

V. Measurement of Exhaled NO

The most widely used method for measurement of exhaled NO is chemiluminescence. This method allows measurement to approximately 1 ppb. [Lundberg JO *et al.* 1996b] However, a new sensor technology has been applied to allow the measurement of exhaled NO with a hand-held device suitable for use in routine clinical practice. [Hemmingson T *et al.* 2004] Joint guidelines by the European Respiratory Society (ERS) and the American Thoracic Society (ATS) recommend a flow rate of 50 mL/s. [American Thoracic Society/European Respiratory Society 2005]

5.1. Clinical Online Analysis

Exhaled NO is usually determined during single-breath exhalation. The recommended technique for adult patients involves inspiration of NO-free air via a mouthpiece to total lung capacity, followed immediately by full exhalation at an even rate through the mouthpiece into the apparatus. [American Thoracic Society/European Respiratory Society 2005]

During exhalation closure of the velum prevents contamination of the sample with nasal air. This is important as nasal air contains high concentrations of NO, probably derived from paranasal sinuses (Figure V.1). [Lundberg JO *et al.* 1995; Lundberg JO *et al.* 1994a]

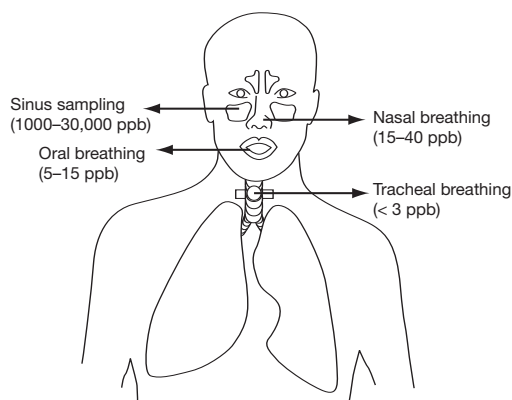


Figure V.1. Schematic drawing showing NO levels measured by chemiluminescence at different levels of the respiratory tract. The subjects were breathing normal tidal volumes through the nose, mouth or through a permanent tracheostomy. Sinus air was aspirated through a catheter placed in the maxillary sinus of healthy subjects [Courtesy of Prof. Jon Lundberg, MD]

Levels of NO in ambient air may be high and have been shown to influence the measurement of exhaled NO, [Baraldi E *et al.* 1998b] although some investigators have not confirmed this. [Piacentini GL *et al.* 1998] It is therefore recommended that NO-free air (< 5 ppb) is inhaled, which can be achieved, for example, by absorption of NO in a scrubber. In the short term, spirometry, hyperresponsiveness test and sputum induction appear

to reduce exhaled NO levels. Therefore, NO should be measured prior to these tests. [Antczak A *et al.* 2005; Barreto M *et al.* 2006; Deykin A *et al.* 1998; Gabriele C *et al.* 2005; Ho LP *et al.* 2000; Kissoon N *et al.* 2002; Piacentini GL *et al.* 2000b; Silkoff PE *et al.* 1999; Tee AK and Hui KP 2005]

The ATS and ERS have developed joint guidelines on how exhaled NO measurements should be performed. [American Thoracic Society/European Respiratory Society 2005] Measurements performed according to these guidelines are called Fractional Exhaled Nitric Oxide ($F_{E_{NO}}$). The guidelines state that exhaled NO measurements must be performed at a controlled and standardized exhalation flow rate as the $F_{E_{NO}}$ value is highly flow dependent.

5.1.1. Practical Aspects

- The patient inhales NO-free air to total lung capacity (TLC) in order to trigger the 10-second exhalation, through the mouthpiece.
- The subject then starts an exhalation against a constant, positive counter pressure of 10–20 cm H₂O to ensure an exhalation flow rate of 50 mL/s according to ATS guidelines. [American Thoracic Society/European Respiratory Society 2005]
- The pressure parameters are controlled by both visible and audible feedback in order to guide the subject in performing a valid exhalation manoeuvre.
- Exhalation pressures outside the specified 10–20 cm H₂O limitation render an erroneous test and an error message is shown on the display.
- A valid breathing manoeuvre at a flow rate of 50 mL/s is considered if the mean exhalation rate is 50 ± 5 mL/s, in accordance with the ATS guidelines, [American Thoracic Society/European Respiratory Society 2005] which is important as the exhaled NO levels are flow dependent [Lundberg JO *et al.* 1996b; Pedroletti C *et al.* 2000; Silkoff PE *et al.* 1997] and may otherwise vary considerably. The results are processed using dedicated software, expressed as the NO concentration in ppb.

