

VII. Exhaled NO in Research

Different methods of assessing exhaled NO are used in the research setting compared with clinical practice. These methods allow exhaled NO levels to be monitored in an extended range of patients (e.g. children aged < 2 years) and conditions (e.g. allergic rhinitis, chronic bronchitis, cystic fibrosis).

7.1. Biochemical Principles

NO elicits many of its physiological actions by activating cytosolic guanylate cyclase, which converts guanine triphosphate (GTP) to cyclic guanine monophosphate (cGMP). In 1998, Drs Robert F Furchgott, Louis J Ignarro and Ferid Murad were awarded the Nobel Prize for Medicine or Physiology for their basic discoveries in this field.

The synthesis of NO is mediated by NO synthases (NOS), which exist in constitutive (cNOS) and inducible (iNOS) isoforms. Constitutively expressed NOS are present particularly in endothelial cells (eNOS) and neural tissue (nNOS). All three forms of NOS, i.e. eNOS, nNOS and iNOS, have been shown to be present in the airways. [Ricciardolo FL *et al.* 2004] However, only the expression of iNOS correlates with levels of exhaled NO. [Lane C *et al.* 2004]

Expression of iNOS is seen particularly in airway epithelial cells and the expression is markedly increased in asthma. [Hamid Q *et al.* 1993; Redington AE *et al.* 2001] Interestingly, the expression of iNOS in airway inflammation, and thus exhaled NO, seems to be dependent on the expression and function of nNOS. Nitric oxide formed by cNOS may lead to cGMP-dependent relaxation of airway smooth muscle, whereas high quantities of NO released by iNOS may be associated with proinflammatory effects. [Barnes PJ and Liew FY 1995; Nijkamp FP and Folkerts G 1994] In addition, endothelial NOS has been localized to the bronchial and pulmonary circulation and a known DNA sequence variant in eNOS is associated with decreased levels of exhaled NO. [Feletou M *et al.* 2001; van's Gravesande KS *et al.* 2003] Guo and colleagues demonstrated that patients with asthma exhibit increased expression of iNOS mRNA in airway epithelium compared with healthy controls, but iNOS mRNA was not detected in alveolar macrophages. [Guo FH *et al.* 2000] Moreover, those patients who were receiving corticosteroids had decreased expression of iNOS protein and mRNA compared with those not receiving this treatment.

iNOS expression can be induced by a variety of stimuli, in particular inflammatory cytokines, but the exact mechanism is not understood even though it has been suggested that the transcription factor STAT-1 is involved. [Guo FH *et al.* 2000] Figure VII.1 illustrates a possible mechanism for this. Another important transcription factor, NF-kappa B, is

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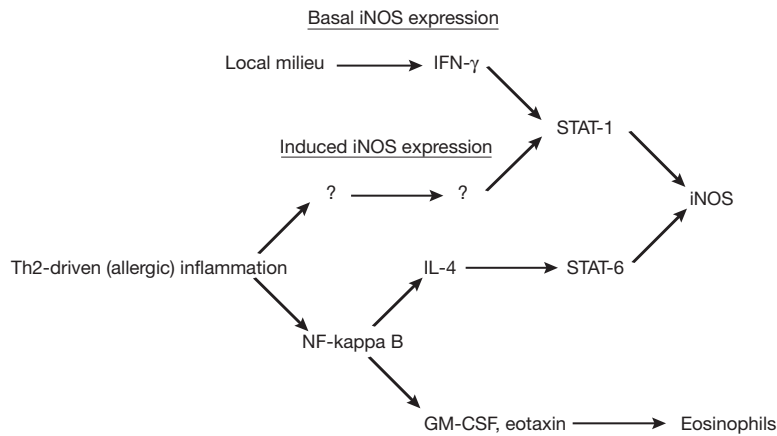


Figure VII.1. Possible mechanism of how airway lung inflammation leads to increased levels of NO in exhaled air. Courtesy of Prof. Kjell Alving

upregulated in allergic inflammation and down-regulated by corticosteroids. [Barnes PJ and Adcock IM 1998] This transcription factor is essential for expression of, for example, eotaxin and granulocyte-macrophage colony-stimulating factor (GM-CSF). These proteins are also expressed in bronchial epithelial cells in asthma. It seems that STAT-1 is up-regulated simultaneously with, or possibly as a consequence of, the up-regulation of NF-kappa B in the asthmatic human airway epithelium.

Some studies indicate that iNOS also plays an important role in pulmonary inflammation. For example, Cuzzocrea and co-workers have shown that iNOS-knockout mice have a less severe pulmonary inflammatory response to carrageenan than wild-type mice. [Cuzzocrea S *et al.* 2000] In humans, the increased levels of Th2 cell-derived cytokines observed in patients with atopic asthma compared with healthy subjects appears to be correlated with levels of exhaled NO. [Shirai T *et al.* 2006] As well as synthesis by iNOS, NO may also be produced by conversion of nitrate at low pH. It has been shown that airway NO is reduced in asthmatic subjects after inhalation of phosphate-buffered saline, supporting the hypothesis of nitrate conversion and airway pH dysregulation in asthma. [Shin HW *et al.* 2007]

Oral treatment with a selective iNOS inhibitor has been shown to reduce exhaled NO by 80–90% in patients with asthma and in healthy controls. [Hansel TT *et al.* 2003]

7.2. Exhaled NO Research Methods

7.2.1. Tidal Measurement

Although the single-breath method is the most reliable way of measuring exhaled NO, the breathing manoeuvre required is too complicated to perform for very young children. Many groups have tried to overcome difficulties by devising methods for measuring exhaled NO from tidal breathing, which requires only passive cooperation.

