that NO is produced in bronchial epithelial cells and alveolar macrophages by i-NOS [24]. Endothelial NO synthase (e-NOS), which controls NO production depending on the intracellular calcium ion concentration, was located in alveolar and bronchial endothelial cells [25]. All these components may have contributed to the measured increase in pulmonary NO excretion during exercise. On the other hand, the activity of i-NOS is not expected to be dependent on any consequences of exercise. Therefore, the observed doubling of the NO release from the lower respiratory tract during moderate exercise probably results from an increased NO synthesis in endothelial cells regulating the pulmonary vessel tone and the homeostasis of the lower airways. Phillips et al. [19] found a similar increase in pulmonary NO excretion during moderate exercise. They also investigated the influence of ventilation and blood flow and suggested that both contribute to changes in pulmonary NO excretion.

In various studies, the NO concentrations in expired gas were found to be dependent upon the smoking habits of the subjects [11, 12, 13, 15]. It is suggested that the exogenous administration of NO in high concentra-

tions from tobacco smoke can suppress the endogenous NO production. We measured less nasal NO in smokers compared to non-smokers on average but, due to the wide variability within each group, this was not statistically significant. Similarly, no significant difference in the orally expired NO levels between the two groups was obtained. By analysis of statistical power we found that a sample size of greater than 70 subjects would have been required to determine adequately whether there were mean differences between smokers and non-smokers. Our results indicate that the individual NO excretion from the respiratory tract allows no conclusion about smoking habits.

NO measurements with time resolution for single breaths have been rare and were commonly performed in exhaled air [9, 11]. The results allow only indirect conclusions about nasal NO release during inspiration. In this study, we present directly measured values for autoinhaled NO concentration during spontaneous nasal breathing for the first time. Our measurements within the nasopharynx demonstrated increasing NO concentrations during inspiration which were followed by a decrease during expiration (see Figs. 4 a and 4 b). This indicates an uptake of nasally released NO by the inspiratory gas and a consecutive absorption within the lower respiratory tract. Moderate exercise considerably reduced the autoinhaled NO concentration to nearly one half of its value at rest, while the minute ventilation approximately doubled (see Table 3). This finding is compatible with a steady NO release rate in the nasal cavity, which is diluted by the inspired air.

Assuming that this nasal NO excretion is not different from that determined during nasal aspiration (NO $Rate_{nasal\ asp}$), the autoinhaled NO concentration (NO $Conc._{autoinh}$) can be calculated by the equation:

$$NO\ Conc._{autoinh}\ [ppb] = NO\ Rate_{nasal\ asp}\ [nl/min] * f_{insp}/MV[l/min],$$

where MV is the minute ventilation and f_{insp} denotes the fraction of inspiration related to the total respiratory cycle time. The ratio MV/f_{insp} represents the mean flow rate occurring during inspiration. In our experiment f_{insp} was near to 0.5. Inserting this value, the quantities for MV from Table 3, and the measured NO $Rate_{nasal\ asp}$ of 358 nl/min in the above relationship, we obtain 19.9 ppb as prediction for the autoinhaled NO concentration at rest. During exercise we expect 8.5 ppb. Although this is only an estimation, the calculated values are in close agreement with our experimental findings. At first sight this seems to be contradicted by the small increase in nasal NO release rate during exercise which results from our measured values (see Table 3). On the other hand, an increase in the autoinhaled amount of NO is expected due to the enlarged fraction of the inspiratory phase on total respiratory cycle time during exercise. Therefore, it seems reasonable to postulate that nasal NO excretion remains approximately constant up to the nasal flow rates occurring during moderate exercise. Nevertheless, we cannot exclude the possibility that nasal NO release increases in absolute value at higher levels of exercise, as indirectly concluded by Phillips et al. [19], or even decreases on heavy exercise above 240 W, as reported by Lundberg et al. [20].

Absorbtion of autoinhaled NO within the alveoli may improve arterial oxygenation by inducing a reduction in intrapulmonary shunt. Although we did not measure blood gases, we can refer to the results of studies concerned with NO administration in patients at very low doses, which are comparable to the mean autoinhaled NO concentrations given in Table 3. To date, only a few studies with small patient populations have addressed the physiological effects of NO in concentrations below 100 ppb. Gerlach et al. [26] reported an improvement of PaO_2 in ARDS patients due to an inhaled concentration of 10 ppb NO. Lundberg et al. [27] found an increase of arterial oxygenation in intubated patients during inhalation of 20–40 ppb NO, which was aspirated from the patient's nostrils and admixed to the inspiratory airstream of the ventilator. Therefore, an improvement in arterial oxygenation caused by NO autoinhalation seems likely. Only one recent study relates to the effects of NO autoinhalation on pulmonary vascular tone. In patients after open heart surgery, Lundberg et al. reported a reduction in pulmonary vascular resistance index by about 10 % due to nasal breathing [28]. Further investigations are required to validate that the low autoinhaled NO levels are able to reduce intrapulmonary shunt.

In summary, we found that the excretion of NO from the respiratory tract is dominated by a steady nasal contribution which is not influenced by moderate exercise. The detectable NO release in the lower respiratory tract is approximately one order of magnitude less than that in the nasal cavity and can be determined quantitatively only when nasal NO is removed. During moderate exercise, NO excretion from the lower respiratory tract increases. The individual NO release allows no conclusion about smoking habits. The nasal NO excretion rate was not changed by variations of nasal flow rates between 1 l/min and 6 l/min. In comparison with at rest, the autoinhaled NO concentration is reduced during moderate exercise due to dilution caused by the increased respiratory gas flow. NO levels in the range of the measured autoinhaled NO concentrations were demonstrated to improve arterial oxygenation in earlier studies [26, 27], although further research is required to validate this effect.

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