

Original article

Characterization of airway nitric oxide in allergic rhinitis: The effect of intranasal administration of L-NAME

Background: Several studies have attempted to assess nasal nitric oxide (NO) levels in allergic rhinitis (AR). However, there seem to be differences in the results obtained. We therefore wanted to investigate this further by studying airway NO in AR and controls at several modalities, and also the effect of intranasal administration of the nitric oxide synthase (NOS) inhibitor N^G -nitro-L-arginine-methyl ester·HCl (L-NAME).

Methods: Airway NO was determined through repeated measurements at three flow rates of air (0.5, 3, and 9 l/min), using a single-breath method and a method of nasal aspiration, in 18 patients with birch pollen AR during season and in 18 controls.

Results: Patients with AR were characterized by no difference in nasal but higher orally exhaled NO and a larger interindividual spread in nasal and orally exhaled NO compared to controls. We also found a greater reduction in nasal NO after L-NAME in patients compared to controls.

Discussion: These results indicate that several factors determine the levels of nasal NO in rhinitis. NO production in the nasal mucosa of patients with AR may be upregulated. On the other hand, this increase could be counteracted by swelling of the mucosa and secretions resulting in impaired NO diffusion from, for example, the paranasal sinuses, where particularly high levels of NO have been found. Also, the high background levels of NO from constitutive sources in the nose may blunt smaller increases in mucosal NO output.

Conclusion: It seems that the methods for measurement of nasal NO need to be improved and standardized before we can consider to use this test in monitoring inflammation in AR.

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Nitric oxide (NO) is present in orally exhaled air (1), but higher levels are found in the human nasal airways (2), and in the paranasal sinuses (3). Orally exhaled NO levels are increased in asthma and measurements of NO are now finding their way into the clinic as a non-invasive means of monitoring airway inflammation (4, 5). Several studies have been carried out in order to assess nasal NO levels in allergic rhinitis (AR). Interestingly, there seem to be differences in the obtained results, some studies showing an increase (6–8) while other show no difference in nasal NO between patients and controls (9–11). Whether these discrepancies are due to methodological variations, different patient selections, or some other factor is not known. In addition, NO production in the nasal mucosa of patients with AR seem to be upregulated according to histopathological studies on NOS (12–15).

There are probably several different sources in the nose contributing to the levels of NO found in nasally exhaled air. Some of these sources are easily reached by topical sprays whereas others, for example, the paranasal sinuses, are not. If this is true, administration of locally acting drugs that interfere with NO production and release

could be useful to study local disturbances in NO release from different compartments in the nose. We wanted to further study nasal and orally exhaled NO in AR without clinical asthma by using several sampling methods at varying flow rates. In addition, we wanted to compare the effects of intranasal administration of an NO synthase inhibitor, N^G -nitro-L-arginine-methyl ester·HCl (L-NAME) on exhaled and nasal NO levels in AR and controls. Our hypothesis being there would be some sampling modality discerning AR from controls.

Materials and methods

Subjects and setting

Eighteen patients (mean age 32 years, range 21–50, nine men) and 18 controls (mean age 30 years, range 20–46, nine men) were included.

After a medical history was taken through telephone interview, patients with ongoing, symptomatic and reportedly skin prick test-positive nonsteroid-treated birch pollen AR, symptomatic strictly during pollen season (mainly April–June) and without diagnosis or

symptoms of asthma or other disease, were preliminarily included. Seven patients who had previously experienced asthmatic reactions, which mainly had ceased during childhood or adolescence, were included. Patients occasionally taking intranasal steroids previous years withheld from steroids the actual year of trial. Oral and topical antihistamines and sodium cromoglycate were abandoned for more than 24 h before measurements. No subject reported any respiratory infections the last 2 weeks. One patient on continuous treatment with levothyroxin was included. All patients were non-smokers.

All controls were reportedly healthy nonsmokers without any history of allergic airway disease (skin prick test was not performed) and without respiratory infections the last 2 weeks.