Visser and colleagues developed an offline method of measuring NO in mixed exhaled gas collected during 5 minutes of tidal breathing in children 4–14 years of age. [Visser M] *et al.* 2000] This method required children to breathe quietly through a mouthpiece, whilst wearing a noseclip. [Rutgers SR *et al.* 1998] NO-free air was inhaled from a Douglas bag and exhaled gas was collected for 5 minutes through a non-rebreathing valve into a separate Douglas bag. Using this method, the group showed that exhaled NO levels are significantly elevated in asthmatic children compared to non-asthmatic children (Figure VII.2), [Visser M] *et al.* 2000] in agreement with other studies. [Artlich A *et al.* 1996; Dotsch J *et al.* 1996; Lundberg JO *et al.* 1996a] Although other groups have used tidal breathing methods, [Baraldi E *et al.* 1997; Baraldi E *et al.* 1999b; Dinarevic S *et al.* 1996; Frank TL *et al.* 1998] this study was the first to collect mixed exhaled air, thus obtaining a mean NO concentration of several breaths and eliminating potential influences of flow changes and breath variability. [Kimberly B *et al.* 1996] One group has reported a method using a facemask (divided by a diaphragm) and a one-way valve that allowed exhalation through the nose. [Daniel PF *et al.* 2005] This method yielded similar results to a standard single-breath method (7.0 vs. 6.9 ppb) and thus seems a useful technique.

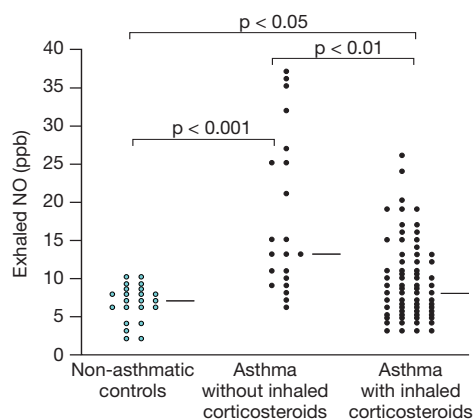


Figure VII.2. Exhaled NO levels are higher in asthmatic children compared with non-asthmatic controls and asthmatics on inhaled corticosteroid therapy [Visser M] *et al.* 2000]

Tidal measurements have been used successfully to differentiate steroid-naïve young children with intermittent asthma from healthy children, from non-asthmatic children with chronic cough, and from asthmatic children treated with inhaled steroids. [Avital A *et al.* 2001] In contrast, subsequent evidence suggested that offline tidal measurements cannot distinguish patients with wheeze and healthy controls. [Franklin PJ *et al.* 2004b] However,

Gabriele *et al.* were able to differentiate respiratory diseases in children younger than two years of age using tidal NO measurements. Exhaled NO in children with recurrent wheeze was higher than in healthy controls ($p = 0.009$), bronchopulmonary dysplasia ($p = 0.038$), and cystic fibrosis ($p < 0.001$). Atopic wheezers had higher exhaled NO compared with non-atopic wheezers ($p = 0.04$). Children with cystic fibrosis had lower exhaled NO compared with healthy controls ($p = 0.003$) and children with bronchopulmonary dysplasia ($p = 0.043$). Latzin *et al.* measured exhaled NO in newborn infants and found a correlation between elevated NO and increased risk of subsequent respiratory symptoms in babies of atopic mothers ($p = 0.007$) and babies of mothers who smoked ($p = 0.001$). [Latzin P *et al.* 2006] Many factors appear to affect these tidal measurements. [Franklin PJ *et al.* 2004c]

An online method of measuring NO during controlled tidal breathing has been described. [Buchvald F and Bisgaard H 2001] Resistance at the exhalation valve was continuously adjusted by the operator with the aim of controlling the exhalation flow. Online tidal measurements have been used successfully to show that exhaled NO levels fall significantly after corticosteroid treatment. [Currie GP *et al.* 2003a]

7.2.2 Offline NO Measurement

Offline methods to collect exhaled air to send to a clinic or laboratory for determination of NO have been investigated [Jobsis Q *et al.* 1999; Paredi P *et al.* 1998] Offline collection of samples for NO estimation offers the potential for samples to be collected at sites remote from the analyser, but it also carries potential disadvantages. These include contamination with gas not derived from the lower airway, sample deterioration during storage and transportation, and the inability to offer immediate feedback and assessment of the technique. [American Thoracic Society/European Respiratory Society 2005; Linn WS *et al.* 2004] The ERS/ATS recommendations addressed offline measurement of NO. Critical considerations for offline analysis include the use of NO-free air for inspiration and the recording of the expiratory flow rate. [American Thoracic Society/European Respiratory Society 2005]

Kissoon *et al.* developed a method whereby online sampling and offline sampling could be done simultaneously at various flow rates, in order to compare NO measurements using the two techniques. [Kissoon N *et al.* 2000] They found a strong correlation between offline and online measurements of NO with flow rates of 46, 31, 23 and 15 mL/s ($p < 0.001$). Similarly, Jobsis and colleagues showed a good correlation between offline and online techniques at a flow rate of 50 mL/s, provided that the first 220 mL of the offline exhalation was excluded. [Jobsis Q *et al.* 2001] Discarding the first 220 mL of the exhalation reduced contamination from 'dead space' air. Other publications have suggested that the use of a prebag to collect exhaled air will initially reduce contamination from ambient air. [Steenberg PA *et al.* 2000] It has been shown that the use of a 1 L prebag may prevent such contamination. However, the use of an NO scrubber is preferred with regard to the reduction of ambient air contamination.