Summary of procedure:

- Empty lungs
- Inhale deeply through disposable filter to total lung capacity
- Exhale through filter
- View results on the display

5.1.2. Reproducibility

A pre-requisite for the routine clinical use of exhaled NO in asthma management is that the method shows good reproducibility. Kharitonov and colleagues have shown that highly reproducible measurements can be obtained using the NIOX® Nitric Oxide monitoring system. [Kharitonov SA *et al.* 2003] The study demonstrated that the mean standard deviation in NO measurements was 2.1 ppb, suggesting that a change in exhaled NO of 4 ppb is likely to result from a change in the status of the inflammation (Figure V.2). In another study, the mean standard deviation of NIOX was

shown to be < 2.0 ppb. [Alving K *et al.* 2006] In this study, data from NIOX were compared with data from NIOX MINO® – the new hand-held airway inflammation monitor. A correlation ($p < 0.001$) was seen between the results from the two devices, with the mean standard deviation in NO measurements with NIOX MINO being < 2.5 ppb. Gill *et al.* found a standard deviation less than 3 ppb for NO measurements < 30 ppb, and mean coefficient of variation < 10% for NO measurements > 30 ppb with the NIOX MINO. [Gill M *et al.* 2006]

It should be noted that inpatient and outpatient variability can affect levels of exhaled NO. [Grasemann H *et al.* 2000; Wechsler ME *et al.* 2000] Exhaled NO levels may also vary over time in patients with stable asthma. [Whelan GJ *et al.* 2003] Stark *et al.* found that exhaled NO levels in healthy subjects were lower in the morning compared with the afternoon, but were highly reproducible day-to-day, week-to-week, and across seasons, indicating that during long-term monitoring, NO levels should be measured at the same time of the day. [Stark H *et al.* 2007]

5.1.3. Reference Values for Healthy Individuals

Data on reference values using the standard exhalation rate of 50 mL/s are rapidly increasing (Table V.1). The studies show that healthy individuals usually have exhaled NO values between 5 and 35 ppb (children slightly lower, 5–25 ppb), if measured according to ERS/ATS guidelines. Analysis of the variation seen in such studies, suggests that 97% of healthy individuals have NO levels of less than 35 ppb (< 25 ppb in children). The lower values reported in children indicate an age dependence of exhaled NO levels. Indeed, it has been

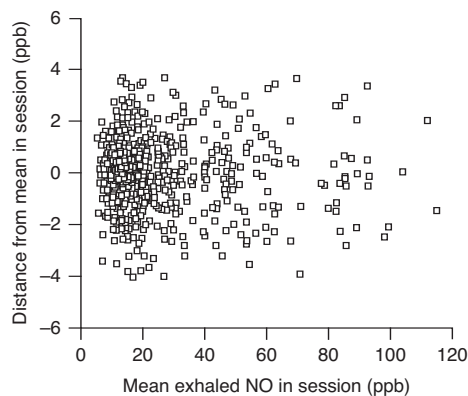


Figure V.2. Bland-Altman analysis for the repeatability of fractional exhaled NO values ($n = 637$ measurements). Measured with NIOX® [Kharitonov SA *et al.* 2003]

Table V.1. Reference values for healthy individuals (50 mL/s unless stated).