On the day of examination, all patients had experienced their maximum level of AR symptoms at some point within the last 2 weeks. Scoring for allergic symptoms [nasal blockage, irritation (itching and sneezing), and secretion] was obtained from the patients by the same examiner after final inclusion and 30 min after intranasal administration of L-NAME. The patients noted their score on a continuous scale ranging from no symptom to absolute maximum imaginable level of the symptom (visual-analog scale) immediately converted to a discrete parameter at steps of 0.5 on a scale ranging from 0 to 10. Scorings for each symptom were recorded, and a mean of the symptom scoring before and after L-NAME was calculated. Anterior rhinoscopy using microscope revealed no anatomical abnormalities other than moderate thickening and reddening of the nasal mucosa. Local pollen levels on a five-grade scale (0 = nonexisting, 1 = low, 2 = moderate, 3 = high, or 4 = very high) were obtained daily from the Swedish Palynological Laboratory at the Swedish Museum of Natural History. The mean regional allergen level during the study was 1.8 (range 0–3).

All measurements of airway NO (described below) were performed before and within 30–50 min after the intranasal administration of L-NAME. All measurements were supervised by the same examiner. The study was approved by the local ethics committee.

Measurements of NO in nasally and orally exhaled air

A chemiluminescence technique was used according to the recommendations recently described for measurements of exhaled NO in the European Respiratory Society Task Force Report and American Thoracic Society Recommendations using a single-breath method for oral NO and an aspiration method for nasal NO (4, 5). Briefly, compressed NO-free air was continuously introduced into a non-diffusing gas collection bag (Hans Rudolph Inc.) at a rate of 2–4 l/min. The bag was connected to a three-way non-rebreathing valve (Hans Rudolph Inc.), which in turn was connected to an antiviral filter. This filter was directly used as a mouthpiece. The subjects were asked to inhale NO-free air via the mouth and immediately exhale via the same route, at various flow rates of air against various resistances resulting in similar oral pressure, for a period of 18 s at 0.5 l/min and 10 s at 3 and 9 l/min. The exhaled air passed through a linear pneumotachymeter (Hans Rudolph Inc.) after which resistors of 50, 500, and 1000–1200 cmH₂O/l/s (Hans Rudolph Inc.) were connected. Exhaled air was continuously sampled, from a point close to the antiviral filter, and fed into the NO analyzer at a flow rate of 0.1 l/min (77 AM; Eco Physics, Switzerland). The signal output from these devices were connected to a computer-based system (Aerocrine NO system, Aerocrine AB, Stockholm, Sweden) yielding instant on-screen display of flow for biofeedback and pressure, NO concentration and NO output of the exhaled air. The NO concentration showed a stable plateau after an initial peak in all measurements except for oral exhalation at 0.5 l/min, where the period of 18 s did not seem sufficient for a

plateau to develop in all subjects. In order to minimise nonenzymatically formed NO from contributing to the levels of orally exhaled NO (16), the mouth was first rinsed during 30 s using 20 ml of 10% sodium bicarbonate and then using 10 ml for 15 s before repeated measurement. For measurements of NO in nasally exhaled air, a tightly fitting mask covering the nose was mounted on the antiviral filter used for oral exhalations and the procedure was repeated likewise.

Measurement of NO in nasally aspirated air

Nasal air was aspirated at flow rates of 0.5, 3, and 9 l/min from one nostril using a nasal olive. The NO analyzer sampled air from close to the nasal olive at a flow rate of 0.1 l/min. During nasal measurements the subjects performed an oral single-breath exhalation as described above at an oral pressure of >6 cmH₂O in order to obtain closure of the soft palate.

L-NAME nasal spray

N^G-nitro-L-arginine-methyl ester-HCl (L-NAME; Alexis Corp.) was dissolved in 0.1 M phosphate buffer (pH 7.2) to a concentration of 100 mg/ml. Final pH was 6.6. Five sprays of L-NAME (5 mg/spray) were administered into each nostril, resulting in a total of 50 mg.

Sodium bicarbonate mouth wash

Sodium bicarbonate was dissolved in distilled water, at 50°C to avoid precipitation, to a concentration of 10%.

