

Exhaled nitric oxide: relation to sensitization and respiratory symptoms

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Summary

Background Conflicting data have been presented as to whether nitric oxide (NO) in exhaled air is merely reflecting atopy rather than airway inflammation.

Objective To investigate the relationship between exhaled NO (eNO) and nasal NO (nNO), respiratory symptoms, and atopy, in the context of a cross-sectional study of the respiratory health of bleachery workers.

Methods Two hundred and forty-six non-smoking bleachery and paper-mill workers answered a questionnaire and were examined by measurements of eNO and nNO and spirometry, outside the pollen season. Blood samples were collected and analysed for specific IgE against common aeroallergens (birch, timothy, cat and house dust mite). Atopy was defined as a positive Phadiatop™ test.

Results The atopic and the non-atopic subjects without asthma or rhinitis had similar levels of eNO. Subjects reporting asthma or rhinitis who were also sensitized to perennial allergens had higher levels of eNO, whereas those sensitized to only seasonal allergens had similar eNO levels as non-atopic subjects with asthma or rhinitis. In multiple linear regression models adjusted for nNO, eNO was associated with asthma and sensitization to perennial allergens.

Conclusion The results indicate that only atopic subjects who have recently been exposed to the relevant allergen have elevated levels of eNO. Atopic subjects who are not being exposed to a relevant allergen or have never experienced symptoms of asthma or rhinitis show normal eNO. These data indicate that eNO relates to airway inflammation in atopic subjects.

Keywords asthma, atopy, breath analysis, exhaled nitric oxide, nasal nitric oxide, rhinitis, sensitization

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Introduction

Exhaled nitric oxide (eNO) is low in the exhaled breath of healthy subjects. The fact that eNO is greater in subjects with atopic asthma, but decreases after inhaled steroid therapy, suggests that NO could be a valuable marker for airway inflammation [1, 2]. eNO has also been shown to correlate with asthma control in adults [3] and asthma severity in children [4, 5]. eNO is also correlated with eosinophilia in sputum [6] but to a lesser extent with eosinophils in bronchial biopsies [7]. Lately, however van der Toorn et al. [8] and Payne et al. [9] have presented a significant correlation between major basic protein, derived from activated eosinophils, in bronchial biopsies and eNO.

Although the knowledge of nasal NO (nNO) is still limited, it has been suggested that increased nNO levels could be a sign of nasal inflammation, since they have been shown to be higher in patients with allergic rhinitis (AR) [10–12] and

during infections of the upper respiratory tract [13]. The increased nNO levels found in patients with AR are generally believed to originate mainly from the nasal mucosa [14], despite a very high production in the paranasal sinuses [15].

Whether eNO reflects atopy rather than airway inflammation is a matter needing investigation. Horvath et al. [16] found elevated levels of eNO among 15 asymptomatic atopic subjects as compared with non-atopic controls. Salome et al. [17] found higher eNO in atopic subjects without airway hyper-responsiveness (AHR) than in non-atopic subjects without AHR, although the method of measuring eNO used in that study did not control for exhalation flow rate, thus making the results less reliable. Ho et al. [18] have described positive associations between eNO and total serum IgE ($r = 0.43$), between eNO and an increasing number of positive skin-prick tests and also the maximum diameter of each skin prick ($r = 0.73$). These data have been interpreted as evidence that eNO merely reflects atopy *per se*, rather than airway inflammation. A positive association between eNO and total IgE ($r = 0.44$) has also been noted by Simpson et al. [19]. In that study, eNO levels were found to be higher in subjects who had been sensitized as well as being exposed to relevant

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allergen than in those who had been sensitized but were not being currently exposed. This may indicate that in sensitized subjects, the exposure is giving rise to airway inflammation and increased eNO. Moreover Moody et al. [20] have found increased levels of eNO in asymptomatic Pacific Islanders sensitized to house-dust mite (HDM). They suggest that the increase in the levels of eNO denotes subclinical airway inflammation, due to persistent exposure to mites.

A non-invasive marker of airway inflammation such as eNO has a potential for adding useful information to respiratory questionnaires and spirometry in studies of large populations. A major limitation of questionnaires is that they are liable to bias, and spirometry is not sensitive enough to identify early events in the respiratory tract. So far NO in exhaled air has only been used in a few population-based epidemiological studies among adults [17, 21] and in surveillance studies of groups exposed occupationally [22–26] to detect inflammation of the airways among adults.