Authors, year	Participants	Exhaled NO (ppb)	Notes
Children			
Jöbbsis <i>et al.</i> , 2001	73 children	10.5 ± 1.1	Online method
Kharitonov <i>et al.</i> , 2003	20 children	15.6 ± 9.2	Measurements in morning and afternoon
Kissoon <i>et al.</i> , 2000	32 adolescents	36.9 ± 25.4	Flow rate of 46 mL/s
Scollo <i>et al.</i> , 2000	23 children	10.1 ± 4.1	
Malmberg <i>et al.</i> , 2003	62 children	5.3 ± 0.4	
Buchvald <i>et al.</i> , 2005a	405 children	9.7	
Wong <i>et al.</i> , 2005	111 Chinese boys 147 Chinese girls 33 Caucasian boys 18 Caucasian girls	25.3 15.8 14.9 10.1	
Malmberg <i>et al.</i> , 2006	114 children	10.3	
Santamaria <i>et al.</i> , 2005	40 children	10.0	
Adults			
Chatkin <i>et al.</i> , 1999	23 adults	28.3	Flow rate of 45 mL/s
Olin <i>et al.</i> , 2001b	202 non-smokers Atopic – without rhinitis – rhinitis Non-atopic – without rhinitis – rhinitis	14.7 ± 2.3 31.1 ± 7.6 15.5 ± 0.9 18.1 ± 1.8	NO measured outside pollen season
ElHalawani <i>et al.</i> , 2003	42 adults	25.6	
Haight <i>et al.</i> , 2006a	23 younger adults (median age 24 years) 25 older adults (median age 72 years)	18.7 36.9	
Olivieri <i>et al.</i> , 2006	204 adults	10.8 ± 4.7	
Olin <i>et al.</i> , 2006a	2295 adults	16.0	

shown that NO levels increase with age in children. [Buchvald F *et al.* 2005a; Malmberg LP *et al.* 2006; Santamaria F *et al.* 2005] Malmberg *et al.* demonstrated that exhaled NO was significantly associated with age and height (both $p < 0.0001$) in healthy children (Figure V.3). [Malmberg LP *et al.* 2006] Height was found to be the best independent variable for the regression equation for exhaled NO, which on average showed an increase in the height range of 120–180 cm from 7 to 14 ppb. Racial differences in exhaled NO were shown in a study by Wong *et al.*, in which healthy Chinese schoolchildren had significantly higher levels compared with Caucasians. [Wong GW *et al.* 2005]

Height correlates with exhaled NO levels, possibly as a result of larger airway calibre. [Olin A-C *et al.* 2005; Tsang KW *et al.* 2001] Studies have shown that males have higher NO levels than females. [Kharitonov SA *et al.* 2003; Olivieri M *et al.* 2006; Tsang KW *et al.*

2001; Wong GW *et al.* 2005] Olin and co-workers found that height, age, atopy, reporting of asthma symptoms in the last month, and reported use of inhaled steroids were positively associated with exhaled NO, while current smokers had lower NO, and gender was not associated with exhaled NO levels (Table V.2). [Olin AC *et al.* 2006b]

Exhaled NO levels vary with age in adults. In a study of healthy individuals by Haight *et al.*, and confirmed by Olin, median exhaled NO values were 18.7 ppb in younger (median age 24 years) and 36.9 in older adults (median age 72 years) ($p < 0.001$). [Haight RR *et al.* 2006]

In addition to age, other physical factors may affect NO levels. de Winter-de Groot *et al.* have reported that exhaled NO levels increase with increasing body mass index (BMI) in adults. [de Winter-de Groot KM *et al.* 2005] They speculate that obesity, which is known to result in low-level systemic

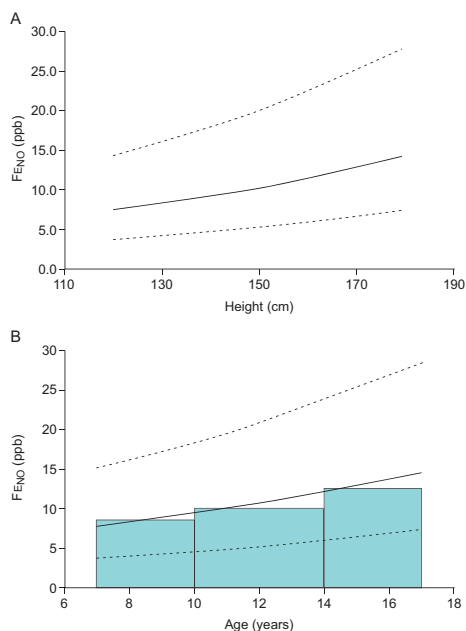


Figure V.3. Predicted $F_{E_{NO}}$ (solid line) as function of height (A) and age (B) and 95% prediction intervals (dashed lines). [Malmberg LP *et al.* 2006] In B, mean data (bars) of healthy non-atopic children are included for comparison. [Buchvald F *et al.* 2005a]