Statistics

The NO output (nl/min) was calculated for all sampling modalities. Nasal NO was investigated using two methods: (i) the subtraction method, where the level of NO in orally exhaled air after mouthwash is subtracted from the level found in nasally exhaled air (17, 18); and (ii) the method of nasal aspiration (4, 5). Lower airway NO was investigated as that in orally exhaled air after mouthwash (4, 5, 16).

Nonparametric statistics with two-sided *P*-values was used throughout the study. For the analysis of repeated measurements Friedman's test was used. For correlation analysis, Spearman rank correlation was used. For analysis of unpaired data Mann-Whitney *U* test was used. Each parameter used for statistics being the mean of two measurements at each situation. A *P*-value of less than 0.05 was considered significant. Results are given as mean ± SD unless otherwise noted. Statistical analyses and figures were made using Graph Pad Prism by Graph Pad Software Inc. (Graph Pad, San Diego, CA).

Results

All subjects completed the study. During all measurements ambient NO was <2 ppb. Patients presented a mean VAS symptom score before L-NAME of 3.1 (range 0–7), with one patient having no symptoms on the day of the study. This score was 3.6 (range 0–8.3) after L-NAME (nonsignificant). One subject experienced daytime somnolence 1–8 h following L-NAME administration nasally (19), otherwise no side effects were noted.

Table 1. NO output obtained through a nasal aspiration method, a nasal subtraction method, and oral exhalation in 18 controls and 18 patients with AR at flow rates of 0.5, 3, 9 l/min

	NO (nl/min), mean $\pm$ SD		Mann-Whitney <i>U</i> test, <i>P</i> -value
Flow (l/min)	Allergic rhinitis	Controls	
Nasal aspiration method			
0.5	160 $\pm$ 75	153 $\pm$ 36	1.0
3	211 $\pm$ 103	198 $\pm$ 45	0.9
9	271 $\pm$ 217	232 $\pm$ 62	0.9
Nasal subtraction method			
0.5	109 $\pm$ 75	97 $\pm$ 56	0.7
3	183 $\pm$ 133	218 $\pm$ 83	0.1
9	297 $\pm$ 149	301 $\pm$ 82	0.5
Oral exhalation			
0.5	27 $\pm$ 18	17 $\pm$ 9	0.06
3	113 $\pm$ 86	41 $\pm$ 19	0.002
9	140 $\pm$ 118	47 $\pm$ 26	0.003

Nasal NO

NO output increased in all subjects with higher flow rates ($P < 0.0001$, Friedman's test) (Table 1). There was no difference in NO output between AR and controls using the subtraction-method or in nasally aspirated air. However, the interindividual variation was greater in patients (Table 1 and Fig. 1.) L-NAME caused a decrease in nasal NO in both groups (Fig. 1). At the flow of 3 l/min using the aspiration method, mean nasal NO decreased from 198 ± 44 to 152 ± 39 nl/min in controls and from 211 ± 103 to 136 ± 77 nl/min in patients. The decrease following L-NAME was greater in patients compared to controls at all modalities. This difference between patients and controls was particularly pronounced at the highest flow rate, being significant both when looking at the absolute (100 ± 125 vs. 37 ± 30 nl/min, $P = 0.03$; Mann-Whitney *U* test) and relative decreases (0.31 ± 0.16 vs. 0.16 ± 0.13 , $P = 0.004$; Mann-Whitney *U* test; Fig. 2). There was no difference in nasal NO between patients who had previously experienced asthmatic reactions and those who had not.

A strong correlation between baseline nasal NO and the reduction following L-NAME was noted in patients (Spearman $r = 0.8$, $P = 0.002$) (Fig. 3A), but with only a tendency towards significance in controls (Spearman $r = 0.4$, $P = 0.07$, not shown). Thus, patients with the highest basal nasal NO levels decreased the most. NO aspirated nasally at 3 l/min did not correlate with symptom score before ($r = 0.22$, $P = 0.37$; Spearman rank correlation), or after L-NAME.

