

III. Physiological Background

3.1. Exhaled NO: Relationships with Eosinophilic Inflammation, Lung Function and Bronchodilators

Asthma is a chronic inflammatory disorder of the airways. But how are NO and inflammation related to the main clinical symptoms of asthma?

3.1.1. Exhaled NO and Eosinophilic Inflammation

The current concept of asthma pathogenesis involves a chronic inflammatory process, which causes the development of airflow limitation and increased responsiveness to allergens. The airway inflammation is characterized by an increased number of activated eosinophils, mast cells and T-lymphocytes in the airway mucosa and lumen. The actions of these cells result in epithelial damage, swelling, mucus secretion and airway smooth muscle contraction. Thus, inflammatory cells and mediators are the cause of asthma symptoms. Amin and co-workers showed an inverse correlation between the concentration of eosinophils and epithelial integrity of the airways in individuals with atopic asthma, indicating that inflammation plays an important role in airway remodelling. [Amin K *et al.* 2000] Levels of NO derivatives in induced sputum correlate with markers of airway remodelling (Figure III.1). [Gabazza EC *et al.* 2000] Such remodelling may increase the thickness of airways not responding to treatment. [Ketai L *et al.* 2005]

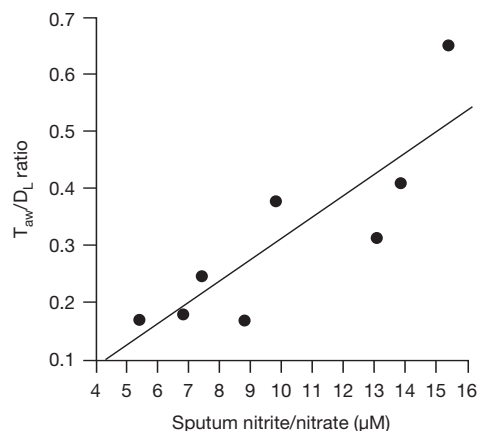


Figure III.1. The concentration of nitrites and nitrates in sputum correlates with the ratio of airway wall thickness (T_{aw}) to lumen diameter (D_L) in asthmatic patients ($r = 0.9$; $p < 0.01$) [Gabazza EC *et al.* 2000]

Eosinophilic inflammation is a hallmark of bronchial asthma. [Bousquet J *et al.* 1990] Berry and colleagues found a significant but non-linear correlation between exhaled NO and sputum eosinophils in 566 adult patients with asthma (Figure III.2). [Berry MA *et al.* 2005] There were no clinically important confounding factors to this model in non-smokers. A good correlation between sputum eosinophils and exhaled NO levels was also found in children by Malmberg *et al.* [Malmberg LP *et al.* 2005]

In addition to sputum eosinophilia, correlation between exhaled NO and eosinophilic cells in biopsy and BAL has also been established. Two studies have provided strong evidence that exhaled NO reflects airway inflammation. van den Toorn and co-workers assessed the quantity of major basic protein in bronchial biopsies of patients with asthma and individuals in clinical remission. [van den Toorn LM *et al.* 2001] Significant correlations between major basic protein density and exhaled NO levels occurred in both groups. Payne and colleagues examined children with 'difficult' asthma, and showed a significant correlation between exhaled NO levels and eosinophil scores in biopsies ($r = 0.54$, $p = 0.03$). [Payne DN *et al.* 2001a]

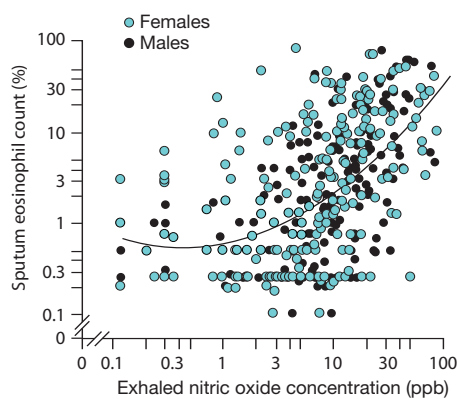


Figure III.2. Exhaled nitric oxide correlated with sputum eosinophil counts in a non-linear model [Berry MA *et al.* 2005]

Lastly, in a study of 27 children with moderate-to-severe asthma by Lex *et al.*, there was a significant correlation between exhaled NO and BAL eosinophils ($r = 0.54$, $p = 0.006$). [Lex C *et al.* 2006]