Table V.2. Values of exhaled NO among 2,200 subjects in a random population study by smoking habits, gender and atopy. [Olin AC *et al.* 2006b]

Variables	Never-smokers (n = 1,090)	Ex-smokers (n = 697)	p-value‡	Current smokers (n = 413)	p-value§
All	17.1 (12.7–23.6)	16.9 (12.2–24.0)	0.63	10.6 (7.6–15.8)	< 0.001
Gender					
Female	15.8 (11.7–21.5)	15.8 (11.1–21.9)	1.0	10.1 (7.0–15.2)	< 0.001
Male	18.8 (13.7–25.5)	17.5 (13.1–25.4)	0.32	11.6 (8.4–16.2)	< 0.001
Atopy†					
Yes	19.6 (13.8–30.1)¶	18.1 (13.0–28.4)¶	0.22	13.2 (9.1–17.2)¶	< 0.001
No	16.6 (12.2–22.0)	16.5 (12.2–23.1)	0.56	10.4 (7.4–15.1)	< 0.001
Physician-diagnosed asthma					
Yes	19.9 (14.6–31.4)¶	14.8 (10.5–27.2)	0.047	10.5 (6.6–18.9)	0.001
No	17.0 (12.7–23.5)	17.0 (12.5–23.9)	1.0	10.6 (7.6–15.4)	< 0.001

* Data are presented as median (25th to 75th percentile).

† Positive Phadiatope response.

‡ Ex-smokers vs. never-smokers.

§ Current smokers vs. never-smokers.

|| Female vs. male subjects $p > 0.05$.

¶ Yes vs. no $p < 0.05$.

inflammation, may cause inflammation in the airways. In contrast, Maniscalco *et al.* reported reduced levels of exhaled NO in severely obese subjects compared with healthy controls, which were reduced to control levels after weight reduction. [Maniscalco M *et al.* 2006] Interestingly, it has been reported that there is no relationship between age-adjusted BMI and exhaled NO levels in children. [Santamaria F *et al.* 2005] This could be due to the possibility that obese children have had relatively less exposure to proinflammatory adipokines than obese adults. Also of note is that the relationship between BMI and NO levels in adults, is lost in patients with asthma. [Kazaks A *et al.* 2005]

5.1.4. Reference Values for Asthmatic Patients

Following the early publications by Alving [Alving K *et al.* 1993] and Kharitonov, [Kharitonov SA *et al.* 1995a; Kharitonov SA *et al.* 1994] many reports have confirmed the increased levels of exhaled NO in patients with asthma.

Table V.3 lists studies that have found NO to be elevated by two- to four-fold compared with matched controls. Considering the data presented in Table V.3, asthma patients tend to have exhaled NO values between 25 and 80 ppb if measured according to ERS/ATS guidelines; higher values may occur in some patients, particularly those with exacerbations.

As the determination of exhaled NO is easy and non-invasive, the method appears to be particularly attractive for paediatric use, and several authors have studied NO levels in children with asthma. [Baraldi E *et al.* 1998b; Frank TL *et al.* 1998; Lundberg JO *et al.* 1996a]

Table V.3. Reference values for asthmatic patients (50 mL/s).

Authors, year	Exhaled NO (ppb)		Ratio
	Asthma patients	Controls	
Kharitonov <i>et al.</i> , 2003	24.9 ± 22.3 (children)	15.6 ± 9.2	1.6
Olin <i>et al.</i> , 2004b	52.5 (adults)	15.7	3.3
Kharitonov <i>et al.</i> , 2003	61.7 ± 48.4 (adults)	17.8 ± 6.8	3.5
Scollo <i>et al.</i> , 2000	76.2 ± 26.2 (children)	10.1 ± 4.1	7.5
Malmberg <i>et al.</i> , 2003	22.1 ± 3.4 (children)	5.3 ± 0.4	4.2
Sacco <i>et al.</i> , 2003	15.8 (children)	9.8	1.6
Silvestri <i>et al.</i> , 2003	16.0 (children)	6.8	2.4
Smith <i>et al.</i> , 2004	52.0 ± 34.0 (both)	15.7 ± 12.9	3.3
Leung <i>et al.</i> , 2005	78 ± 59 (children)	40 ± 43	2.0
Baraldi <i>et al.</i> , 2000	31.4 ± 2.5 (children)	9.6 ± 0.7	3.3
Olin <i>et al.</i> , 2001b	43.5 (adults)	20.4	2.1
Zietowski <i>et al.</i> , 2006	84.0 ± 51.4 (allergic adults)	12.9 ± 4.6	6.5
	45.8 ± 32.6 (non-allergic adults)		3.6