If eNO is to be a useful marker of airway inflammation in epidemiological studies, it is important that it should add to the information obtained from questionnaires and spirometry. Further it should relate to airway inflammation rather than to atopy. The ideal marker should show an increase in the case of asthma in both atopic and non-atopic subjects.

The aim of the present study has been to investigate how NO in exhaled and nasal air can be associated with asthma and rhinitis among atopic and non-atopic subjects.

Materials and methods

The study was carried out in the context of a longitudinal study of the respiratory health effects of bleaching agents [25]. All process workers ($n = 261$) in the bleaching departments of three pulp mills and all the process workers ($n = 75$) from two adjacent paper mills were invited to participate in the study. The pulp mills had been using chlorine/chlorine dioxide as bleaching agents since the 1950s, replacing them by ozone between 1992 and 1993. The bleachery workers had, in varying extent, been exposed to high peaks of bleaching agents during a period of years, while those working in the paper mill had been exposed to low levels of paper dust. The results concerning exposure and eNO are presented elsewhere [26].

In this study, we present data on the relation between nitric oxide in exhaled and nasal air and atopy, sensitization, lung function, and respiratory symptoms. The study was performed outside the pollen season between October 1998 and March 1999. Of all the invited subjects ($n = 336$), 39 declined to participate or had an infection of the upper respiratory tract at the time for examination and hence were excluded. Fifteen subjects were unable to perform acceptable measurements of eNO and consequently were also excluded. Nine subjects were either unable to carry out measurements of nNO or declined to participate, and were, therefore, excluded in the presentation from nNO, but not in the other analyses. Because smoking is known to markedly decrease the levels of eNO [27], and knowledge is lacking as to whether NO in exhaled air can be used as a marker of inflammation in the case of smokers, the data presented have been confined to subjects who were either ex-smokers ($n = 98$) or never

smokers ($n = 148$). Altogether 246 subjects were included in the final analyses.

All were asked to refrain from nitrate-rich food for 4 h prior to the NO measurements and kept fasting for 1 h before the measurements took place [28]. All subjects who had reported upper respiratory infection within the previous 3 weeks were, when possible, examined later.

All subjects answered a questionnaire with items similar to those in previous ones [29, 30]. Altogether 49 subjects could be defined as having asthma on account of affirmative answers to 'physician-diagnosed asthma' ($n = 22$) and/or current asthma (wheezing, shortness of breath, or nocturnal dyspnoea within the previous 12 months) ($n = 36$). From the 49 subjects classified as having asthma, 22 subjects also had rhinitis. Twenty-seven subjects (exclusive of those with asthma) were defined as having rhinitis on the basis of affirmative answers to two questions: 'Have you, after the age of 15 years, ever had nasal symptoms such as nasal stuffing and/or sneezing without having a cold?' and 'Have these symptoms had a duration of more than one month?' [31, 32].

eNO was measured during a slow single exhalation against an oral pressure of 5 cmH₂O at an exhalation flow-rate of 47.5–52.5 mL/s, in accordance with American Thoracic Society recommendations [33]. The mean concentration (ppb) during the plateau phase was registered. The mean NO concentration that is presented is from three measurements, within 10% deviation from the lowest value.

nNO was measured when the analyser was sampling nasal air from one nostril at a constant sample rate of 10 mL/s, while the subject was holding his/her breath for 20 s and closing the velum voluntarily. A stable plateau of NO level on the on-line computer recording confirmed the closure of the velum. The nNO concentration during the plateau phase was registered. One measurement was performed in each nostril and the mean NO concentration calculated.

A chemiluminescence analyser was used to measure nitric oxide (Ecophysics Breath Analyzer CLD 77 AL, Switzerland). A special system was used for regulating the exhalation flow (Exhalation Breath AnalyzerTM, Aerocrine AB, Stockholm, Sweden). A two-point calibration of the analyser was carried out each day with a certified NO calibration gas (AGA-gas, Stockholm, Sweden). The variability of the method has been examined on 25 healthy subjects on four different occasions 4 weeks apart. The intra-individual coefficient of variation was 9.2% (Andersson, unpublished data).

All subjects underwent spirometry with a dry-wedge spirometer (Vitalograph[®], Bucks, UK). They went through at least three technically acceptable trials and the highest values for vital capacity (VC), forced vital capacity (FVC) and the forced expiratory volume in 1 s (FEV₁) were registered and compared with predicted values [34].