Orally exhaled NO

NO output increased in all subjects with higher flow rates ($P < 0.0001$, Friedman's test) (Table 1). Oral NO output was more than 2.5 times greater in AR than in controls at

3 and 9 l/min (both $P < 0.005$, Mann-Whitney *U* test) (Table 1 and Fig. 4). L-NAME caused a decrease in orally exhaled NO in both groups (Fig. 4). At the flow of 3 l/min, mean orally exhaled NO decreased from 41 ± 19 to 33 ± 18 nl/min in controls and from 113 ± 86 to 93 ± 79 nl/min in patients. There was no difference in orally exhaled NO between patients who had previously experienced asthmatic reactions and those who had not.

A significant correlation between baseline orally exhaled NO and the reduction following L-NAME was noted in patients (Spearman $r = 0.52$, $P = 0.03$) (Fig. 3B), but not in controls (Spearman $r = 0.3$, $P = 0.2$, not shown). Thus, patients with the highest basal orally exhaled NO levels decreased the most. NO exhaled orally at 3 l/min did not correlate with symptom score before ($r = 0.10$, $P = 0.69$; Spearman rank correlation) or after L-NAME.

Discussion

Earlier studies on nasal NO in allergic rhinitis have shown varying results. Some authors have reported higher nasal NO in patients with AR (6–8). Also, it has been shown that the elevated nasal NO in AR decreases following treatment with topical nasal steroids (7, 20). In contrast to these results, other groups have failed to find any differences in nasal NO output between AR and controls (9–11) or following treatment with topical nasal steroids (21). Again, in the present study there was no difference in nasal output between AR and controls. One could argue that the patients in this study had only moderately active disease and that this would explain the lack of difference between the groups. If, however, the degree of symptoms would determine nasal NO levels one would perhaps expect a correlation between nasal NO and symptom score, which was not the case neither in the present study, nor in another study investigating the relation between nasal NO and nitrate in nasal lavage and nasal symptoms (22). Moreover, orally exhaled NO was much higher indicating an ongoing inflammatory process in the ('united') airways, and there was a significant correlation between baseline nasal and orally exhaled NO and the reduction following L-NAME in the AR patients in our study (Fig. 2).

Despite the lack of difference in nasal NO between patients and controls, we did observe some interesting patterns that may shed some new light on this subject. The interindividual spread in nasal NO was greater in the patients. One way of interpreting this is that NO production in the nasal mucosa is generally increased but at the same time passage of NO from, for example, the sinus mucosa to the nasal cavity is impaired in some patients. This could be due to secretions or mucosal swelling partly occluding the sinus ostiae (23). If this is true, an increase in NO production in the nasal mucosa could easily be counteracted. The idea of a diffusion