Zietkowski *et al.* assessed exhaled NO in patients with allergic and non-allergic asthma. [Zietkowski Z *et al.* 2006a] Compared to the healthy control group, exhaled NO in both groups of asthma patients was significantly elevated (12.9 ppb, $p < 0.0001$) (Figure V.4). Exhaled NO in the allergic asthma group was significantly higher than in patients with non-allergic asthma (84.0 ppb vs. 45.8, $p = 0.0001$). A relationship between NO levels and asthma severity was shown in the allergic asthma group, where higher exhaled NO levels were found in patients with moderate asthma than in those with mild asthma (109.0 ppb vs. 75.66 ppb, $p = 0.03$).

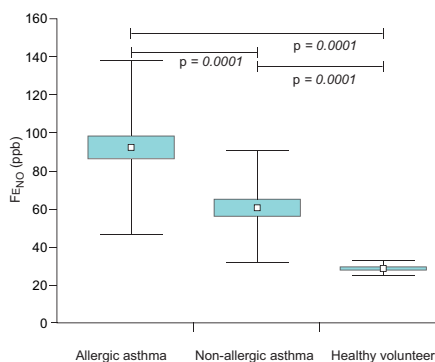


Figure V.4. Exhaled NO in healthy controls and subjects with allergic and non-allergic asthma [Zietkowski Z *et al.* 2006a]

5.2. Clinical Relevance

5.2.1. Exhaled NO in Clinical Guidelines

Respiratory societies have produced clinical guidelines on the use of exhaled NO in the management of asthma. Joint guidelines from the ATS and ERS provide detailed recommendations on standardized procedures for the online and offline measurement of exhaled and nasal NO in adults and children. [American Thoracic Society/European Respiratory Society 2005] An ATS workshop was held in September 2005 specifically to review exhaled NO measurement and its clinical application. [Silkoff PE *et al.* 2006] ATS/ERS guidelines have also been developed for use in paediatric medicine, including recommendations for procedures that are suitable for infants and young children. [Baraldi E and de Jongste JC 2002]

The Global Initiative for Asthma (GINA) guidelines for asthma management and prevention recognize that exhaled NO is increasingly used to monitor the effectiveness of asthma treatment, but provide no specific recommendations on procedures or interpretation. [Global Initiative for Asthma 2006] The potential clinical value of monitoring exhaled NO levels is also anticipated to be discussed in the 2007 update of the US National Heart Lung and Blood Institute (NHLBI) guidelines on the diagnosis and management of asthma, though at the time of writing this report was yet to be published (a draft form had been temporarily available).

5.2.2. Clinical Guide for Interpretation

Taylor *et al.* developed two algorithms for interpreting exhaled NO results in day-to-day practice – one for diagnostic use (Table V.4) and one for ongoing asthma management in patients treated with anti-inflammatory medications (Table V.5). [Taylor DR *et al.* 2006]

Table V.4. Exhaled NO as an aid to the diagnosis of chronic respiratory symptoms. [Taylor DR *et al.* 2006] (Modified after personal communication with the author).

			Interpretation (as an aid to diagnosis of chronic respiratory symptoms)	
FE _{NO} (ppb)	Classification	Eosinophilic airway inflammation	Adults	Children
Adults: 5–25 Children (< 12 years old): 5–20	Low	Unlikely	Consider: Neutrophilic asthma Anxiety/hyperventilation Vocal cord dysfunction Rhinosinusitis Gastro-oesophageal reflux Cardiac disease	Consider: Wheezy bronchitis Gastro-oesophageal reflux ENT disorders Cystic fibrosis Primary ciliary dyskinesia (FE _{NO} < 5 ppb), (check nasal NO) Congenital abnormalities, e.g. airway malacia Other immunodeficiencies
Adults: 25–50 Children: 20–35	Intermediate	Present but mild	Interpretation based on clinical presentation	Interpretation based on clinical presentation
Adults: > 50 Children: > 35	High	Significant	Consider: <i>Atopic asthma if the history is appropriate.</i> If FEV ₁ < 80% predicted, diagnosis of asthma is very likely Eosinophilic bronchitis Churg-Strauss syndrome A positive response to a trial of inhaled or oral steroid is likely. In ex-smokers with COPD this may also be true	If combined with any objective evidence of reversible airway obstruction, asthma is very likely and a positive response to a trial of inhaled or oral steroids is likely