Blood samples were taken from all subjects, and serum was stored at -20°C . Atopy was defined as a positive PhadiatopTM (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) [35], with class 0 being regarded as non-atopic and class 1 as atopic. Specific IgE to birch, timothy grass, HDM (*Dermatophagoides pteronyssinus* and *D. farinae*) and cat allergen were measured by the Pharmacia CAP system (Pharmacia & Upjohn Diagnostics). Specific IgE levels equal to or greater than 0.35 kU/L were considered positive.

Table 1. Descriptive data on subjects

	No rhinitis or asthma	Rhinitis	Asthma
Subjects, <i>n</i>	170	27	49
Age (years)*	44.5 ± 0.69	41.5 ± 1.51	45.0 ± 1.07
Female/male, <i>n</i>	11/159	2/25	1/48
Ex-smokers, <i>n</i> (%)	65 (38)	12 (44)	21 (43)
Never smokers, <i>n</i> (%)	105 (62)	15 (56)	28 (57)
Atopy, <i>n</i> (%)	33 (19)	10 (37)	24 (49)
VC (% pred)	100.2	100.9	97.5
FEV ₁ (% pred)	105.3	104.3	99.3

VC, vital capacity; pred, predicted; FEV₁, forced expiratory volume in 1 s.

*Mean ± standard error of the mean.

The local committee of ethics approved the study and informed consent was obtained from all volunteers prior to their being included in the study.

Statistical methods

Statistical analyses were carried out with SAS, version 6.12 (SAS Statistical package, Cary, NC, USA). Mean values for the basic data are presented in Table 1. Because the eNO and nNO concentrations were not distributed normally, the results that have been given are those for the median and inter-quartile range of eNO and nNO concentrations (ppb) (Table 2). Significance testings have been based on non-parametric tests such as the Kruskal–Wallis and the Wilcoxon signed-rank test. The associations between the log-transformed values of eNO as the dependent variable and different explanatory (independent) variables were tested in multiple linear regression models using PROC GLM. Independent categorical variables (yes/no) were asthma, rhinitis, atopy, sex, and occupational exposure (heavy gasings to ozone).

Results

Descriptive data on the subjects are presented in Table 1. As may be seen from Table 2, the eNO levels were higher in subjects with asthma than in subjects with no asthma (22.5 vs. 15.8 p.p.b., $P = 0.0004$). In a multiple linear regression model, using ln eNO as the dependent variable, adjusted for atopy, nNO, sex, smoking and occupational exposure, asthma was

Table 2. Median exhaled NO (interquartile range) (p.p.b.), and respiratory disease among non-atopic and atopic subjects

	All (<i>n</i> = 246)	No atopy (<i>n</i> = 179)	Atopy (<i>n</i> = 67)
Without rhinitis or asthma (<i>n</i> = 170)	15.8 (11.4–22.4)	15.8 (11.9–21.4)	16.5 (11.3–27.6)
Rhinitis only (<i>n</i> = 27)	20.6 (14.1–30.8)	19.2 (14.1–22.0)	31.0* (16.0–50.5)
Asthma (<i>n</i> = 49)	22.5*** (16.0–31.2)	18.8 (14.6–26.5)	29.8** (18.2–47.8)

The tests of significance are based on Kruskal–Wallis test. NO, nitric oxide. * $P = 0.06$, ** $P = 0.004$; atopic subjects without rhinitis/asthma vs. atopic subjects with rhinitis/asthma. *** $P = 0.004$; asthma vs. no asthma or rhinitis.

independently associated with eNO (estimate 0.47, $P = 0.002$).

When the subjects were divided according to atopic status, only the atopic ones with asthma were found to have significantly higher eNO, see Table 2. The atopic and the non-atopic subjects without asthma or rhinitis had similar levels of eNO. The atopic subjects reporting rhinitis (but not asthma) had higher eNO with only borderline significance. The relations between the median eNO among atopic and non-atopic subjects reporting no asthma or rhinitis, or rhinitis and/or asthma, are shown in Fig. 1. The data were analysed further in multiple linear regression models, dividing the subjects into atopic and non-atopic subjects. In these models, asthma was found to be independently associated with eNO among the atopic subjects (estimate 0.96, $P = 0.01$) but not among the non-atopics.