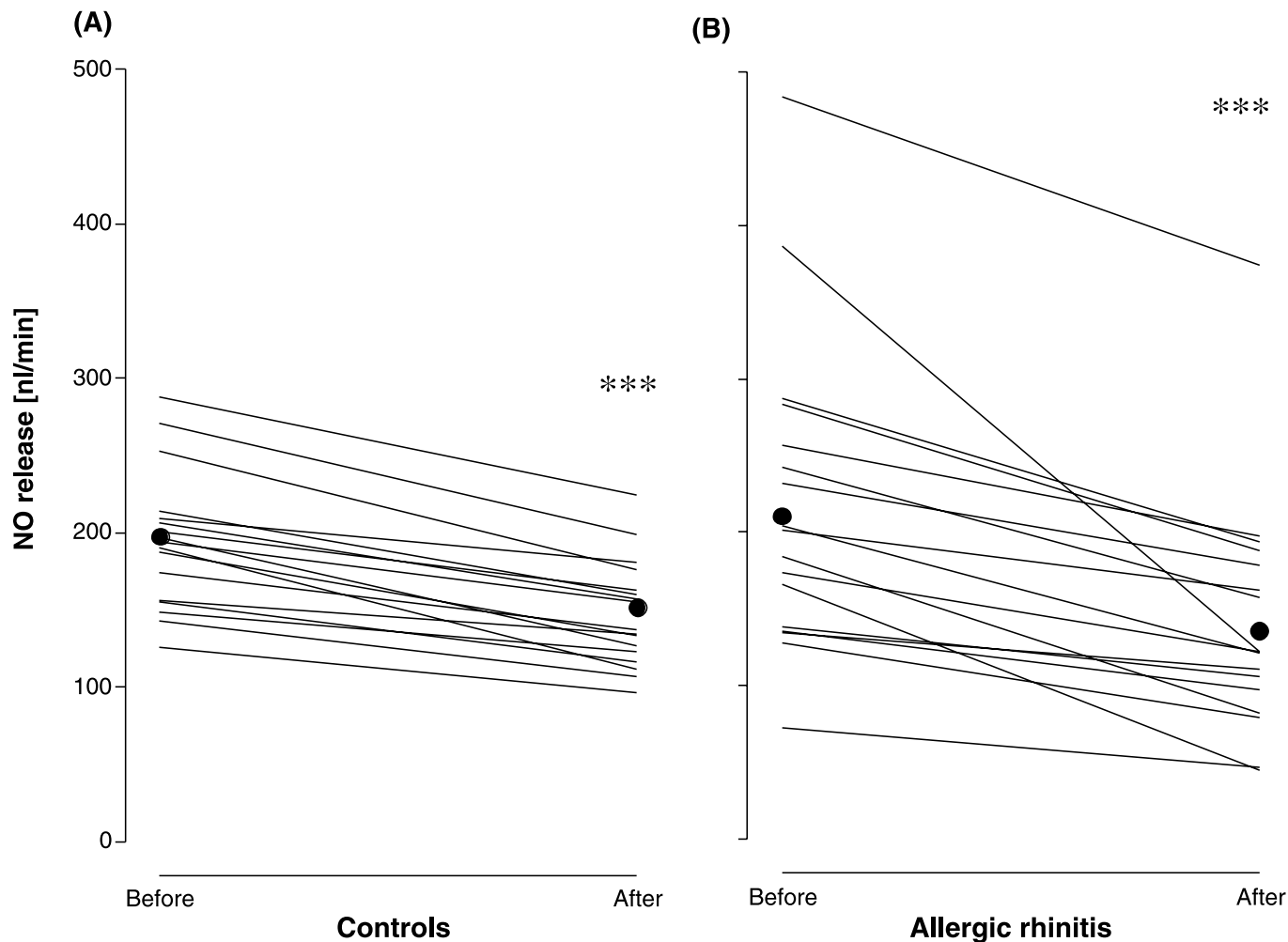


Figure 1. Nasal NO in 18 controls (A) and 18 patients with AR (B) before and after intranasal L-NAME. Air was aspirated nasally at 3 l/min. Closed circles represent means. There was no difference between patients and controls before or after L-NAME ($P = 0.9$ and $P = 0.2$, respectively; Mann–Whitney U test). The mean reduction after L-NAME was 26% in controls and 37% in patients ($***P < 0.001$; Wilcoxon signed rank test).

barrier for NO in AR patients is further supported by earlier studies looking at the effects of nasal decongestants on nasal NO. Nasal NO is decreased in healthy subjects by topical decongestants (24). However, when using the same drug in patients with symptomatic AR, nasal NO actually increases (6). Another finding in the present study was that nasal NO decreased more in the AR patients than in the controls following intranasal L-NAME. This indicates that NO release in the patients is in fact increased in those parts of the nasal mucosa that are reached by the drug. Altogether, nasal NO seems determined not only by induction of NO synthesis in the inflamed nasal mucosa but also by the passage of NO from a distant production site to the nasal cavity where it is sampled. This fact may explain the varying results obtained in different studies.