Table V.5. Exhaled NO as an aid to the management of asthma. [Taylor DR *et al.* 2006] (Modified after personal communication with the author).

Interpretation (as an aid to the management of asthma)				
FE _{NO} (ppb)	Classification	Eosinophilic airway inflammation	Adults	Children
Adults: 5–25 Children (< 12 years old): 5–20	Low	Unlikely	<p>If <i>symptomatic</i>, review diagnosis</p> <p>Neutrophilic asthma Anxiety/hyperventilation Vocal cord dysfunction Rhinosinusitis Gastro-oesophageal reflux</p> <p>If <i>asymptomatic</i> and taking ICS: Implies good compliance with treatment. Reduce dose or, in case of low ICS dose, even withdraw ICS altogether</p>	<p>If <i>symptomatic</i>, review diagnosis</p> <p>Consider also: Wheezy bronchitis Cystic fibrosis Congenital abnormalities, e.g. airway malacia Primary ciliary dyskinesia</p> <p>If <i>asymptomatic</i> and taking ICS: as for adults</p>
Adults: 25–50 Children: 20–35	Intermediate	Present but mild	<p>If <i>symptomatic</i>, consider: Infection as reason for worsening High levels of allergen exposure Adding in other therapy apart from ICS (e.g. long acting β_2-agonist) Consider ICS dose increase</p> <p>If <i>asymptomatic</i>: No change in ICS dose if patient is stable</p>	<p>If <i>symptomatic</i> (besides considerations in adults), consider: Possible inadequate ICS treatment (1) check compliance (2) check for poor inhaler technique and consider metered dose inhaler and spacer if patient is currently using a dry powder device</p> <p>If <i>asymptomatic</i>: as for adults</p>
Adults: > 50 Children: > 35	High (or rise of 60% or more since previous measurement)	Significant	<p>If <i>symptomatic</i>, consider: Inadequate ICS treatment: (1) check compliance (2) check for poor inhaler technique (3) inadequate ICS dose Continuous high level allergen exposure Imminent exacerbation or relapse depending on history of individual patient. More likely if ICS dose is zero Steroid resistance (rare)</p> <p>If <i>asymptomatic</i>: No change in ICS dose if patient is stable</p>	<p>If <i>symptomatic</i> (besides considerations in adults) consider: Metered dose inhaler and spacer if patient is currently using a dry powder device</p> <p>If <i>asymptomatic</i>: as for adults</p>

The Taylor article uses knowledge from clinical research work performed by the authors and suggests a guide for how exhaled NO values should be interpreted in a clinical setting. However, the cut-off points outlined in the Thorax article were unfortunately printed incorrectly. The correct ranges are: Normal: 5–25 ppb (adults), or 5–20 ppb (children); Intermediate: 25–50 ppb (adults), or 20–35 ppb (children); and High: > 50 ppb (adults), or > 35 ppb (children). These values are from personal communication with the authors.

Elevated NO levels in a symptomatic asthma patient indicate uncontrolled eosinophilic airway inflammation. This is most likely due to poor compliance with anti-inflammatory treatment or poor inhaler technique rather than inadequate ICS dosing. Taylor *et al.* postulate that persistently high exhaled NO levels despite a seemingly adequate inhaled drug regime may be due to allergen exposure, overexpression of constitutive steroid resistant NOS, or may be derived from alveolar NO rather than bronchial NO. In the latter scenario, a better clinical response may be achieved using oral rather than inhaled anti-inflammatory treatment.

A low level of exhaled NO implies the absence of eosinophilic airway inflammation. In non-smokers, an alternative or additional diagnosis to atopic asthma should be considered if the patient has respiratory symptoms with low exhaled NO, such as non-atopic asthma, gastro-oesophageal reflux disease, rhinosinusitis with postnasal drip, or left ventricular dysfunction.