The results regarding sensitization were also analysed in a multiple linear regression model with ln eNO as the dependent variable, see Table 3. Sensitization to perennial allergens and asthma were the two most important factors determining the eNO levels.

The atopic subjects sensitized only to seasonal allergens, who were reporting rhinitis and/or asthma, had levels of eNO within the range of non-atopic subjects, with the measurements taking place outside the pollen season. The subjects with rhinitis or asthma who were sensitized to cat allergen or HDM had higher eNO than non-atopic subjects with rhinitis or asthma (44.2 vs. 19.2 ppb, $P = 0.001$ and 34.4 vs. 18.8 ppb, $P = 0.02$).

The subjects sensitized to cat allergen but without asthma or rhinitis had eNO levels similar to those of non-atopic subjects (20.8 vs. 15.8 ppb, $P = 0.2$). Those being sensitized to HDM but without respiratory symptoms had elevated levels of eNO, as compared with non-atopic subjects without asthma and rhinitis (22.6 vs. 15.8 ppb, $P = 0.043$).

Ten of the 49 subjects with asthma reported using inhaled glucocorticoids. Their median (inter-quartile range) NO level was 27.9 (23.6–38.5) ppb compared with 20.0 (14.6–31.1) ppb for asthmatics not reporting medication with inhaled steroids.

We did not find any correlation between eNO and FEV₁ or nNO and FEV₁.

nNO was not associated with nasal symptoms or with atopy.

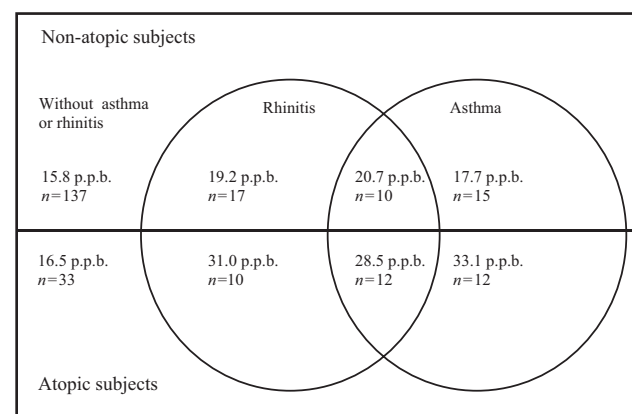
**Fig. 1.** Median exhaled nitric oxide among non-atopic and atopic subjects with no symptoms of asthma or rhinitis and such subjects reporting rhinitis and/or asthma.

Table 3. Variables included in a multiple linear regression model with ln eNO as the dependent variable adjusted for rhinitis, occupational exposure and nasal NO

Variable	Estimate	SEM	P-value
Intercept	2.4	0.12	
Asthma	0.21	0.09	0.019
Sensitization to perennial allergens	0.43	0.14	0.003
Sensitization to seasonal allergens	0.10	0.12	0.41

eNO, exhaled nitric oxide; SEM, standard error of the mean.

Discussion

We found no difference in eNO levels between atopic and non-atopic subjects with no reported asthma or rhinitis. Atopic subjects with asthma or rhinitis had higher eNO levels than those without atopy. Among the atopic subjects reporting rhinitis or asthma, those sensitized to cat allergen or HDM had higher eNO, whereas those sensitized to seasonal allergens had normal eNO. This indicates that the eNO levels are associated with airway inflammation in atopic subjects who have been sensitized and possibly exposed to a particular allergen.

Thus, there is reason to believe that eNO reflects the current status of inflammation in the airways. As regards the extent to which airway inflammation and eNO vary over time, knowledge is very limited. In this study, we have compared the measurements of eNO in one occasion with answers to questions such as 'Has a doctor diagnosed you as having asthma? If 'yes', in what year?' or 'Did you in the last twelve months have symptoms of asthma?' or 'Have you after the age of 15 years ever had symptoms of rhinitis for more than one month?' Consequently, this implies that among the subjects we have been classified as having asthma or rhinitis are also those who have either improved or are in full remission of disease. Although this kind of misclassification will tend to decrease the differences between those reporting the disease and those who did not, we nevertheless found a significant increase in eNO among subjects answering in the affirmative.

