Rhinology, 36, 117–121, 1998

Nasal nitric oxide and its relationship to nasal symptoms, smoking and nasal nitrate*

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SUMMARY

Nitric oxide (NO) is produced in the nasal mucosa and in the paranasal sinuses. Increased nasal NO concentrations have been found in patients with asthma and/or rhinitis, and nasal NO has been suggested to be a marker of nasal inflammation. Measuring the stable end products of NO, nitrate and nitrite in nasal lavage fluid have been proposed as an indirect method for measuring NO concentration. The aim of this study was to measure nasal NO concentration, and to find out its relationship to nasal nitrate concentration and clinical parameters. 73 paper-mill workers were investigated with nasal and exhaled NO, nitrate in nasal lavage fluid and were given a respiratory questionnaire. Nasal air was sampled directly from a nasal mask and NO concentration was measured with a chemiluminescence analyser. Exhaled NO was measured with the subjects breathing tidal volumes and wearing nose clips. The nitric oxide metabolites were analysed as nitrate, after reduction of nitrite to nitrate.

Smokers had lower nasal NO concentration (264 ppb) as compared to NO concentrations of 340 ppb among non-smokers (p=0.02). There was no statistically significant relationship between nasal NO concentration and nitrate in nasal lavage fluid or nasal symptoms. Nasal NO concentration was significantly related to FVC (p=0.047) and there was a relationship with borderline statistical significance (p=0.06) to FEV_1.

In conclusion, we found no relationship between nitrate in nasal lavage and nasal NO, and neither of these were correlated to nasal symptoms or to nasal PIF. Nasal NO was significantly lower among smokers. Further controlled studies on subjects with rhinitis are needed, to evaluate the relation between nasal NO and nasal inflammation. In addition, there is also a need to develop methods for measuring nasal NO that minimise contamination from sinuses.

Key words: nitric oxide, upper airways, biomarkers

INTRODUCTION

In the nasal airways, nitric oxide (NO) is produced in paranasal sinuses and, to a lesser extent by the nasal mucosa (Furukawa et al., 1996). In the paranasal sinuses, very high concentrations of NO have been found, ranging between 300 and 23 000 ppb (Lundberg et al., 1994b, 1995a). The biological significance of NO in the nasal region remains unclear. It is believed to take part in the local host defence system keeping the sinuses sterile due to its antiviral and bacteriostatic properties (Moncada et al., 1991). NO is known to increase the mucociliary beat frequency and hence increase the mucociliary clearance (Jain et al., 1993, Runer, 1996). NO is also a vasodilator and might contribute to mucosal swelling in, for example, rhinitis. Studies on guinea-pig and rat (Kuo et al., 1992; Bernareggi et al., 1997) indicate that NO also is involved in plasma exudation in the airways. Nitric oxide seems thus to be involved in the inflammatory response, and nasal NO might be used for assessing nasal inflammation (Kharitonov et al., 1997a, 1997b).

NO reacts with water in the respiratory tract lining fluid (RTLF), and this results in the formation of nitrate/nitrite. Measuring the concentration of the nitric oxide metabolites nitrate/nitrite in nasal lavage has therefore been suggested as an indirect method for measuring NO concentration (Garrelds et al., 1995).

* Received for publication October 10, 1997; accepted April 30, 1998

We are not aware of any studies in humans measuring both nasal NO and nitrite/nitrate in nasal lavage.

The aims of the present paper are, firstly, to test a method for measuring nasal NO and relate it to nitrite/nitrate in nasal fluid. Secondly, we further wanted to elucidate the clinical significance of nasal NO and nitrite/nitrate by relating them to clinical parameters from the nasal tract.

SUBJECTS AND METHODS

This study was performed on 73 employees from a paper mill, during two weeks in February, 1996. The employees were office workers (n=36) and process workers with low exposure to paper dust (n=37). All subjects answered a respiratory questionnaire containing questions about current nasal symptoms and symptoms from the lower airways (Torén et al., 1993). In the study, nitric oxide measurements were performed first, followed by nasal function tests and lung function measurements. The lung function (FVC and FEV_1) was measured with a dry spirometer (Vitalograph) according to ATS (American Thoracic Society, 1979). The spirometric values were expressed in percent of predicted, based on reference material not adjusted for smoking habits (Berglund et al., 1963).

The nasal function tests included measurement of nasal transit time (s) with a saccharine test (Anderssen et al., 1974), nasal lavage (Naclerio et al., 1983) and nasal peak inspiratory flow (Hellgren et al., 1997). The lavage fluid was centrifuged, and the supernatant was frozen immediately at -20°C.