It is recommended that individualized exhaled NO targets are devised for patients. [Taylor DR *et al.* 2006]

5.3. Factors Influencing Levels of Exhaled NO

Airway inflammation, asthma and atopy are the major causes of increased levels of exhaled NO (see Chapter III. Physiological Background). Levels of exhaled NO can also be influenced by other factors. The daily clinically relevant factors are airway viral infection, allergic rhinitis and recent intake of a nitrate-rich diet, all of which may increase the level of exhaled NO. Conversely, spirometric manoeuvres, exercise, bronchoconstriction and smoking may decrease levels of exhaled NO (Figure V.5). [Henriksen AH *et al.* 1999; Murphy AW *et al.* 1998; Olin AC *et al.* 2001a; Piacentini GL *et al.* 2002; Silkoff PE *et al.* 1999; Terada A *et al.* 2001; Verleden GM *et al.* 1999] In most instances, however, the differential diagnosis is not difficult.

The finding that NO levels are reduced immediately after bronchoconstriction caused by histamine or methacholine challenge shows that hyperresponsiveness tests should be performed after NO measurements. [Ho LP *et al.* 2000; Piacentini GL *et al.* 2002] In addition, spirometry and measurement of maximal expiratory pressures reduces NO levels

and should also be avoided before NO measurement. [Barreto M *et al.* 2006] Another interesting finding is that NO levels increase in the period after a nitrate-rich meal is eaten. [Olin AC *et al.* 2001a] Furthermore, exhaled NO levels remain high up until 20 hours after consumption of a nitrate-rich meal. [Verges S *et al.* 2005] Although further studies are needed to confirm this finding, physicians may consider recommending avoidance of high-nitrate foods within a day of NO measurement.

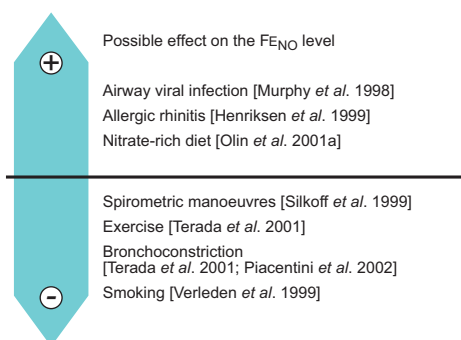
5.3.1. Diurnal variation

Ambulatory measurement of exhaled NO in asthmatic individuals shows that morning levels are higher than those in the evening. [Pijnenburg MW *et al.* 2006]

Healthy subjects also have some degree of diurnal variation in exhaled NO levels (lower levels in the morning compared with the afternoon), while the reproducibility from day-to-day is high. [Stark H *et al.* 2007] Exhaled NO levels are slightly but significantly lower during the night than during the day in patients with nocturnal asthma. [Georges G *et al.* 1999] The mean value of 77.2 ± 8.2 ppb fell to 68.4 ± 8.7 ppb at 22:00 h and to 66.0 ± 8.5 ppb at 04:00 h. In another study involving patients with nocturnal asthma, iNOS levels in bronchial biopsies increased during the day, but not during the night. [ten Hacken NH *et al.* 2000] Patients in this study with non-nocturnal asthma did not show any significant diurnal variation. Kharitonov and co-workers found no evidence of diurnal variation in exhaled NO levels in healthy and asthmatic children and adults. [Kharitonov SA *et al.* 2003] However, in an earlier study, in which exhaled NO was measured every third hour in children with asthma, exhaled NO demonstrated a cosine-like circadian rhythm with lowest levels at 19:00 h and highest at 07:00 h. [Mattes J *et al.* 2002]

5.3.2. Smoking

Smokers have an increased risk of respiratory infections and pulmonary-vascular complications, in addition to chronic respiratory disorders. A lack of endogenous NO may play a role in the increased risk of some of these disorders. For example, Kharitonov and



Always perform exhaled NO measurements first, prior to any other respiratory test.

Always check if the patient:

- has an upper or lower airway infection
- is a smoker
- has consumed food or liquid in the last hour

Smoking reduces exhaled NO. Smokers normally have FE_{NO} levels between 2 and 10 ppb. Increased FE_{NO} levels in smokers are still significant for ongoing eosinophilic inflammation. However, near-normal levels are difficult to analyse. Results should be interpreted with great caution.

