

Review article

Nasal nitric oxide and nasal allergy

Measurements of nasal nitric oxide (nNO) are attractive because they are completely noninvasive and can easily be performed. The measurements may be useful in the early diagnosis of patients with chronic airway disorders such as Kartager's syndrome and cystic fibrosis. The possible use of nNO measurements in the diagnosis and treatment of allergic rhinitis still needs to be further evaluated because of the variable and also contradicting findings of nNO concentrations in this disease. In this review we will discuss the origin, production and measurement of nNO as well as the effect of allergic rhinitis, nasal allergen challenge and medication on nNO. Subsequently, we examine published data on allergic rhinitis and nNO, and summarize the effect of treatment of rhinitis on nNO. Finally, we discuss the potential future role for nNO in the diagnosis and management of allergic rhinitis.

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Nitric oxide (NO) was initially described as an endothelium-derived relaxing factor due to its action as a vasodilator (1, 2). We now know that NO has a regulatory role in a wide variety of cellular functions and tissues. Gustafsson et al. (3) first demonstrated that NO is present in exhaled air. Alving et al. (4) observed the presence of NO in the nasal cavity in even much higher concentrations compared with the lower airways. In addition, it was demonstrated that nasal NO (nNO) is affected by inflammation of the upper airways (5–8). These findings lead to the question whether nNO can be used in a clinical setting as a diagnostic tool of airway inflammation, or more specific of allergic rhinitis. This is mainly the result of the lack of consensus on measurement techniques, consequently leading to different findings of nNO concentrations in different airway illnesses as sinusitis, polyposis nasi and (allergic) rhinitis. Exceptions are primary ciliary dyskinesia (9) and cystic fibrosis (10, 11). It is well known that nNO levels in these diseases are quite low, independent of measurement method, and can be used as a noninvasive screening tool. In this review, we try to answer the question whether nNO is helpful in the diagnosis and management of patients with allergic rhinitis.

Origin of nasal NO

Nitrogen is unique among the elements in forming no fewer than seven molecular oxides, all thermodynamically unstable. Nitric oxide may react by electron gain to form nitroxyl anion (NO^-) and by electron loss to form NO^+ , the nitrosonium molecule (12). In biological systems, the mode and rate of NO metabolism is dependent on its own concentration, its diffusibility and the surrounding concentration of other bioreactants (13–15). The mode and rate of NO degeneration also varies in the gaseous and the aqueous phase; NO produced by airway epithelium is quickly metabolized in oxygenated aqueous environments by the binding of intravascular haemoglobin, leading to rapid removal of NO from the lungs without systemic side effects (12). *In vivo* NO is formed by several isoforms of the enzyme NO synthase (NOS), using the amino acid L-arginine and oxygen as a substrate (16, 17). Different isoforms of NOS exist (16). The neuronal (nNOS) and endothelial (eNOS) isoforms are constitutively expressed and are calcium dependent. Inducible NOS (iNOS) is calcium independent and is expressed in an inflammatory context in response to certain cytokines or bacterial lipopolysaccharides. Cells potentially capable of generating nitric oxides in the lungs include macrophages

(18, 19), neutrophils (20), vascular smooth cells, endothelial cells and pulmonary epithelial cells (2). On the other hand, the exact origin of nitric oxides in the nose is not exactly known. However, most studies indicate that the main production of nNO is within the mucosal epithelium of the paranasal sinuses (21–23).

Nasal NO: production in health and in allergic rhinitis

There are several studies assessing the difference in the expression of NOS in human nasal epithelial cells between normal subjects and patients with allergic rhinitis. Kulkarni et al. (24) localized immunoreactivity for nNOS in healthy humans in the olfactory mucosa, in olfactory receptor neurons, in numerous nerve fibres, in glands and in surface epithelial cells. Olthoff et al. (25) localized nNOS immunoreactivity in healthy persons and in patients with allergic rhinitis in the muscular layer of nasal vessels, in the basal portion of submucosal glands, in the periost and in the osteocytes of the inferior turbinate bones. They also found elevated nNOS immunoreactivity around glands in patients with allergic rhinitis. Takeno et al. (26) found that nasal epithelial cells of allergic patients overall produce higher levels of NO through concomitant expression of different isoforms (i and eNOS). Kawamoto et al. (27, 28) also found an increased expression of iNOS in epithelial cells of allergic patients. In accordance, others (29) have shown a higher iNOS expression in allergic patients as well, especially in the submucosal glands of the nose. The increased iNOS expression of epithelial cells in patients with allergic rhinitis may likely be explained by an increase in iNOS activity due to persistent mucosal inflammation (28). Moreover, Andersson et al. (30) demonstrated that an ostial occlusion as seen in upper airway allergy or infection, caused by mucosal swelling, would result in a slowly increasing negative pressure inside the sinus cavity. In parallel, the oxygen pressure in the sinus will decrease, resulting in hypoxia, which in turn, is a powerful inducer of NOS. The group concluded that reduction in sinus pressure therefore might result in an increase in nNO production. Another source of nNO in allergic rhinitis was shown by Hanazawa et al. (31). In an experiment, they showed a significant increase of nNO in patients with allergic rhinitis after the recruitment of eosinophils induced by eotaxin, which causes chemotaxis of eosinophils with a clinically symptomatic inflammatory response of the nasal mucosa. This effect is accompanied by an increase in nNO contributing to oxidative stress.