7.3. Other Indications in the Research Setting

7.3.1. Overview of Factors Affecting Exhaled NO Levels

Airway inflammation such as asthma appear to be the most important cause of increased levels of exhaled NO. Treatment with inhaled corticosteroids reduces the amount of exhaled NO (see Section 4.4. *Response to Anti-inflammatory Treatment*). Other diseases and factors that have been demonstrated to decrease or increase the levels of exhaled NO include*: ↓ = decrease in level, ↑ = increase in level.

7.3.1.1. Diseases and Conditions

- Airway viral infection [Antus B *et al.* 2005; Gentile DA *et al.* 2002; Sanders SP *et al.* 2004] no change or ↑
- Allergic rhinitis [Aronsson D *et al.* 2005; de Kluijver J *et al.* 2003; Jouaville LF *et al.* 2003; Malmberg LP *et al.* 2006; Olin AC *et al.* 2001b] ↑ or no change
- Alveolitis [Lehtimaki L *et al.* 2001; Paredi P *et al.* 1999] ↑
- Asbestosis [Sandrini A *et al.* 2006] ↑
- Atopic dermatitis [Malmberg LP *et al.* 2006] ↑
- ATT deficiency [Malerba M *et al.* 2001] ↓
- Bronchiectasis [Ho LP *et al.* 1998; Tsang KW *et al.* 2002] no change or ↑
- Bronchoconstriction [Ho LP *et al.* 2000; Piacentini GL *et al.* 2002] ↓
- Chronic bronchitis [Delen FM *et al.* 2000] ↑
- Chronic cough [Avital A *et al.* 2001; Chaudhuri R *et al.* 2004; Li AM *et al.* 2003; Nogami H *et al.* 2003] ↑
- Chronic lung disease [Baraldi E *et al.* 2005; Leipala JA *et al.* 2004] ↑ in infants, but ↓ in school children
- Ciliary dyskinesia [Corbelli R *et al.* 2004; Horvath I *et al.* 2003; Narang I *et al.* 2002] ↓ (particularly nasal NO)
- COPD [Ansarin K *et al.* 2001; Delen FM *et al.* 2000; Ferreira IM *et al.* 2001; Papi A *et al.* 2000] no change or ↑
- Cystic fibrosis [Grasemann H *et al.* 2005a; Grasemann H *et al.* 2004; Lim AY *et al.* 2003; Ojoo JC *et al.* 2005] ↓, but ↑ during respiratory exacerbation
- Heart failure [Katz SD *et al.* 2005] ↓
- HIV [Palm J *et al.* 2000] no change or ↓
- Hypersensitivity [Franklin PJ *et al.* 1999; Moody A *et al.* 2000] ↑
- Hypertension [Donmez G *et al.* 2002; Schilling J *et al.* 1994] ↓
- Idiopathic pneumonia syndrome [Qureshi MA *et al.* 2004] ↑

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- Lung transplant rejection (bronchiolitis obliterans syndrome [BOS]) [Brugiere O *et al.* 2005; Gabbay E *et al.* 2000; Mora BN *et al.* 2000; Verleden GM *et al.* 2003a; Verleden GM *et al.* 2004] ↑
- Nasal polyposis [Prieto L *et al.* 2004] no change
- Obesity [Maniscalco M *et al.* 2006; McLachlan CR *et al.* 2006] no change or ↓
- Pneumonia [Adrie C *et al.* 2001] ↑
- Premenstrual asthma [Oguzulgen IK *et al.* 2002] ↑
- Pulmonary hypertension [Cremona G *et al.* 1994; Forrest IA *et al.* 1999; Girgis RE *et al.* 2002; Kharitonov SA *et al.* 1997a; Machado RF *et al.* 2004; Rolla G *et al.* 2000] ↓ or no change
- Pulmonary sarcoidosis [Moodley YP *et al.* 1999; Ziora D *et al.* 2004] ↑
- Sickle cell anaemia [Girgis RE *et al.* 2003; Morris CR *et al.* 2003; Sullivan KJ *et al.* 2001] ↓
- Systemic sclerosis [Moodley YP and Laloo UG 2001; Rolla G *et al.* 2000] ↑

7.3.1.2. Other Factors

- Age [Haight RR *et al.* 2006; Malmberg LP *et al.* 2006] ↑
- Alcohol consumption [Jones AW *et al.* 2005; Yates DH *et al.* 1996] small ↓ in healthy individuals, possibly larger ↓ in patients with asthma
- Altitude [Brown DE *et al.* 2006; Karagiannidis C *et al.* 2006] ↓
- Caffeine [Bruce C *et al.* 2002; Taylor ES *et al.* 2004; Warke TJ *et al.* 2003b] ↓ or ↑, no change in patients with asthma
- Cardiac pulmonary bypass [Alexiou C *et al.* 2004; Tornberg DC *et al.* 2005] ↑ or ↓
- Exercise [Bonsignore MR *et al.* 2003; Clini E *et al.* 2001; Clini E *et al.* 2002; El Halawani SM *et al.* 2003; Gabriele C *et al.* 2005; Henriksen AH *et al.* 2002; Verges S *et al.* 2005] no change or ↑ or ↓
- Nitrate-rich diet [Olin AC *et al.* 2001a; Vints AM *et al.* 2005] ↑
- Passive smoking [Franklin P] *et al.* 2006; Maniscalco M *et al.* 2002; Warke TJ *et al.* 2003a; Yates DH *et al.* 2001] no change or ↓
- Smoking [Baur X and Barbinova L 2005; Franklin PJ *et al.* 2004a; Hogman M *et al.* 2002a; Horvath I *et al.* 2004; Malinovschi A *et al.* 2006a; Marteus H *et al.* 2004; McSharry CP *et al.* 2005] ↓
- Spirometric manoeuvres [Barreto M *et al.* 2006; Gabriele C *et al.* 2005; Kisson N *et al.* 2002; Tee AK and Hui KP 2005] ↓ or no change
- Sputum induction [Antczak A *et al.* 2005; Beier J *et al.* 2003; Piacentini GL *et al.* 2000b] ↓ or ↑

*Only a selection of articles are included that typify results.