Figure V.5. Factors affecting exhaled NO levels

co-workers showed that exhaled NO levels were more than 50% lower in smokers compared with non-smokers. [Kharitonov SA *et al.* 1995b] Others have reported similar results. [Hogman M *et al.* 2002a; Marteus H *et al.* 2004; Robbins RA *et al.* 1997] Nasal NO levels are also reduced in smokers. [Robbins RA *et al.* 1997] Malinovschi *et al.* demonstrated that the reduction in exhaled NO levels seen in ex- and current smokers was associated with a lower total airway NO in ex-smokers and reduced airway and alveolar nitric oxide concentrations in current smokers. [Malinovschi A *et al.* 2006a] Interestingly, exhaled NO levels are higher in smokers with asthma than in healthy smokers, suggesting that exhaled NO may still be a useful marker of airway inflammation in smokers. [Horvath I *et al.* 2004]

Passive smoking may slightly reduce exhaled NO levels. Maniscalco *et al.* reported that exhaled NO levels in healthy individuals fell from 16.7 ± 1.4 ppb to 13.9 ± 1.33 after short-term exposure to environmental cigarette smoke. [Maniscalco M *et al.* 2002] However, the decrease was transient, recovering within 30 minutes. Yates and colleagues also found a temporary 24% decrease in NO levels after exposure to environmental cigarette smoke. [Yates DH *et al.* 2001] Notably, active cigarette smoking was associated with continuously low exhaled NO. One study involving children, however, suggested that exhaled NO levels were not reduced in healthy individuals exposed to tobacco smoke. [Warke TJ *et al.* 2003a] In children with asthma, passive smoking was associated with a decrease in exhaled NO. [Warke TJ *et al.* 2003a]

The cause of the decrease in NO levels associated with smoking and smoke exposure requires further investigation. Research suggests that cigarette smoke decreases the activity of iNOS in lung epithelial cells [Hoyt JC *et al.* 2003] and eNOS in pulmonary artery endothelial cells. [Su Y *et al.* 1998] Cigarette smoke reduces NO levels produced in the oropharyngeal tract. However, it has been shown that iNOS expression is not reduced in oropharyngeal biopsies, nor is there evidence of reduced non-enzymatic formation of NO in this region. [Marteus H *et al.* 2004] Ryttilä *et al.* demonstrated higher levels of iNOS-positive cells in the sputum of smokers than non-smokers. [Ryttilä P *et al.* 2006] In a study by Högman *et al.*, diffusion modelling showed that NO flux from the airways was reduced in smokers, but alveolar NO was increased. [Hogman M *et al.* 2002a]

Whatever the mechanism affecting NO levels in smokers is, it does appear to be reversible. In the Högman study, airway NO flux increased to levels similar to non-smokers in patients who stopped smoking for 4 weeks. [Hogman M *et al.* 2002a] Another study showed that exhaled NO levels increased after just 1 week of not smoking and had increased again by 8 weeks (Figure V.6). [Robbins RA *et al.* 1997] In contrast, individuals who failed to stop smoking did not show an increase. These reports suggest that measurement of exhaled NO levels may have a role in smoking cessation programmes. An increase in NO levels may

SCIENTIFIC BACKGROUNDER

be a simple method of demonstrating improvement to an individual and thus encourage continued abstinence. In addition, NO levels may highlight lack of compliance with a cessation programme.

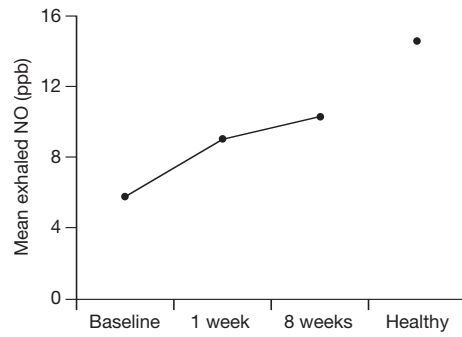


Figure V.6. Mean exhaled NO levels (10 mL/s) in smokers following cessation of smoking and in healthy controls [Robbins RA et al. 1997]

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