Nasal NO: measurement

The chemiluminescence technique for detecting NO in exhaled nasal air is currently the preferred method of measurement (6, 32). Measurement of nNO requires

generation of airflow through nasal cavities aspirating or insufflating air via one nostril while the velum is closed or in parallel, e.g. exhaling via one or both nostrils, aspirating via the mouth with air entrained into both nostrils during breath holding, or aspirating from one (Fig. 1) or both nostrils during breath holding. Closing of the pharynx by the velum is always required to block off oral air. This can be done in a variety of ways. One is, to exhale against a resistance, another is breath holding with the velum elevated or by voluntarily elevating the soft palate (6). With all these methods, constant transnasal flow produces a washout phase followed by the establishment of a steady NO plateau (Fig. 2). Nasal NO concentration is inversely related to the transnasal airflow (6, 33) and thus, the aerodynamics of the flow affect nNO. Besides nNO measurement during quiet exhalation, aspiration or during breath hold, nNO can also be measured while 'humming' (34–37). Humming is an exhalation while phonating the 'm'. In this case there is vibrating or oscillating exhalation flow through the nose. This leads initially to high NO peak levels gradually decreasing into a plateau in healthy subjects, in comparison with nNO measurement without humming (Fig. 3).

Nasal NO in healthy subjects and in patients with allergic rhinitis

Kharitonov et al. (38) compared nNO in 46 healthy subjects and in 72 subjects with seasonal allergic rhinitis, some of who had concomitant asthma (Fig. 4). Nasal NO was measured during the pollen season. Seasonal rhinitis was defined by a clinical history of seasonal allergic rhinitis for at least 2 years. However, skin prick tests were performed and not all allergic subjects were sensitized. Nitric oxide was measured in one nostril during breath



Figure 1. Example of nasal nitric oxide measurement by aspirating air from one nostril during breath hold.

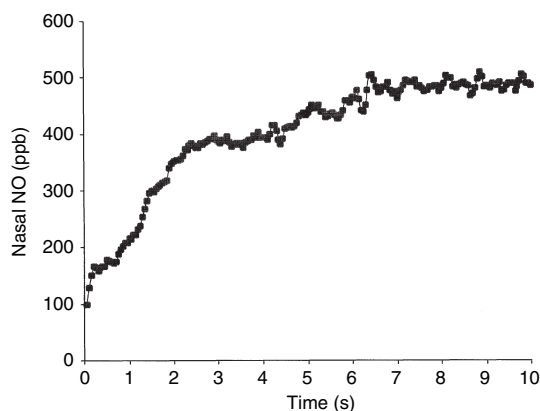


Figure 2. Example of on-line nasal nitric oxide measurement during 10 s of breath hold.

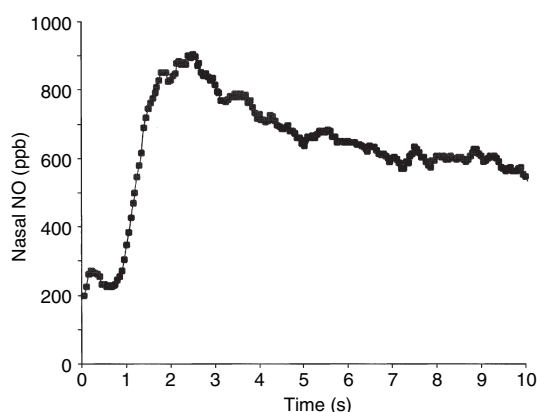


Figure 3. Example of an on-line measurement in a healthy subject during 10 s of humming as loud as possible with the mouth closed.