7.3.2. Home Use

Measurement of exhaled NO by patients in their own home provides the opportunity to monitor airway inflammation on a daily basis. In a study by Pijnenburg *et al.*, asthma patients used the handheld NIOX MINO[®] device to measure exhaled NO twice daily for 14 consecutive days. [Pijnenburg MW *et al.* 2006] There was a significant diurnal variation in exhaled NO with geometric mean morning levels 14% higher than evening levels ($p = 0.013$) (Figure VII.3). Individual subjects showed marked fluctuation of exhaled NO, with a mean intrasubject coefficient of variation of 40% for morning and 36% for evening values. Exhaled NO and cumulative symptom scores did not correlate. In another study, eleven children with mild asthma and allergy to birch pollen performed daily home measurements of exhaled NO for 6 weeks before and during the birch pollen season by using the NIOX MINO[®] monitor, and these measurements were compared with those obtained in the clinic (NIOX[®] device). [Vahlkvist S *et al.* 2006] Daily exhaled NO measured at home increased significantly with increasing pollen count ($p < 0.001$) (see Figure III.7 in Section 3.2. Relationship Between Exhaled NO and Atopy). Home and clinic values exhibited a correlation coefficient of 0.98, although the home measurements were significantly higher than values obtained at the clinic ($p < 0.01$).

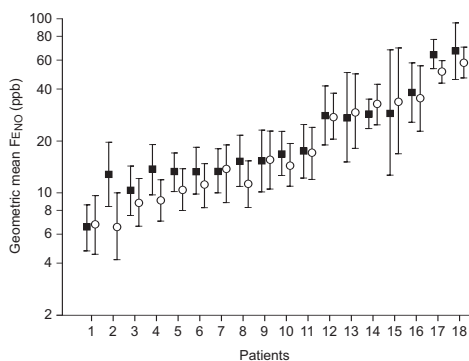


Figure VII.3. Individual diurnal variability of geometric mean exhaled NO; morning values (closed symbols) and afternoon values (open symbols) ± 1 s.d. for each patient during 2 weeks [Pijnenburg MW *et al.* 2006]

7.3.3. Early Marker of Asthma

Several publications have indicated that exhaled NO levels may be increased before asthma symptoms develop. Moody and co-workers examined NO levels and skin-prick tests in 64 asymptomatic Pacific Islanders – a racial group known to have a high risk of developing asthma. [Moody A *et al.* 2000] Individuals sensitive to house dust mites had high levels of exhaled NO, which correlated with the severity of their sensitivity. The authors concluded that raised NO levels in this population may represent subclinical airway inflammation.

de Kluijver and colleagues have shown that ‘silent’ chronic allergen exposure can induce and maintain airway inflammation, which can be prevented with anti-inflammatory treatment. [de Kluijver J *et al.* 2002] Repeated low-dose allergen exposure to house dust mites resulted in a significant increase in sputum eosinophils, ECP and exhaled NO, compared with the budesonide-treated group.

Ilhre *et al.* demonstrated an increase in exhaled NO after allergen exposure in the absence of impairment in lung function. [Ilhre E *et al.* 2006] In patients with mild atopic asthma who had no asthma symptoms 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. (See Figure III.6 in Section 3.2. *Relationship Between Exhaled NO and Atopy*).

In a population-based study, Nordvall *et al.* showed that higher NO levels were associated with asthma, wheeze and allergy symptoms. [Nordvall SL *et al.* 2005] In this study, most of the children who had asthma had only mild disease and the authors thus suggest that exhaled NO is a sensitive marker of asthma and allergy. In contrast FEV₁ values did not show strong correlations with asthma and allergy symptoms.

The relationship between exhaled NO levels and subclinical airway inflammation has been investigated by Franklin and colleagues who measured NO levels in healthy children and the level of formaldehyde in their homes. [Franklin P *et al.* 2000] Formaldehyde has been associated with adverse respiratory symptoms in both children and adults. NO levels were significantly higher in children living in homes with formaldehyde levels above 50 ppb than in those living in homes with less formaldehyde. Notably, there was no correlation between formaldehyde levels and spirometry results.

Asthma symptoms often decline during puberty and some patients experience clinical remission in early adulthood. However, a proportion of those who experience remission have relapses later in adult life. van den Toorn and colleagues have shown that adults in remission can have airway inflammation that correlates with NO levels. [van den Toorn LM *et al.* 2001] The authors speculated that this subclinical inflammation may be a risk factor for asthma relapse in later life.

As well as being elevated in asthma, some evidence suggests that exhaled NO levels are also elevated, though to a lesser extent, in non-asthmatic rhinitis. [Lopuhaa CE *et al.* 2003] This finding suggests that exhaled NO levels may help identify patients at risk of developing asthma. However, it should be noted that not all studies in patients with rhinitis have shown increased exhaled NO levels. [Aronsson D *et al.* 2005] Similarly, Heffler *et al.* considered prediction of the risk of asthma in patients with allergic rhinitis. [Heffler E *et al.* 2006] The authors concluded that measuring exhaled NO 'may be useful' in this regard. (See also Section 4.12.2. *Rhinitis*).