In summary, the symptoms of asthma result from the activation of eosinophils and other inflammatory cells. Evidence shows there are links between exhaled NO, eosinophilic inflammation, and asthma. Exhaled NO measurements correlate well with eosinophilic airway inflammation. [Taylor DR 2006]

3.1.2. Exhaled NO and Lung Function

Lung function tests represent the standard method for assessing asthma, although it is increasingly recognized that such tests do not reflect inflammation or patient self-assessment of asthma status. Accordingly, Stirling and co-workers found no correlation between exhaled NO levels and lung function tests. [Stirling RG *et al.* 1998] These findings were also confirmed by Langley *et al.* who found there was no correlation between exhaled NO and FEV₁ in a cross-sectional, hospital-based study of 392 patients with varied asthma severity. [Langley SJ *et al.* 2003b] Sippel *et al.* found only a weak correlation between FEV₁ and NO levels. [Sippel JM *et al.* 2000]

Piacentini and colleagues monitored NO levels in a group of children with atopic asthma who were placed in an Alpine home away from their allergens. [Piacentini GL *et al.* 1999b]

NO levels fell during the 3 months in the Alpine home and remained stable even when glucocorticoids were withdrawn. Patients' exhaled NO levels were increased after three weeks of returning to their non-Alpine homes, showing a marked inflammatory status. In comparison, spirometry results responded more slowly and continued to improve after the patients returned to their non-Alpine home (Figure III.3).

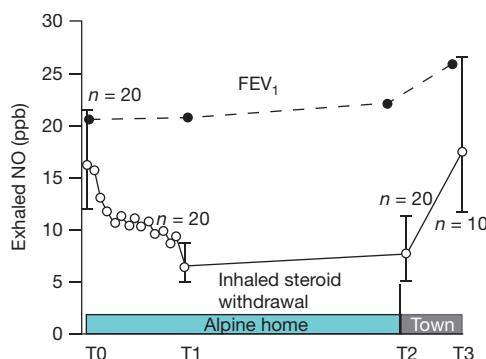


Figure III.3. Exhaled NO levels respond faster than spirometry results to changes affecting airway inflammation. FEV₁ = Forced expiratory volume in 1 second; T0 = before admission to residential home; T1 = 2 weeks after T0; T2 = 3 months at Alpine home; T3 = 2 weeks after return to home [Piacentini GL et al. 1999b]

Overall, most studies indicate that there is little or no correlation between exhaled NO levels and standard pulmonary function tests in patients with asthma. Furthermore, it appears that exhaled NO may respond more rapidly than spirometry to changes in allergen exposure, thus making it a more sensitive marker of disease state. [Baraldi E et al. 1999a; Piacentini GL et al. 1999b] However, it should be noted that a correlation between exhaled NO and residual volume has been reported. [Mappa L et al. 2005] The authors considered this to reflect a correlation between airway inflammation and trapping of air within the lungs. Other spirometry measures, such as small airway function tests, may correlate with exhaled NO. [Battaglia S et al. 2005]

In summary, lung function tests and exhaled NO measure different aspects of asthma: there is little correlation between lung function tests and exhaled NO.

3.1.3. Exhaled NO and Bronchodilators

Short- and long-acting β_2 -agonists do not generally appear to affect the levels of exhaled NO in patients with asthma. [Aziz I et al. 2000; Fuglsang G et al. 1998; Leme AS et al. 2002; Yates DH et al. 1997] Although one study showed a 10–20% increase in NO levels within 20 minutes of albuterol treatment, the effect was transient. [Kissoon N et al. 2002]

In summary, β_2 -agonists treat symptoms and **not** inflammation. Short- and long-acting β_2 -agonists do not generally affect the levels of exhaled NO in patients with asthma.

3.2. Relationship Between Exhaled NO and Atopy

Atopy is a genetically determined condition and is a frequent characteristic of asthma. From a clinical point of view, it is important to differentiate atopic and non-atopic asthma as the

differentiation will impact the therapeutic consequences. For example, Piacentini *et al.* demonstrated an increase in exhaled NO following allergen exposure in asthmatic children; and treatment with inhaled corticosteroids prevented this increase. [Piacentini GL *et al.* 2000a]

Alving *et al.* were the first to report increased levels of exhaled NO in asthma. [Alving K *et al.* 1993] Eight atopic patients with documented allergy towards at least rat allergen, and with mild symptoms of asthma and rhinitis, had two- to three-fold increased NO levels compared with 12 healthy, non-smoking control individuals. A relationship between an increase in exhaled NO and allergen exposure was also documented by Kharitonov *et al.* [Kharitonov SA *et al.* 1995a]