Generally, the NO output increased with the flow rate in all subjects at all sampling modalities as previously

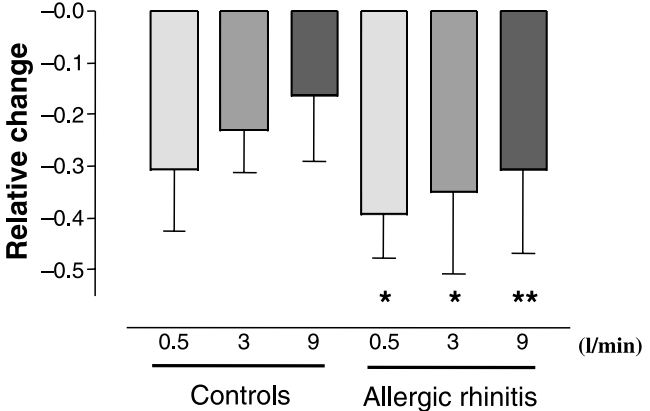


Figure 2. Relative change in nasal NO following intranasal L-NAME in 18 controls and 18 patients with AR. Air was aspirated nasally at 0.5, 3, and 9 l/min (* $P = 0.01$; ** $P = 0.004$; Mann–Whitney U test).

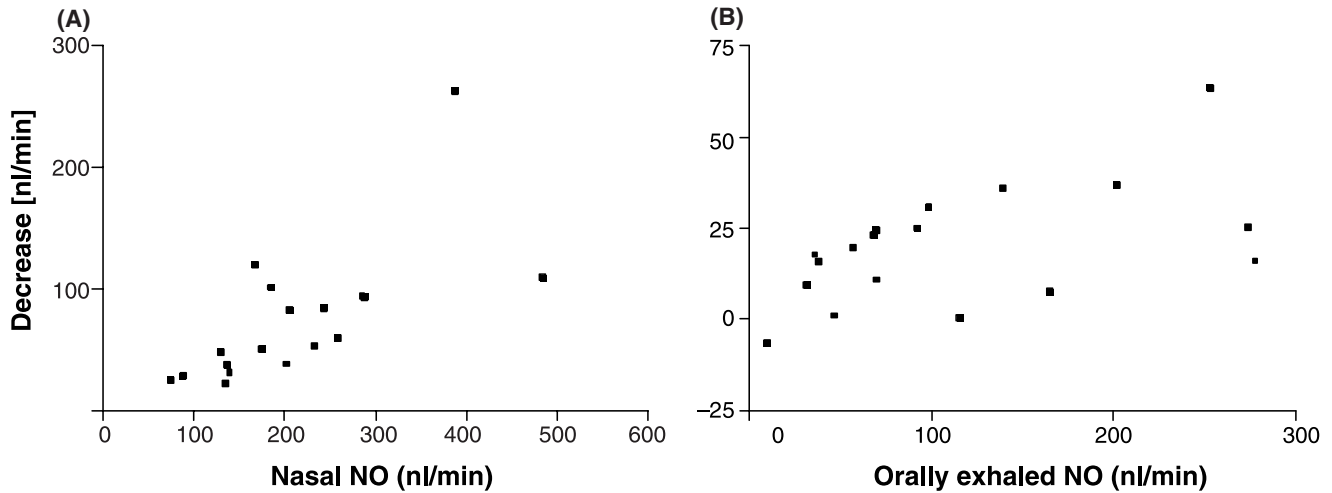


Figure 3. Correlation between baseline nasal NO (A) and orally exhaled NO (B) and the reduction following L-NAME in 18 patients with AR. Air was aspirated nasally or exhaled orally at 3 l/min ($r = 0.77$, $P = 0.002$ and $r = 0.52$, $P = 0.03$, respectively; Spearman rank correlation).