The concentration of NO in nasally exhaled air was measured with a chemiluminescence NO/NOx analyser (Monitor Labs 9841, Eaglewood Co, USA). Subjects used a nasal mask and breathed normal tidal volumes through the nose with their mouths closed. Subjects with moustaches (n=17) not allowing proper closure of the nasal mask used a nasal olive. NO was sampled (=0.64 litres/min) over 180s, and the concentrations were registered each 15 s. The concentrations (parts per billion, ppb) of nasal nitric oxide in relation to the sampling time are shown in Table 1. After 90 s, the mean levels were stabilised at a plateau above 300 ppb. The mean value of the samplings (105 s to 180 s) for each person was taken as an average for the nasal NO concentration and termed nasal NO.

Table 1. Concentrations (ppb) of NO in the nose at different sampling times.

Sampling time	Concentration (SD)	N
60 s	276.7 (113.8)	72
75 s	292.3 (118.2)	73
90 s	298.8 (127.8)	71
105 s	318.4 (155.8)	73
120 s	309.6 (126.8)	73
135 s	310.6 (121.3)	73
150 s	312.2 (122.8)	73
165 s	312.8 (129.2)	73
180 s	328.9 (141.4)	73
Mean (105-180)	*315.4 (123.3)*	73

The within-subject variation was calculated (Bland and Altman, 1996). Nasal NO was measured at two different days in 27 healthy subjects selected from the staff of the Section of Occupational Medicine. The mean standard deviation for the duplicate measurements was 92.2 ppb, and the coefficient of variation (C.V.) was 23.2%. The repeatability was 255.3 ppb.

Exhaled NO was measured with the tidal breathing method (Lundberg et al., 1996). The same chemiluminescence NO/NOx analyser was used. Briefly, orally exhaled NO was measured while the subjects were seated wearing a nose-clip during normal tidal breathing over a 4-min period. No internal restrictor was used; that is, the subjects were breathing with an open soft palate, permitting contamination of the exhaled NO by NO from the upper airways (Silkoff et al., 1997). The exhaled amount of NO was calculated in relation to body surface area and expressed as

$$\text{nmol/min*m}^2.$$

The nitric oxide metabolites were analysed as nitrate, after reduction of nitrite to nitrate, by an automated nitrogen segmented continuous flow system, the Technicon autoanalyzer π system (Technicon, Solna, Sweden). Samples were injected into the carrier stream of ammonia buffer at a rate of 20 samples an hour. After passing an online copper-coated cadmium reductor, which reduces nitrate to nitrite, the carrier was mixed with a phosphoric acid solution. The intensity of the azo dye that then was formed was measured at 540 nm. Concentration was evaluated by peak height and was expressed in μM NO.

The statistical analyses have been performed with t-test and Spearman's rank correlation coefficient (r_s). The significance of the slope in the univariate and multivariate regression models have been based on t-distribution. For the linear regression models PROC REG from the SAS statistical package was used.

RESULTS

Age, gender, spirometric values and smoking habits of all subjects are presented in Table 2.

Table 2. Sex, age, smoking habits and pulmonary function among the investigated.

Sex		
	Males (N)	31
	Females (N)	42
Age		
	Males (yrs)	50.3 (36-63)
	Females (yrs)	48.5 (39-63)
Smoking habits		
	Smokers (N)	23
	Former smokers (N)	21
	Never smokers (N)	28
	Not known (N)	1
FEV_1 (% predicted)		102.7 (15.9)
FVC (% predicted)		101.1 (12.6)

The mean concentration of nasal NO was 315.4 ppb, with no statistically significant (p=0.52) differences between men (325.9 ppb) and women (307.6 ppb). The concentration of nasal NO among never-smokers was 338 ppb, among former smokers 341 ppb, and among smokers 264 ppb. The difference between never/former smokers and smokers was significant p=0.02). There was no difference with regard to nasal NO in relation to exposure to paper dust (exposed 300 ppb vs. unexposed 330 ppb, p=0.31). Nasal NO concentration was significantly related to FVC (p=0.047) and there was a relationship with borderline significance to FEV_1 (Table 3). There was no significant relationship to nasal transit time, nasal PIF or age (Table 3).