Patients with asthma experience an early and late inflammatory response when challenged with allergens. [Holgate S 1993] The early response is transient and characterized by an increase in neutrophils. In contrast, the late response is sustained and associated with an increase in eosinophils and T cells. It is generally considered that this late response is associated with an increase in NO levels. However, one study concluded that neutrophils also contribute to NO production in asthma. [Ramesh G *et al.* 2001]

Studies have shown that patients with atopic asthma have higher levels of exhaled NO than other patients with asthma. [Brussee JE *et al.* 2005; Chng SY *et al.* 2005; Frank TL *et al.* 1998; Franklin PJ *et al.* 2003; Gratziau C *et al.* 1999; Henriksen AH *et al.* 2000; Ludviksdottir D *et al.* 1999; Sacco O *et al.* 2003; van Amsterdam JG *et al.* 2003a] Indeed, some authors report no difference in exhaled NO levels between non-atopic asthma patients and healthy controls. [Frank TL *et al.* 1998; Henriksen AH *et al.* 2000] Moreover, there is evidence that atopic individuals without asthma have abnormally high NO levels. For example, Horváth and Barnes showed that healthy, non-smoking individuals who were atopic had significantly higher exhaled NO levels than those who were non-atopic. [Horvath I and Barnes PJ 1999] Similarly, children with atopy but not asthma have higher NO levels than controls or children with non-atopic asthma. [Prasad A *et al.* 2006]

Studies have shown a correlation between exhaled NO levels and skin-prick test reactivity to house dust mites. [Barreto M *et al.* 2001; Moody A *et al.* 2000; van Amsterdam JG *et al.* 2003b] Leuppi *et al.* have shown that sensitization to house dust mites was associated with raised exhaled NO levels in the winter season. The NO values correlated significantly with airway hyperresponsiveness to histamine, independently of whether the children had symptoms or not. [Leuppi JD *et al.* 2002] Barreto *et al.* also showed that exhaled NO levels were significantly greater in children with atopic asthma compared with non-atopic asthma, and that NO levels were particularly high in atopic asthmatics who were sensitized to house-dust mite allergen compared with other

common allergens. [Barreto M *et al.* 2001] Chronic exposure to allergens creates an inflammatory status detected by exhaled nitric oxide.

Inhalation of grass pollen has been shown to increase the level of exhaled NO in sensitized children. [van Amsterdam JG *et al.* 2003a] The effect of sensitization to indoor allergens is analogous: in a study of 450 children, levels of exhaled NO were 1.5-times higher in sensitized versus non-sensitized children ($p < 0.05$; Figure III.4). [van Amsterdam JG *et al.* 2003b] IgE sensitization has also been associated with increased NO levels in children. [Cardinale F *et al.* 2005; Janson C *et al.* 2005] In adults with asthma, Langley *et al.* showed higher levels of exhaled NO among those who had been sensitized and exposed to high levels of indoor sensitizing allergen; moreover, the increased NO levels coincided with a more severe form of the disease. [Langley SJ *et al.* 2003a] Others have shown that NO levels increase in response to allergen challenge in patients with allergic asthma, [Diego PG *et al.* 2006; Erpenbeck VJ *et al.* 2005] and that the degree of NO increase is associated with the number of allergens the subject is exposed to (Figure III.5). [Spanier AJ *et al.* 2006]

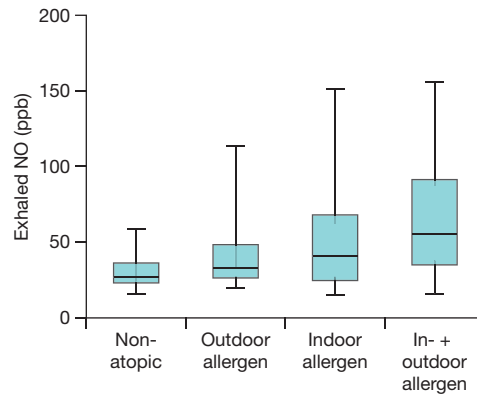


Figure III.4. Sensitization to indoor allergens is associated with higher levels of exhaled NO compared to non-sensitized children [van Amsterdam JG *et al.* 2003b]