hold with a 250 ml/min flow. Untreated rhinitis patients with and without asthma had significantly 1.5-fold higher nNO concentrations compared with healthy controls ($n = 19$). With the same method of measurement, Djupesland et al. (33) found, in a study on the aerodynamic influences on nNO output measurements, substantially higher NO outputs in symptomatic seasonal allergic patients ($n = 5$) compared with healthy controls ($n = 8$) at different aspiration flows (0.2–3.7 l/min). As opposed to Kharitonov and Djupesland, Henriksen et al. (39) found no significant difference in nNO levels (aspiration method) in the pollen season between controls ($n = 12$) and allergic rhinitis subjects ($n = 46$; Fig. 4). There was also no difference between only seasonal ($n = 19$) and both seasonal and perennial sensitization ($n = 27$). Nasal NO was also measured in a smaller group during the pollen season; all subjects were symptomatic and on treatment with antihistamines. They found an increase in nNO, but this was not statistically significant. When

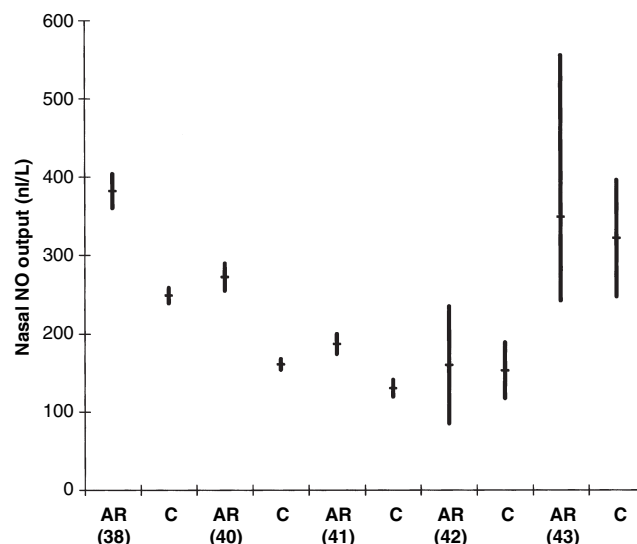


Figure 4. Mean nasal nitric oxide output (with range) in allergic rhinitis (AR) vs healthy controls (C) reported by different authors (38, 40–43).

comparing baseline nNO concentrations between allergic patients and controls in intervention studies we found that Arnal et al. (40) found significantly higher nNO concentrations in patients with perennial and/or seasonal rhinitis ($n = 36$) with and without active symptoms compared with healthy controls ($n = 19$). Nasal NO was measured from each nostril during breath hold with an aspiration flow of 0.7 l/min. Another group (41) measured nNO in 21 children with perennial rhinitis out of therapy for at least 3 weeks. Nasal NO was measured during aspiration flow of 700 ml/min from one nostril during 25-s breath hold, which seems quite long for children. Basal mean NO concentrations in the nose were significantly higher in the affected children compared with the healthy controls ($n = 21$), 267 ppb (± 18) vs 186 (± 15) respectively. There was no significant correlation between nasal symptoms and nNO values. In contrast (39, 42, 43) two studies showed that there are no differences in nNO concentrations at baseline in allergic patients compared with healthy controls. Both studies used the same measurement method (aspiration during breath hold), but employed different aspiration flows.

Of interest is the report by Moody et al. (44). They analysed the association of nNO and skin prick reactivity to house dust mite. Sixty-four nonasthmatic Pacific Islanders were skin prick tested for eight common allergens: 56.3% were positive for house dust mite, 25% for cockroach and 12.5% for grass pollen. The sensitivity of house dust mite reactivity was independently associated with higher nNO concentrations. There was no relation between nNO and a positive skin prick tests for other allergens.

'Humming' during nNO measurement initially increases nNO levels considerably in healthy subjects (34, 35). This increase is abolished in patients with an obstructed osteomeatal complex including those with allergic rhinitis, nasal polyps and sinusitis. The postulated hypothesis is that humming causes a rapid washout of NO from the sinuses. Indeed, such a mechanism was confirmed by a model study (36). One study indicated that absence of an nNO peak during humming is associated with endoscopic findings suggestive of sinus ostial obstruction in patients with allergic rhinitis (45).