Although more studies are required, it is possible that exhaled NO levels may prove useful in identifying individuals at risk of developing asthma.

7.3.4. Epidemiology

The epidemiology of asthma is of great interest, as researchers seek insights into the reasons behind the general increase in the prevalence of the condition. Epidemiologists have generally relied on symptoms and lung function tests to gain information on factors that affect asthma risk, but to fully understand the natural history of asthma, information is needed on factors that affect airway inflammation. Exhaled NO, particularly with the advent of a portable device, now offers a convenient method of assessing airway inflammation in such studies. Saito and colleagues have tested the potential of exhaled NO in an epidemiologic study involving children. [Saito J *et al.* 2004] They found that exhaled NO was the best predictor of recurrent wheeze, when compared with IgE and pulmonary function tests. Given this and the convenience of the test, the authors suggest that exhaled NO is a valuable marker of inflammation that is suitable for epidemiological investigations. Similar conclusions were drawn by Thomas *et al.*, who found that exhaled NO was as good as other established methods for detecting asthma. [Thomas PS *et al.* 2005] Arora *et al.* investigated the potential for using exhaled NO measurement to screen for asthma in US Air Force military trainees. [Arora R *et al.* 2006] This study was inconclusive since an optimal NO cut-off level could not be found.

One epidemiological study in the Netherlands investigated the differences in allergic sensitization and NO levels between children of different ethnic origin. [van Amsterdam JG *et al.* 2004] Exhaled NO levels were higher in children with allergic sensitization than in those without any sensitization. Children of Moroccan origin who were sensitized to indoor allergens had the highest levels of NO. A community study in Australia also showed that allergic sensitization was associated with increased NO in levels in schoolchildren. [Franklin PJ *et al.* 1999] With the growing use of exhaled NO measurements in epidemiology, one can expect to see further insights into the factors that affect the risk of developing asthma. One potential risk factor of great interest is air pollution.

7.3.5. Air Pollution

Air pollution is known to affect the health of patients with asthma, but its role in the development of the condition is more controversial. A birth cohort study in Norway (the Environment and Childhood Asthma study) found no correlation between outdoor air pollution and the occurrence of bronchial obstruction in the first 2 years of life. [Lodrup Carlsen KC 2002] However, no assessment of the effects of pollution on airway inflammation was made in the initial part of this study (a follow-up involving exhaled NO measurements is ongoing). A number of studies have assessed exhaled NO in relation to air pollution. Steerenberg and co-workers found that exhaled NO levels in children were directly associated with environmental levels of black smoke, nitrogen dioxide, NO and

particulate matter < 10 µm. [Steenberg PA *et al.* 2001] Fischer *et al.* also reported that exhaled NO levels in children increased (by as much as 31%) with increasing air pollution levels. [Fischer PH *et al.* 2002] Notably, there was no association between changes in lung function and air pollution. Given this, and the fact that exhaled NO levels correlated with mild respiratory symptoms (e.g. sore throat, runny nose), the authors concluded that exhaled NO was a more suitable measure of the health effects of air pollution. Jansen *et al.* drew similar conclusions from their study, which showed that NO levels correlated with exposure to particulate matter and black carbon in patients with asthma, whereas other health markers (including spirometry) did not. [Jansen KL *et al.* 2005] Steenberg and colleagues noted a 67–78% increase in exhaled NO in adults on days with high levels of ambient NO. [Steenberg PA *et al.* 1999] A significant increase in exhaled NO levels in association with a 17.7 µg/m³ increase in levels of particulate matter < 2.5 µm has been reported in elderly individuals. [Adamkiewicz G *et al.* 2004] Importantly, the association between NO and particulate matter was stronger in patients with COPD. It has been shown that NO levels correlate with ambient exposure to particulate matter, but not to indoor particulate matter levels, suggesting that ambient pollution levels are a key factor in airway inflammation. [Koenig JQ *et al.* 2005] Others have shown that NO correlates with exposure to ozone and trichloramine (a gas found in indoor chlorinated swimming pools). [Bernard A *et al.* 2005] Mar and co-workers have shown that NO levels correlate with exposure to particulate matter < 2.5 µm up to 10–12 h after exposure, providing valuable information for researchers wishing to use NO levels to monitor pollution exposure. [Mar TF *et al.* 2005]

In all of the studies described here, response to environmental air pollution was assessed. However, a laboratory study failed to find significant changes in NO levels in response to increasing exposure to carbon ultrafine particles in both healthy individuals and patients with asthma. [Pietropaoli AP *et al.* 2004] The reason for this apparently contrasting finding is not known, but could be due to the contrast between the relatively acute exposure in the laboratory setting and the chronic exposure in the epidemiological setting.