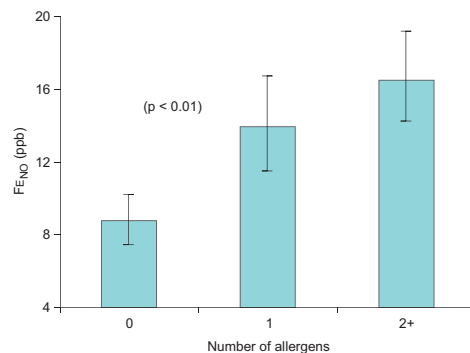


Figure III.5. Asthmatic children who were sensitized to two or more allergens had higher exhaled NO than children who were sensitized to only one allergen, in a dose-dependent fashion (geometric mean NO levels [95% CI]) [Spanier AJ *et al.* 2006]

Ihre *et al.* demonstrated an increase in exhaled NO after allergen exposure in the absence of impairment in lung function. [Ihre E *et al.* 2006] In patients with mild

atopic asthma who were symptom-free at the time of the study, daily challenge with low-dose inhaled allergen resulted in an early and gradual increase in exhaled NO levels compared with challenge with placebo diluent, while pulmonary function remained unchanged (Figure III.6). Similarly, in a study by Vahlkvist *et al.* involving children with

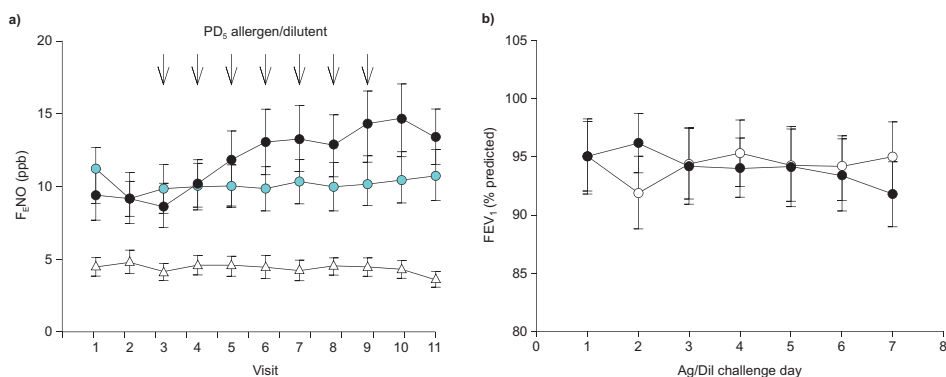


Figure III.6. a) Exhaled NO measurements taken before allergen or placebo diluent inhalation (●: allergen in asthmatic subjects; ●: diluent in asthmatic subjects; △: diluent in healthy control subjects). b) FEV_1 during diluent (○) and repeated low dose-allergen (●) challenge period, respectively [Ilhre E et al. 2006]

mild asthma and birch-pollen allergy, exhaled NO levels increased as the pollen season progressed, although pulmonary function measures remained unresponsive and few symptoms were recorded (Figure III.7). [Vahlkvist S et al. 2006]

The relationship between exhaled NO and airway responsiveness was shown to be evident only in atopic children. [Franklin PJ et al. 2003; Steerenberg PA et al. 2003] Such evidence has led some experts to conclude that increased NO levels are a feature of atopy rather than asthma. Raised exhaled NO levels appear to be associated with an underlying mechanism linking atopy and airway responsiveness but not necessarily respiratory symptoms. [Franklin PJ et al. 2004a; Franklin PJ et al. 2003]

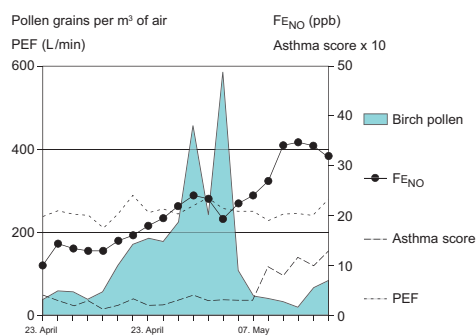


Figure III.7. Daily home measurement of exhaled NO and pulmonary function compared with natural pollen counts during the birch pollen season [Vahlkvist S et al. 2006]