Table 3. Univariate regression coefficients in simple regression models

Dependent variable	Independent variable	Slope (SE)	p-value
Nasal NO (ppb)	FEV_1[1]	1.73 (0.90)	0.06
Nasal NO (ppb)	FVC	2.28 (1.13)	0.047
Nasal NO (ppb)	Age	–0.31 (2.05)	0.88
Nasal transit time (s)	Nasal NO (ppb)	0.48 (0.316)	0.12
Nasal NO (ppb)	Nasal-PIF (1/s)	–0.075 (0.25)	0.76

[1] Percent predicted (Berglund et al., 1963)

The mean concentration of nitrate in nasal lavage fluid, expressed as NO, was 30.4 μM. For men it was 34.1 μM and for women it was 27.8 μM (p=0.16). Nitrate in nasal lavage and nasal NO was not related (r_s=0.20, p 0.09, nasal nitrate =24.1 + 0.02*nasal NO, 95% CI for the slope was –0.016 - 0.056). There neither was a significant relation between nasal nitrate and nasal NO in a multiple regression model adjusting for exhaled NO; 95% CI for the coefficient for nasal NO was –0.014 -0.072. There were no significant differences in nasal NO nor nasal nitrate in relation to nasal symptoms (Table 4).

Table 4. Nasal nitric oxide concentrations (ppb) and nitrate concentrations, expressed as NO (μM), according to nasal symptoms

Current symptom	Nasal NO (p-value)	Nasal nitrate (p-value)
Sneezing		
Yes	328.0 (0.42)	30.8 (0.82)
No	304.4	29.3
Nasal blockage		
Yes	325.6 (0.57)	28.2 (0.38)
No	308.7	31.9
Itching in the nose		
Yes	324.2 (0.77)	27.6 (0.50)
No	313.2	31.1
Runny nose		
Yes	314.7 (0.95)	29.1(0.55)
No	316.2	31.9
Any nasal symptom		
Yes	314.8 (0.94)	30.8 (0.82)
No	317.3	293

In exhaled air, the NO concentration was 1.74 nmol/min/m^2. In a univariate regression model, a significant (p=0.0001) relationship between oral and nasal NO was found. In a multiple linear regression model, when controlling for smoking and pulmonary function, the orally exhaled NO was significantly (p=0.001) related to the nasal NO, r^2 for exhaled NO was 0.31, for the whole model it was 0.37.

DISCUSSION

A universal method for measuring nasal NO has not yet been established, but direct nasal sampling during breathholding has been proposed (Kharitonov et al, 1997b). So far, the methods for measuring nitric oxide concentrations from the nose have varied (Table 5). The main differences in nasal NO between different studies may be explained by different sampling rates.

Table 5. Summary of studies reporting measurements of nasal NO (ppb)

Author	Sampling method	Sampling rate	Normal subjects	Asthmatic subjects	Subjects with untreated allergic rhinitis
Imada et al., 1996	Nasal sampl[1]	1 1/mm	323	–	–
Lundberg et al, 1996	Nasal exhaled Nasal sampl	0.8 1/min	21 239	27 254	29[2]
Martin et al., 1996	Nasal sampl	Reservoir	25	–	35
Runer, 1996	Nasal sampl	0.66 1/min	273	–	–
Kharitonov et al., 1997a	Nasal sampl	0.25 1/min	996		1527
Arnal et al., 1996	Nasal sampl	0.7 1/min	230[3]	–	389

[1] Nasal olive
[2] Children
[3] Mean of right and left nostril

Most authors are interested in revealing a NO induction in the nasal mucosa, but it seems difficult to avoid that NO derived from the sinuses influences the results, as these concentrations are much higher.
It is most probable that the sampling of nasal NO during tidal breathing includes measurements of NO derived from both the lower and upper respiratory tract and sinuses, as our method does not imply closure of the soft palate. NO from the lower airways is much lower than the nasal NO concentration, as mean orally exhaled NO was 9 ppb compared to nasal NO 315 ppb. Hence, air with low NO concentration from the lower respiratory tract probably dilutes the higher NO concentration in the nasal area, causing a registration of a too low NO value, It has been recommended that in adults nasal NO sampling should be performed during breathholding (Kharitonov et al, 1997b). A dilution would, however, take place even if the soft palate is closed, as the nasal air would be diluted with ambient air.

Investigators sampling nasal NO during breathholding and using a similar sampling flow rate as in our study, seem to obtain nasal NO concentrations similar to what we find (Lundberg et al, 1994a, 1996a). In these studies nasal air is sampled from one nostril, and ambient air (or NO free air) is continuously drawn through the contralateral nostril. Studies with higher nasal NO concentrations use a lower sampling flow rate (Kharitonov et al, 1997a). Probably the sampling flow rate is an important predictor for the obtained nasal NO concentrations, which also has been stated by others (Kharitonov et al, 1997b).