Whereas subjects with positive PhadiatopTM and no symptoms of asthma or rhinitis, as a group, had eNO levels similar to those with a negative PhadiatopTM, there were two subjects sensitized to HDM and one subject sensitized to cat allergen with eNO levels similar or in excess of 39.7 p.p.b. (90th percentile of eNO), without respiratory symptoms. The significance of the elevated eNO in these subjects is unclear but may indicate that these subjects have subclinical airway inflammation. The presence of airway inflammation in atopic subjects without clinical asthma has been demonstrated by Djukanovic [36].

Whereas our finding that adult atopic subjects without symptoms of rhinitis or asthma, as a group, have normal eNO is in accordance with a previous study by Gratziau et al. [37], it contradicts data from Horvath et al. [16]. The differences between the results could maybe be explained by the selection of subjects. The study of Horvath et al. was of limited size, having included only 15 asymptomatic atopic subjects, and a few of them possibly could have had subclinical airway inflammation and thereby, to a high degree, influenced the results. Accordingly, Moody et al.

[19] found increased levels of eNO, among non-symptomatic Pacific Islanders sensitized to HDM, and conclude that they might have subclinical airway inflammation.

We found that atopic subjects who were both sensitized to cat or HDM and were reporting asthma had increased NO levels, whereas those sensitized to seasonal allergens had normal eNO. The measurements were done outside the pollen season. These results were also confirmed in multiple linear regression models. These models were adjusted for nNO, which was also significantly associated with eNO.

The results further support the idea of eNO as a sensitive marker for airway inflammation, in atopic subjects, since it seems that exposure to the relevant allergen can, in sensitized subjects, be associated with airway inflammation. Our results are in agreement with two other studies [9, 38], where the highest eNO levels were found in subjects sensitized to cat and presenting symptoms of rhinitis and asthma. Conversely, Gratziau et al. [39] recently presented a study where asymptomatic atopic subjects with seasonal rhinitis ($n = 9$) had elevated levels of eNO also outside the pollen season, as compared with non-atopic controls. The highest eNO levels were those found in subjects with symptoms of seasonal rhinitis and concomitant hyper-responsiveness to methacholine. Again, one assumption could be that they had asymptomatic airway inflammation.

There are previous studies [40] showing bronchial inflammation among subjects with AR. We have also shown that rhinitis increases the risk of developing asthma [41]. One might speculate that eNO could help the identification of subjects who are at risk.

In non-atopic subjects with asthma we found levels of eNO similar to those among non-asthmatic subjects. This is in agreement with a study by Henriksen et al. [21] who did not find any increase of eNO in 35 subjects with suspected non-atopic asthma, and one by Ludvigsdottir et al. [42] who found similar levels of eNO in 20 subjects with non-allergic asthma and 13 healthy control subjects.

This study was originally designed to investigate health effects among bleachery workers exposed to irritant gases. The subjects were, therefore, workers who had been exposed to ozone or to paper dust, not a random population sample. This introduces a selection bias (towards healthy workers), which reduces the external validity, since the subjects were probably selected from a healthier part of the population. One might suspect that the number of subjects with severe asthma or rhino-conjunctivitis was less than in a population sample. The prevalence of atopy, which was 26% both among workers exposed to ozone and among the controls, is lower than reported from population samples in Sweden [43]. As a group the exposed subjects had, however, normal VC and FEV₁ in comparison with reference values.

We have previously shown that workers exposed to ozone have a higher prevalence of respiratory symptoms than healthy controls, and that high exposure to ozone increases eNO, and this might also bias our results. Analysis of the data from which the most exposed workers had been excluded gave similar results, although with lower degrees of significance. Because our aim was to study the relation between atopy and eNO, it is difficult to see how these kinds of selection bias could influence or distort our results. There were very few women among the subjects, and none with

atopy and asthma; hence, we can only claim that the results are valid for men.

To conclude, the results indicate that only atopic subjects who have recently been exposed to the relevant allergen have elevated levels of eNO. Atopic subjects who are not being exposed to a relevant allergen or have never experienced symptoms of asthma or rhinitis show normal eNO. These data indicate that eNO relates to airway inflammation in atopic subjects. The results support the view that NO in exhaled air can be of interest for epidemiological studies of asthma and rhinitis. The method has several advantages over present methods, among them being quick and easy to perform. It seems to be useful in studies of atopic asthma, but whether it is a useful measure of inflammation in non-atopic asthma needs to be further evaluated.

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