Indoor exposure to airborne microbes is well known to increase the risk of respiratory conditions and this also appears to be caused by inflammation. One study has shown that exhaled NO levels rapidly increase in individuals exposed to *Aspergillus fumigatus*. [Stark HJ et al. 2005] Airway inflammation in asthma may represent a favourable environment for respiratory viral infections, augmenting virus-induced exacerbations in asthma. de Kluijver et al. showed that RV16 infection and repeated low-dose allergen exposure induce distinct inflammatory profiles within the airways of patients with mild-to-moderate asthma. Exhaled NO increased significantly during allergen exposure ($p < 0.001$), whereas it did not change significantly after RV16 infection (either initially [$p = 0.8$], or following allergen exposure [$p = 0.9$]) (Figure III.8). [de Kluijver J et al. 2003] The lack of apparent interaction between

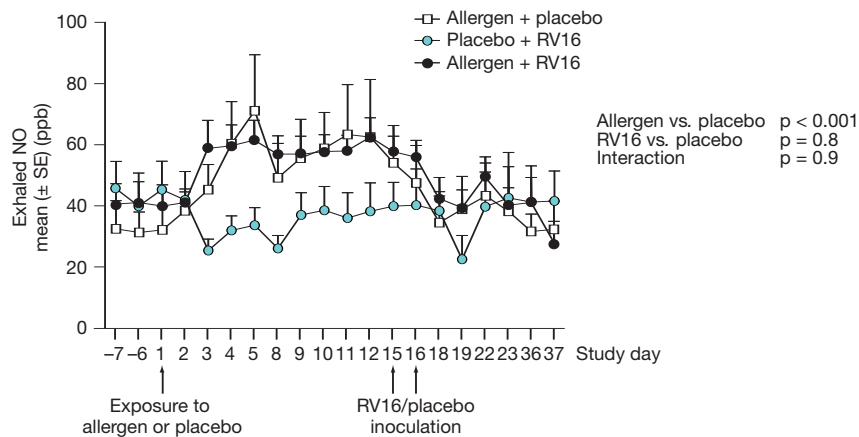


Figure III.8. Exhaled NO increased significantly ($p < 0.001$) during allergen exposure compared with placebo, but did not change significantly after RV16 infection or successive allergen exposure and RV16 infection [de Kluijver] et al. 2003]

these two environmental triggers suggests that priming airways with repeated low-dose allergen does not aggravate patients' response to RV16 infection, in terms of airway obstruction and inflammation.

The evidence linking atopy and increased NO levels is, however, controversial. Gratziou *et al.* reported no difference in exhaled NO levels between atopic and non-atopic healthy individuals. [Gratziou C *et al.* 1999] Similar results were described by Berlyne and co-workers. [Berlyne GS *et al.* 2000] Important insights were published by Olin and co-workers, who showed that only atopic patients who had recently been exposed to the relevant allergen had elevated levels of exhaled NO. Atopic patients who had not been exposed to a relevant allergen or who had never experienced symptoms of asthma or rhinitis showed normal exhaled NO. [Olin AC *et al.* 2004a; Olin AC *et al.* 2001b]

In a study involving patients with persistent rhinitis, sensitization to pollen was associated with seasonal variation in exhaled NO levels. [Downie SR *et al.* 2004], Similarly, in patients with seasonal allergic rhinitis, there was a strong correlation between exhaled NO and pollen counts two weeks previously. [Roberts G *et al.* 2004] The results suggest that NO levels rise 8–14 days after pollen exposure. Furthermore, Pedroletti and co-workers have recently reported that nasal exposure to an allergen does not increase exhaled NO levels in children with atopic asthma. [Pedroletti C *et al.* 2005] Gratziou *et al.* reported that allergic rhinitis is associated with increased NO. [Gratziou C *et al.* 1999] However, Lopuhaa and colleagues showed that although baseline exhaled NO is significantly lower in non-asthmatic rhinitis compared with asthma ($p < 0.006$), the difference in exhaled NO at baseline is abolished after allergen exposure, due to a significantly greater increase in exhaled NO in non-asthmatic rhinitis. [Lopuhaa CE *et al.* 2003] These findings underline the similarities in bronchial changes in allergic patients with and without asthma.

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In considering this issue, one must remember that exhaled NO is a marker for inflammation. Thus, it is likely that increased exhaled NO levels in an atopic individual with rhinitis indicate general airway inflammation and possibly an increased risk of developing asthma.

In summary, exhaled NO is a marker of increased inflammation triggered by allergen exposure. Raised NO levels appear to be linked to atopy and airway hyperresponsiveness, even if the patient may appear to be asymptomatic. Further investigations are still required to determine fully the relationship between NO, atopy and asthma.

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