From our samplings of orally exhaled NO we obtained information about the expiratory flow during one minute tidal breathing (through the mouth). There was a positive association ($p=0.05$) between nasal NO and the oral expiratory flow during one minute tidal breathing. This was also found when controlling for nasal PIF, meaning that subjects with high expiratory flow had high nasal NO, even when adjusting for nasal congestion (measured as nasal PIF).

Nitric oxide metabolites in nasal lavage has been proposed as an indirect method for measuring NO production in the nose (Garrelds et al., 1995). It is, however, unclear to which extent NO from the sinuses is influencing the levels of nitrate/nitrite. So far, no data have been presented allowing a comparison of levels of nasally exhaled NO and nitrate/nitrite in nasal lavage. In this study we did not find a statistically significant relation between nasal NO and nasal nitrate. If such a relation exists, our failure to find it, is hard to explain by a dilution of nasal NO, as the inter-individual differences would persist, but at a lower level. Furthermore, we failed to find any significant relation between nasal NO and nitrate even if we adjusted for both exhaled NO and expiration flow rate.

There was a positive association between nasal NO and FVC (and FEV_1), i.e. subjects with low FVC (and FEV_1) have low nasal NO and vice versa. Whether this is a random finding or has any biological significance is hard to judge. Despite multiple regression modelling residual confounding in relation to smoking may exist, as smokers had a lower nasal NO than never smokers, and smokers may have a decreased lung function. However, in this study FVC for never-smokers was 100.2% and for smokers/former smokers it was 101.6%, making a smoking effect less probable. Of interest is, however, that Lundberg (1996) has proposed NO as an aerocrine messenger. NO produced in the upper airways will follow the air-stream to the lower airways and the lungs with every inhalation. It is not clear whether this NO has any biological effects, but inhaled NO as low as 100 ppb significantly improves arterial oxygenation in subjects with pulmonary disease (Puybasset et al., 1994). Furthermore, it has been shown that PaO_2 increased in intubated patients when nasal air from healthy volunteers was added to the inspired air (Lundberg et al, 1995b).

Recently Kimberley et al. (1996) have shown that even the orally exhaled NO is partly derived from the nose, especially when nose clip is used, as we did in this study. It has also recently been shown that exhaling against an internal restrictor reduces the contamination of exhaled NO by nasal NO (Silkoff et al., 1997).
We also found that the exhaled amount of NO was related to the NO concentration in the upper airways, indicating such an contamination. About 30 % of the variability of exhaled NO was explained by contribution from the upper airways.

It must be pointed out that even if the multiple linear regression models showed statistically significant associations, they have a low explanatory power, meaning that most of the variability was not explained by the models. The model with nasal transit time as the dependent variable had an r^2 of 0.19.

In conclusion, we found no relationship between nitrate in nasal lavage and nasal NO, and neither of these were correlated to nasal symptoms or to nasal PIF. Nasal NO was significantly lower among smokers. Further controlled studies on subjects with rhinitis are needed, to evaluate the relation between nasal NO and nasal inflammation. In addition, there is also a need to develop methods for measuring nasal NO that minimise contamination from sinuses.

ACKNOWLEDGEMENTS
The study was supported by the Swedish Council for Worklife Research, and by the Torsten and Ragnar Söderberg Foundation for Scientific Research.

REFERENCES
1. Anderssen I, Camner P, Jensen PL, Philipsson K, Proctor DF (1974) A comparison of nasal and tracheobronchial clearance. Arch Environ Health 29:290-293.
2. Amal JF, Didier A, Rami J, Charlet JP, Serrano E, Besombes JP (1997) Nasal nitric oxide is increased in allergic rhinitis. Clin Exp Allergy 28:358-362.
3. American Thoracic Society (1979) Workshop on standardization of spirometry. Am Rev Respir Dis 199:831-838.
4. Berglund E, Birath G, Bjure J, Grimby (1963) Spirometric studies in normal subjects. Acta Med Scand 173:185-191.