Nasal NO and nasal allergen challenge

There are two studies on the effect of allergy challenge with grass pollen on nNO. Kharitonov et al. (38) studied five seasonal allergic rhinitis patients outside the pollen season. One hour after the start of the challenge when symptoms were at a peak, there was a maximal decrease in nNO concentration. Nasal NO concentrations were back at baseline after 4 h. Another study challenged nine seasonal allergic patients outside the pollen season (46). Nasal challenge was done with increasing concentrations of grass-pollen extract in each nostril. The challenge did not modify the nNO levels. There is no information on nasal symptoms. Both the studies measured nNO from one nostril, but with different flows (250 and 700 ml/min). The difference in the effect of the challenge on the nNO concentration might depend on the administered concentration of allergen extract. In Kharitonov's study, it can be assumed that administering a high dose of grass-pollen extract at once will have a larger potential leading to blockage of sinuses due to mucosal swelling and thus influencing nNO compared with administering a lower total allergen dose in several steps in Maniscalco's study. The differences in allergen administration might also influence these findings.

Nasal NO and medication in allergic rhinitis

Palm et al. (42) and Maniscalco et al. (43) explored the effect of topical administration of L-NAME, an NOS inhibitor, in subjects with allergic rhinitis. As the expression of NOS in allergic patients is increased (25–29) it seems fair to expect that L-NAME will reduce nNO. The results showed a decrease of nNO in the nose of both allergic patients ($n = 18$) and healthy controls ($n = 18$). The decrease following the L-NAME was larger in patients with the highest 'base line' nNO levels and higher in all patients compared with controls.

However, the dose of L-NAME (50 mg) was rather high and only resulted in about 26–37% decrease in nNO. These findings are similar to what has been observed in two other studies using similar doses (22, 43). Arnal et al.

(40) compared nNO in allergic subjects before and after administration of a nasal vasoconstrictor. Some subjects with pollen allergy were measured during the pollen season, whereas other patients were tested after the pollen season. Allergic patients without symptoms had higher mean nNO levels than patients with symptoms. Accordingly, there was an inverse correlation between mean nNO and symptom score. After inhalation of a vasoconstrictor, nNO increased in allergic patients with symptoms, but decreased in the group without symptoms leading to similar high NO levels after vasoconstriction in both the patient groups. This can be explained on the basis of sinus obstruction, as the decongestant in the symptomatic patients resulted in an increase in nNO. There was no correlation between nNO and symptom score after nasal vasoconstriction. In healthy controls, nNO decreased with about 25% after a nasal vasoconstrictor. The hypothesis that sinus obstruction results in lower nNO values is supported by the study from Colantonio et al. (47). They explored nNO values in allergic patients with and without nasal polyps. Nasal NO values were significantly lower in patients with polyps and the NO levels correlated positively with the extent of the polyposis. Arnal et al. (5) also found lower nNO levels in subjects with nasal polyposis, but this was only in case of nonallergic polyposis. Healthy controls and subjects with allergic polyposis had similar nNO values. Nasal NO concentration in untreated nonallergic polyposis (no steroids) was significantly higher in untreated allergic polyposis. Humming nNO values in a study by Maniscalco et al. (35) showed that posthumming nNO output was lower in healthy subjects, and subjects with allergic rhinitis than in subjects with allergic nasal polyposis. The described findings suggest that nasal allergy results in higher nNO levels independent of nasal obstruction or ostium obstruction. However, this is not confirmed by nasal allergen challenge (see above). Antihistamines in allergic rhinitis patients (41, 48) do not affect nNO in subjects with perennial or seasonal allergic rhinitis. Similarly, leukotriene antagonists did not influence nNO (49) in seasonal allergic rhinitis. On the contrary, there are some studies that showed that topical administrations of nasal steroids do affect nNO (38, 41, 49, 50) by decreasing the nNO concentration. This might be the result of downregulation of the transcription of iNOS (51).

Conclusion

Nasal NO concentration seems to be elevated in allergic rhinitis in some studies, but is similar in others in comparison with healthy controls and seems to be modified by corticosteroids. Hence, there is no unambiguous answer to the question whether nNO reflects allergic rhinitis and whether there is a future role for nNO in the diagnosis of this disease. It is surprising to us that there are no clear

reports on possible apparent difficulties when measuring nNO during (allergic) rhinitis. After all, if there is a certain amount of retained mucous and/or mucosal swelling in the nose and thus the accessibility is limited, measurement of nNO with the aspiration technique will result in a vacuum, and thus impaired measurements. In summary, we are of opinion that collective standardization of nNO measurement and further studies in well-defined populations are imperative to know whether there is a future role for nNO in the diagnosis and management of patients with allergic rhinitis.

Summary of main findings

- The main production of nNO is the mucosa of paranasal sinuses.
- Expression of NOS is increased in allergic rhinitis.
- There is no consensus on how to measure nNO.
- Season apparently does not influence nNO.
- The nNO concentration seems to be elevated in allergic rhinitis, but this is still not convincingly confirmed.

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