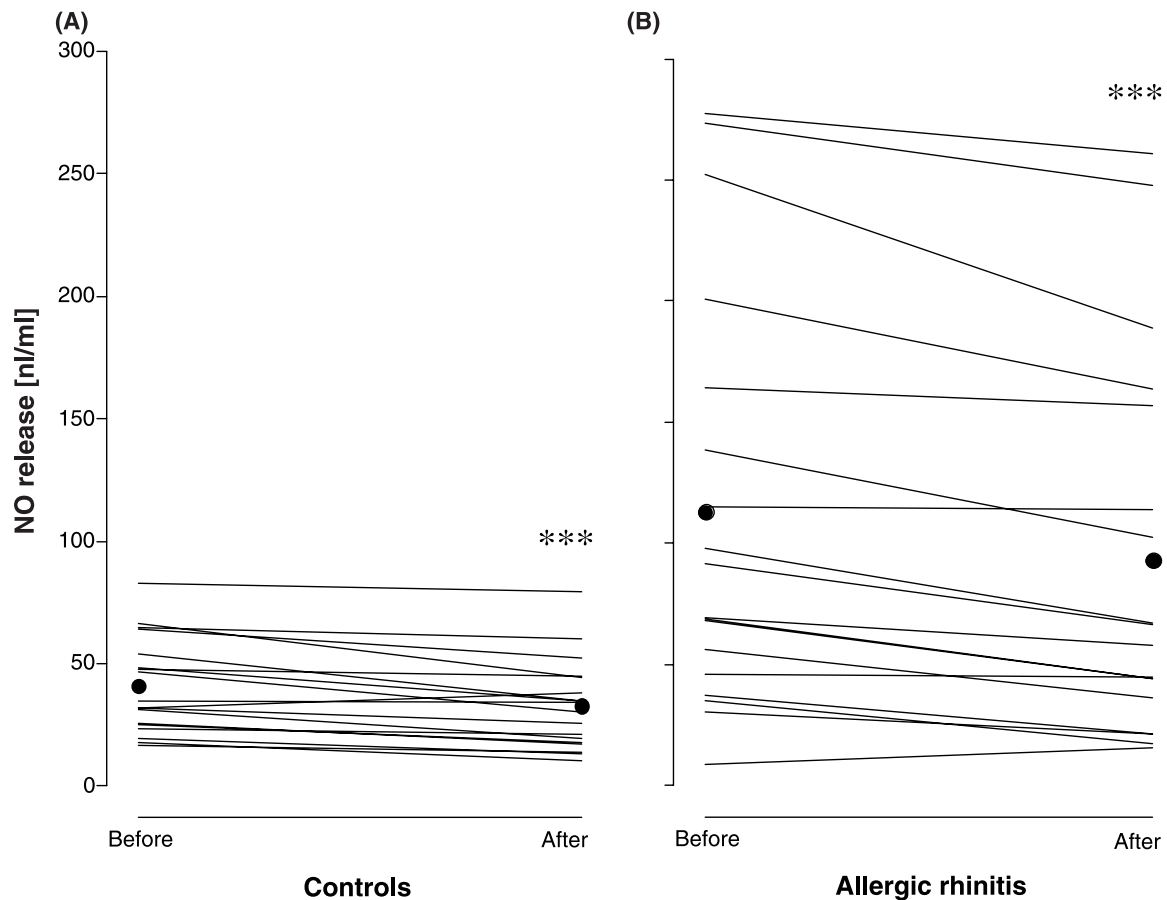


Figure 4. Orally exhaled NO in 18 controls (A) and 18 patients with AR (B) before and after intranasal L-NAME. Air was exhaled at 3 l/min. Closed circles represent means. Higher levels were found in patients compared to controls before and after L-NAME ($P = 0.002$ and $P = 0.006$, respectively; Mann–Whitney U test). The mean reduction after L-NAME was 20% in controls and 18% in patients ($***P < 0.001$; Wilcoxon signed rank test).

described (8). Concerning nasal NO, there could be at least two factors responsible for this. One being an increased concentration gradient between the mucosa and the lumen similar to what has been suggested for the lower airways (25). However, the nasal cavity may not be considered a single NO-producing compartment and an increased flow rate, causing higher turbulence, may therefore also result in recruitment of NO from distal parts of the nasal cavity. Perhaps these two mechanisms have different chances to act in normal and in inflamed nasal mucosa.

In both groups, a consistent decrease in nasal NO was seen after L-NAME treatment. However, despite the rather high dose (50 mg) of L-NAME used, this decrease was only 26–37% at 3 l/min. This is similar to what has been observed in earlier studies using similar doses (3, 11). As proposed earlier (3), this may be explained by minimal penetration of topically administered L-NAME into certain NO-producing compartments of the nose, for example, the sinuses. Indeed, when administering high doses of NOS inhibitors intravenously, nasal NO decreases approximately 65% (26) and when given directly into the sinuses, local NO decreased by 80% (3). Alternatively, the dose of L-NAME given was too low to produce a maximum inhibition of NO release. The reduction in nasal NO following L-NAME was greater in AR than in controls at all modalities, the difference being particularly marked at higher transnasal flow rates of air. This could perhaps indicate a higher concentration gradient having a positive influence on sensitivity of nasal NO measurements in AR in the methods used in this study. Intranasal L-NAME caused a decrease also in orally exhaled NO. This could have several explanations. The drug could be absorbed systemically to a certain degree. Alternatively, it is transported to NO-producing cells in the oro- and hypopharynx by mucociliary activity.