7.3.6. Occupational Health

Occupational asthma is a continuing problem, and exhaled NO is potentially useful for identifying workers at risk of developing asthma after exposure to occupation-related stimuli. [Allmers H *et al.* 2000]

In a study in Scotland, asthmatic bar workers showed a reduction in exhaled NO from 34.3 ppb to 27.4 ppb one month after smoking was banned in their workplaces. [Menzies D *et al.* 2006b]

Isocyanates have become a principal cause of occupational obstructive airway diseases in industrial countries, and exhaled NO is increased in individuals exposed to isocyanate. [Barbinova L and Baur X 2006]

Barbinova *et al.* assessed exhaled NO in 55 workers with isocyanate-related respiratory complaints. [Barbinova L and Baur X 2006] An increase in exhaled NO occurred 22 hours after an isocyanate challenge in two-thirds of responders and in approximately half of non-responders with bronchial hyperreactivity but only rarely in those without hyperreactivity. The authors conclude that the combination of hyperreactivity and increase in exhaled NO in non-responders may offer a diagnostic tool to identify subjects with an increased risk of occupational asthma. Sandrini *et al.* measured exhaled NO in subjects with asbestos-related disorders (asbestosis, pleural plaques, or asbestos-related diffuse pleural thickening). [Sandrini A *et al.* 2006] Exhaled NO was increased in subjects with asbestosis (7.9 pbb, $p = 0.001$) and pleural plaques (6.3 pbb, $p = 0.03$) compared with normal controls (4.6 pbb, all at 200 mL/s). Subjects with diffuse pleural thickening had similar NO levels to healthy control. The authors suggest that exhaled NO could be useful for monitoring progression of asbestos-related diseases and response to treatment.

Lund *et al.* reported increased exhaled NO levels in 99 non-smoking aluminium pot-room workers compared with controls. [Lund MB *et al.* 2000] Only 12 of the workers with high NO levels had asthma-like symptoms. In another study, exhaled NO was assessed in workers after challenge with 4,4'-diphenylmethane di-isocyanate, natural rubber latex or methacholine. [Allmers H *et al.* 2000] Individuals with substance-specific IgE antibodies and a bronchial response to stimulants had a tendency to develop increased NO levels shortly after (22 h) the challenge.

Olin *et al.* have shown that exhaled NO levels are higher in pulp mill workers who have experienced ozone gassing incidents than in those who have not reported such incidents. [Olin AC *et al.* 1999] The authors suggested that the high NO levels may have been due to chronic airway inflammation. In a follow-up study 3 years later, those workers who had the highest exposure to ozone had significantly higher NO levels than controls and an increased prevalence of adult-onset asthma. [Olin AC *et al.* 2004b] Sundblad *et al.* found that bronchial responsiveness and exhaled NO increased after exposure to a swine confinement facility. [Sundblad BM *et al.* 2002] Exhaled NO levels have also been shown to increase (by 40%) in shoe and leather workers during their working day, probably as a result of exposure to organic solvents. [Maniscalco M *et al.* 2004] These studies suggest that NO levels may prove useful as a means of early detection of subclinical inflammation in individuals who are working in environments that may increase their risk of asthma.

7.3.7. Cystic Fibrosis

Exhaled NO, elevated in most inflammatory airway diseases, is decreased in cystic fibrosis (CF), suggesting either decreased production or accelerated metabolism. [Balfour-Lynn IM *et al.* 1996; Lundberg JO *et al.* 1996a] Morrissey *et al.* have shown that, despite confirmation of subnormal iNOS in the CF airway epithelium, the alternative isoforms nNOS and eNOS

were present, and inflammatory cells in the CF airways expressed abundant iNOS. Increased immunohistochemical staining for nitrotyrosine was demonstrated in the lung tissue from patients with CF compared with controls. [Morrissey BM *et al.* 2002] Using a 50 mL/s flow rate, exhaled NO in CF patients in this study was found to be 56% of that in normal volunteers. In a genetic study, Texereau and co-workers showed that patients with CF who had a NOS₁ genotype associated with high NO production had a slower decline in lung function over 5 years. [Texereau J *et al.* 2004] This suggests that the low NO levels seen in CF may be pathophysiologically related to poor lung function. It has also been shown that sputum arginase activity is higher in patients with CF experiencing an exacerbation and this may explain reduced NO levels. [Grasemann H *et al.* 2005b] However, Everard and Donnelly have shown that NO levels do not increase in response to treatment with L-arginine. [Everard ML and Donnelly D 2005] Reduced NO levels are also associated with poor airway ion transport found in patients with CF. [Texereau J *et al.* 2005]

Exhaled NO levels have also been shown to be significantly lower in infants with CF compared with controls. [Elphick HE *et al.* 2001] However, another study in young children found no difference in NO levels compared with controls. [Wooldridge JL *et al.* 2004] In this study, iNOS expression decreased as airway inflammation increased; possibly indicating that low NO levels may be associated with worsening disease.

Grasemann and colleagues have shown that changes in exhaled NO levels parallel changes in pulmonary function in most patients treated with recombinant human DNase I, suggesting that NO levels may be a useful marker of efficacy. [Grasemann H *et al.* 2004] This group also demonstrated that inhaled L-arginine, a precursor for NO formation, significantly increased exhaled NO levels (Figure VII.4), resulted in a sustained improvement of FEV₁, and increased oxygen saturation in patients with CF. [Grasemann H *et al.* 2006] While nebulized saline resulted in a small but significant increase in exhaled NO, there was a decrease in FEV₁ in patients with CF. In control subjects inhalation of L-arginine increased exhaled nitric oxide concentrations, but FEV₁ decreased. Augmentation of NO pathways may provide a new therapeutic target in CF.