5. Bernareggi M, Mitchell JA, Barnes PJ, Belvesi MG (1997) Dual action of nitric oxide on plasma leakage. Am J Respir Crit Care Med 155: 869-874.
6. Bland JM, Altman DG (1996) Measurement error. Br Med J 313:744.
7. Furukawa K, Harrison DG, Saleh D, Shennib H, Chagnom FP, Giad A (1996) Expression of nitric oxide synthase in the human nasal mucosa. Am J Resp Crit Care Med 153:847-850.
8. Garrelds IM, van Amsterdam JGC, de Graaf-in 't Veld C, van Wijk G, Zijlstra FJ (1995) Nitric oxide metabolites in nasal lavage fluid of patients with house dust mite allergy. Thorax 50:275-279.
9. Hellgren J, Jarlstedt J, Dimberg L, Torén K, Karlsson G (1997) A study of some current methods for assessment of nasal histamine reactivity. Clin Otolaryngol (in press).
10. Imada M, Iwamoto J, Nonaka S, Kobayashi Y, Unno T(1996) Measurement of nitric oxide in human nasal airway. Eur Respir 39:556-565.
11. Jain B, Rubenstein I, Robbins R, Leise R, Sisson J (1993) Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. Biochem Biophys Res Comm 191:83-88.
12. Kharitonov SA, Rajakulasingam K, O'Connor B, Durham SR, Barnes PJ (1997a) Nasal nitric oxide is increased in patients with asthma and allergic rhinis and may be modulated by nasal glucorticoids. J Allergy Clin Immunol 99:58-64.
13. Kharitonov SA, Alving K, Barnes PJ (1997b) Exhaled and nasal nitric oxide measurements: recommendations. Eur Respir J 10:1683-1693.
14. Kimberley B, Nejadnik B, Giraud GD, Holden WE (1996) Nasal contribution to exhaled nitric oxide at rest and during breathholding in humans. Am Respir Crit Care Med 153: 829-836.
15. Kuo HP, Liu S, Barnes PJ (1992) The effects of endogenous nitric oxide on neurogenic plasma exudation in guinea-pig airways. Eur J Pharmacol 221:385-388.
16. Lundberg JON, Weizberg E, Nordvall SL, Kuylenstierna R, Lundberg JM, Alving K (1994a) Primary nasal origin of exhaled nitric oxide and absence of Kartagener's syndrome. Eur Respir J 7:1501-1504.
17. Lundberg JON, Rinder J, Weitzberg E, Lundberg JM, Alving K (1994b) Nasally exhaled nitric oxide in humans originates mainly in the paranasal sinuses. Acta Physiol Scand 152: 431-432.
18. Lundberg JON, Farkas-Szallasi T, Weizberg E, Rinder J, Lindholm J, Änggård A, Alving K (1995a) High nitric oxide production in human paranasal sinuses. Nature Med 1:370-373.
19. Lundberg JON, Lundberg SM, Settergren G, Alving K, Weizberg E (1995b) Nitric oxide produced in the upper airways, may act in an "aerocrine" fashion to enhance pulmonary uptake in humans. Acta Physiol Scand 155:467-468.
20. Lundberg JON, Weitzberg E, Rinder J, Lundberg JM, Alving K (1996a) Calcium-dependent and steroid-resistant nitric oxide synthase activity in human paranasal sinus mucosa. Eur Respir J 9:1344-1347.
21. Lundberg JON (1996b) Airborne nitric oxide: inflammatory marker and aerocrine messenger in man. Acta Physiol Scand 157:suppl 633.
22. Martin U, Breygden K, Devoy M, Rowarth P (1996) Allergens, IgE, mediators, inflammatory mechanisms: Increased levels of exhaled nitric oxide during nasal and oral breathing in subjects with seasonal rhinitis. J Allergy Clin Immunol 97:768-72.
23. Moncada S, Palmer RMS, Higgs EA (1991) Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol Rev 43:3051-3064.
24. Naclerio RM, Mejer HL, Kagey-Sobotka A, Adkinson NF, Meyers DA, Norman PS (1983) Mediator release after nasal airway challenge with allergen. Am Rev Respir Dis 128:597-602.
25. Puybasset L, Rouby J, Mourgeon E, Stewart TE, Cluzel P, Arnaud M, Poete P (19940 Inhaled nitric oxide in acute respiratory failure: dose response curves. Intensive Care Med 20:319-327.
26. Runer T (1996) Studies of mucociliary activity and blood flow in the upper airways, with special reference to endothelins and nitric oxide. Thesis, University of Lund, Sweden.
27. Silkoff PE, McLean PA, Slutsky AS, Furlott HG, Hoffstein E, Wakita S, Chapman KR, Szalai JP, Zamel N (1997) Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. Am J Respir Crit Care Med 155:260-267.
28. Torén K, Brisman J, Järvholm B (1993) The assessment of asthma and asthmalike symptoms by questionnaires. Chest 104:600-608.

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