Interestingly, mean oral NO was much higher in the AR patients, despite the absence of symptoms suggestive of asthma. This phenomenon has been described earlier (10, 27–29) and may reflect an ongoing subclinical inflammation also in the lower airways in atopic individuals with AR. It is a well-known fact that AR is a major risk factor for asthma. Whether or not high oral NO in AR patients indicates a greater risk of developing asthma later in life is yet to be proven, however. Again, in the present study, there was no difference in orally exhaled NO between patients who had previously experienced asthmatic reactions and those who had not.

Exhaled NO has been proposed as a useful noninvasive marker of lower airway inflammation and specific guidelines have been developed for standardized measurements in the clinic (4, 5). The intention is to use exhaled NO as a diagnostic tool and to monitor disease activity. Whether or not nasal NO can be clinically useful in monitoring rhinitis is more questionable in

view of the present findings and earlier reports. One general problem with nasal NO measurements in AR is the high background levels of NO from constitutive sources in the nose and sinuses (3). A small increase in nasal NO output may therefore easily be blunted. Thus, we believe that NO measurements in their present form might be less useful in diseases associated with moderate alterations in nasal NO output such as rhinitis. Also, in order to discern a true variation of NO release from noise of measurements the alteration of total nasal NO level would according to one study have to be 20–25% (30). If nasal NO is increased in rhinitis patients, as shown in some studies (6–8), how large increase is then to be expected? If one assumes that NO production is upregulated to a similar degree in the nasal mucosa during rhinitis as in the lower airway mucosa in asthma, the following calculation can be made. The surface area of the nasal airways is somewhere around 100–200 cm² (31), which is similar to the estimated NO producing area in the central lower airways of healthy subjects (32). In this study, mean nasal NO output was more than 4.5 times higher than oral output in the controls (200 vs. 40 nl/min). In untreated asthma, lower airway NO output is increased about threefold (33). A similar absolute change in nasal NO output in AR would result in a total increase of only around 40% because of the much higher background levels. Even if these calculations and assumptions represent rough estimations they may help to explain why nasal NO cannot be expected to be greatly increased in rhinitis. On the other hand, in other airway disorders such as primary ciliary dyskinesia or cystic fibrosis where nasal NO is extremely low (9, 34), this test may give valuable information. If specific standardised techniques are developed where nasal NO can be measured from different compartments of the nose it may also be possible to better separate AR patients from controls. Concerning nasal NO, several techniques have been used but a satisfactory standard method is yet to be developed. It is likely that several possible ways of sampling is necessary depending on the situation. For example, measurements in infants and young children may require other techniques than those performed in adults. According to one study, the fixed flow nasal exhalation appeared to be the preferred method in adults (35). In this study, we used several sampling techniques for estimation of nasal NO output at three different flow rates; a passive aspiration method and an exhalation method (17) where nasal NO was estimated by subtracting orally exhaled levels after a mouthwash procedure from those obtained during a nasal exhalation. Nasal NO was similar in AR and controls in all methods used. Therefore, it is unlikely that the lack of difference is explained by the method.

We conclude that in this material orally exhaled NO, but not nasal NO, was increased in patients with AR during the pollen season. The interindividual spread in nasal and orally exhaled NO and the decrease following

topical L-NAME was greater in the patients. These results indicate that nasal NO is determined not only by induction of NO synthesis in the inflamed nasal mucosa, but also by the passage of NO from a production site distal to the lumen from where it is sampled. This fact may explain the varying results obtained in different studies looking at nasal NO in rhinitis.

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