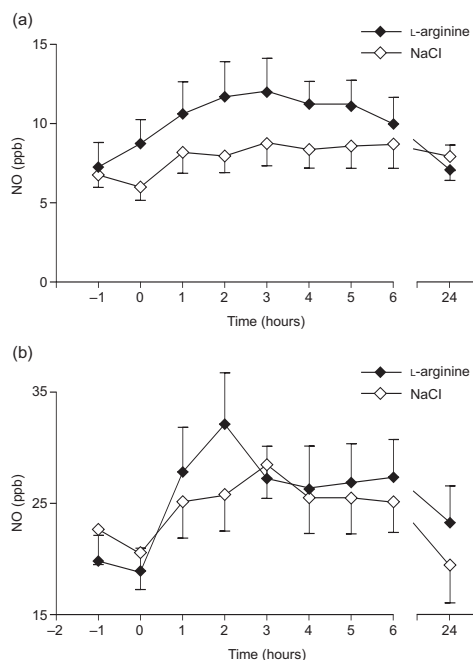


Figure VII.4. Effects of inhalation of a 7% L-arginine hydrochloride solution or 1.7% saline on exhaled NO in (a) 13 patients with CF and (b) 9 healthy subjects [Grasemann H *et al.* 2006]

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity reaction to *Aspergillus fumigatus* (Af). Patients with CF may be prone to Af colonization because of their highly viscous sputum. Untreated ABPA may lead to lung damage, including proximal bronchiectasis, and segmental, lobar, or whole lung collapse. Furthermore, diagnosis of ABPA is difficult as the signs and symptoms are similar to those of CF itself. Lim *et al.* have shown that exhaled NO levels were lower in CF patients on glucocorticoids with a high risk of developing ABPA than in those with a lower risk. [Lim AY *et al.* 2003] Therefore, exhaled NO levels may be a useful predictor of CF patients who are at risk of developing ABPA.

7.3.8. Transplantation

Bronchiolitis obliterans (BOS), a major mid- and long-term complication of lung transplantation is a chronic inflammatory disorder affecting predominantly the terminal and respiratory bronchioles. It causes an obstructive syndrome that subsequently leads to graft failure. Diagnosis of obliterative bronchiolitis (OB) and BOS depends on changes in pulmonary function after lung transplantation (a gradual decrease in FEV₁ or FEF in midexpiratory phase), with or without pathological proof of OB. [Cooper JD *et al.* 1993] Using these criteria, BOS can be diagnosed only when a loss of $\geq 20\%$ of the initial best postoperative FEV₁ has occurred. Consequently much research is now focused on the establishment of early markers for chronic rejection after lung transplantation. Exhaled NO is also being investigated as a potential marker.

An increase in exhaled NO is observed in lung transplant recipients with chronic rejection. [Fisher AJ *et al.* 1998; Verleden GM *et al.* 1998] A preliminary study involving lung and cardiac transplant patients showed that single-breath exhaled NO levels were strikingly different in patients suffering from BOS. [Zegdi R *et al.* 1999] In these patients, the decrease from peak-to-end expiratory NO concentration was slower. At mid-expiration, NO levels were approximately three times higher in the BOS group. The increase in mid-expiratory NO levels did not appear to be related to the syndrome itself. High exhaled NO levels are a strong diagnostic marker for BOS. Thirty-two patients were followed for up to 2 years after receiving a lung transplant and 13 developed BOS. [Verleden GM *et al.* 2004] All but one of the patients who developed BOS had two consecutive NO measurements of ≥ 15 ppb (at 200 mL/s) in the months preceding the diagnosis. In comparison, only three of the 19 patients who did not develop BOS had such NO measurements – resulting in a diagnostic accuracy of 88%.

Gabbay and colleagues showed that exhaled NO levels, although normal, reflected the degree of airway inflammation in 20 stable lung transplant recipients (mean age 49 ± 3 years). [Gabbay E *et al.* 1999] Using regression analysis they showed that the percentage of bronchiolar lavage neutrophils ($r_2 = 0.82$; $p < 0.0001$) and iNOS expression in the bronchial

epithelium ($r_2 = 0.75$; $p < 0.0001$), but not in the lamina propria ($r_2 = 0.16$; $p = 0.08$), were positively predictive of exhaled NO levels. The same group went on to show that in BOS, exhaled NO levels were increased in association with even greater airway neutrophilia and enhanced expression of iNOS in the bronchial epithelium. [Gabbay E *et al.* 2000] Exhaled NO has also been shown to be higher in patients with unstable BOS than patients with stable BOS (18.4 vs. 9.7 ppb at 50 mL/s). [Brugiere O *et al.* 2005] Verleden and colleagues demonstrated that a switch from cyclosporine to tacrolimus therapy stabilized FEV₁ in patients with chronic rejection levels accompanied by a decrease in exhaled NO (Figure VII.5). [Verleden GM *et al.* 2003b] These results suggest that exhaled NO level measurements can be valuable

in guiding the treatment of chronic rejection after lung transplantation. The same group also showed that there was no significant difference in exhaled NO levels between patients with chronic rejection who underwent single lung transplantation and those who underwent sequential single lung transplant and heart/lung transplant. [Verleden GM *et al.* 2003a]

7.3.9. Pulmonary Hypertension

It is known that NO is a potent vasodilator and therefore reduced NO levels may have a pathological role in pulmonary hypertension. Indeed, several studies suggest that pulmonary hypertension may be associated with low exhaled NO levels. For example, Cremona and colleagues showed that the rate of NO production in patients with primary pulmonary hypertension was 2.85 ± 0.7 nM/min compared with 4.69 ± 0.35 nM/min in healthy individuals. [Cremona G *et al.* 1994] Exhaled NO has been shown to decrease in response to a decrease in pulmonary blood flow [Tworetzky W *et al.* 2000] and positive correlations between blood flow and NO have been shown. [Hoit BD *et al.* 2005] Kharitonov and co-workers have shown that concomitant pulmonary hypertension (PHT) in patients with systemic sclerosis (SSc) is associated with low exhaled NO levels, whereas systemic sclerosis without this complication is associated with increased NO (Figure VII.6). [Kharitonov SA *et al.* 1997a]

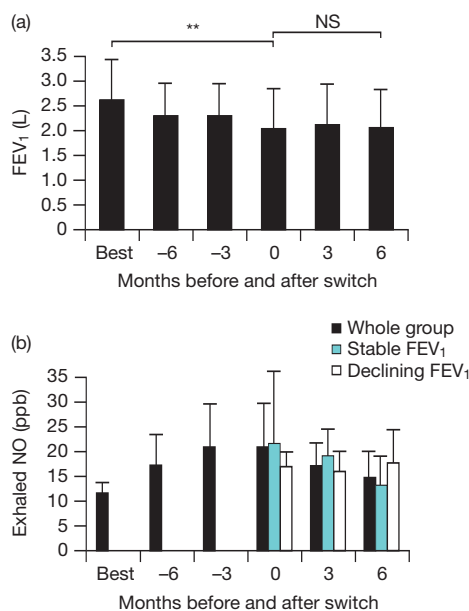


Figure VII.5. After switching treatment from cyclosporine to tacrolimus there was (a) a significant decline in FEV₁, with stabilization after Time 0 (** $p = 0.0047$); (b) a gradual decline in exhaled NO levels after Time 0 in the whole group [Verleden GM *et al.* 2003b]

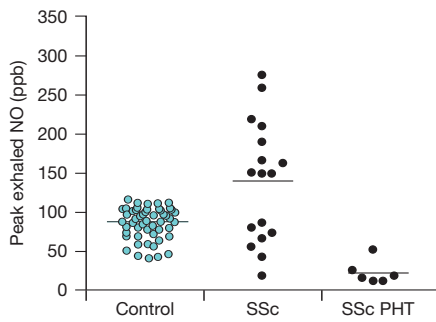


Figure VII.6. Exhaled NO levels (at 500 mL/min) in patients with systemic sclerosis and pulmonary hypertension [Kharitonov SA et al. 1997a]

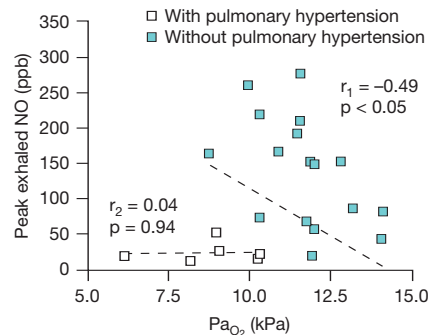


Figure VII.7. Correlation between exhaled NO and arterial oxygen tension in patients with systemic sclerosis with or without pulmonary hypertension [Kharitonov SA et al. 1997a]

In addition, there was a negative linear correlation between NO and arterial oxygen tension (Figure VII.7). Similar results have been found by others. [Rolla G *et al.* 2000] Girgis and colleagues have also reported that NO levels are low in patients with pulmonary arterial hypertension. [Girgis RE *et al.* 2005]

Not all studies have, however, shown that pulmonary hypertension is associated with low NO levels. No differences between controls and patients with the condition have been reported [Forrest IA *et al.* 1999; Girgis RE *et al.* 2002] and Gölbası *et al.* reported that concomitant pulmonary hypertension was associated with an increase in NO levels in patients with rheumatic heart disease. [Golbası Z *et al.* 2001] In this study, patients without pulmonary hypertension had exhaled NO levels similar to healthy controls. Rolla and co-workers found that exhaled NO levels did not correlate with pulmonary arterial pressure in patients with mitral stenosis. [Rolla G *et al.* 2003] Interestingly, Özkan *et al.* showed that patients with primary pulmonary hypertension (PPH) not treated with prostacyclin had lower exhaled NO levels than controls (4.7 ppb vs. 6.6 ppb), whereas those with secondary pulmonary hypertension (SPH) had NO levels similar to controls (7.0 ppb vs. 6.6 ppb) (Figure VII.8). [Ozkan M *et al.* 2001] In patients with primary pulmonary hypertension, it has been shown that exercise does not lead to the usual increase in rate of NO production. [Riley MS *et al.* 1997] Clearly, further insights are required.

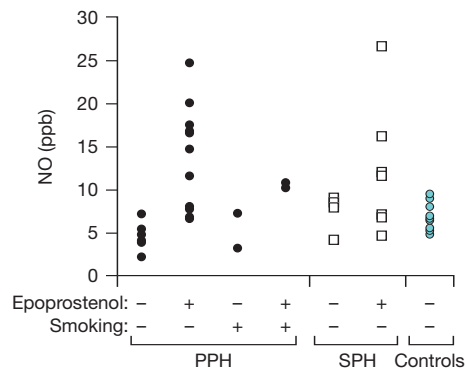


Figure VII.8. Exhaled NO levels in patients with primary and secondary pulmonary hypertension [Ozkan M et al. 2001]

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Nevertheless, monitoring NO levels in patients with pulmonary hypertension may be of value. Machado and co-workers assessed NO levels in patients with pulmonary hypertension over 2 years. [Machado RF *et al.* 2004] They found that in those patients who survived the 2-year follow-up, exhaled NO levels doubled during the study. Moreover in these survivors, NO levels at entry in the study correlated with the change in pulmonary arterial pressure during the follow-up. Exhaled NO levels tended to decrease in those patients who died during the study. Thus, exhaled NO may predict worsening of pulmonary hypertension. Most of the patients in this study were receiving prostacyclin therapy. This treatment has been shown to increase NO levels in patients with pulmonary hypertension. [Forrest IA *et al.* 1999; Ozkan M *et al.* 2001] Beghetti *et al.* have reported that exhaled NO levels decrease by 27% following cardiopulmonary bypass indicating a risk of postoperative pulmonary hypertension. [Beghetti M *et al.* 1998]

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