

Endogenous nitric oxide in allergic airway disease

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There has been intense research into the role nitric oxide (NO) plays in physiologic and pathologic mechanisms. The presence of NO in exhaled breath and the high concentrations in nasal airways stimulated many studies examining exhaled and nasal NO as potential markers of airway inflammation, enabling repeated monitoring of airway inflammation not possible with invasive tests (eg, bronchoscopy). In airway inflammation, NO is not merely a marker but may have anti-inflammatory and proinflammatory effects. Nasal NO measurement may be used in the noninvasive diagnosis and monitoring of nasal disease. This review was compiled by speakers who gave presentations on NO at the annual meeting of the American Academy of Allergy, Asthma, and Immunology in 1999 on exhaled and nasal NO, in vitro studies of NO, the chemistry of airway NO formation, and standardized measurement of exhaled mediators. (*J Allergy Clin Immunol* 2000;105:438-48.)

Key words: Nitric oxide, nasal, asthma, rhinitis

The action of endothelial-derived relaxing factor, a vascular smooth muscle relaxant, was found to depend on nitric oxide (NO) synthesis in 1987, leading to much research into this mediator. There are now thousands of publications about NO in diverse fields of medicine, including allergic pulmonary disease. NO is produced by NO synthases (NOS) of constitutive forms (cNOS) mediating physiologic functions, and an inducible form (type II NOS) (iNOS) is expressed in pathologic states (eg, inflammation) (Table I). The cNOS forms include membrane-bound endothelial NOS (eNOS or type III NOS), which produces NO in response to shear stresses in the systemic and pulmonary circulation, and neuronal NOS (nNOS), which is involved in central and peripheral neurotransmission. NO from cNOS diffuses to smooth muscle and activates soluble guanylate cyclase, increasing intracellular cyclic guanosine monophosphate (cGMP), mediating relaxation.

Abbreviations used

BALF:	Bronchoalveolar lavage fluid
bNOS:	Brain NOS
CF:	Cystic fibrosis
cGMP:	Cyclic guanosine monophosphate
cNOS:	Constitutive NOS
eNOS:	Endothelial NOS
FE _{NO} :	Fractional concentration of exhaled nitric oxide
iNOS:	Inducible NOS
nNOS:	Neuronal NOS
NO:	Nitric oxide
NO ₂ ⁻ :	Nitrite
NO ₃ ⁻ :	Nitrate
NOS:	Nitric oxide synthase
NOx:	Nitrogen oxides
OONO ⁻ :	Peroxynitrite

NONINVASIVE METHODS TO ASSESS AIRWAY INFLAMMATION

Asthma is characterized by bronchial hyperresponsiveness, airway epithelial shedding, and inflammation. Currently the only direct methods for assessing airway inflammation, bronchial biopsy specimens, and bronchoalveolar lavage fluid (BALF) are invasive. Noninvasive tests are needed, and candidates include induced sputum analysis and measurement of exhaled mediators. Of the volatile mediators, exhaled¹ and nasal NO² have been studied the most extensively, but studies of other exhaled gases (eg, carbon monoxide) are also emerging. The fractional concentration of exhaled NO (FE_{NO}) is high in asthmatic compared with nonasthmatic subjects and falls after administration of inhaled or oral corticosteroids.³ This has led to the use of FE_{NO} as an investigative and clinical tool in asthma and elucidation of the role of NO in allergic inflammation (Fig 1).

BIOCHEMISTRY OF AIRWAY NO PRODUCTION B. Gaston

Nitric oxide synthases in the lung.

De novo synthesis of nitrogen oxides (NOx) in the lung requires at least one isoform of NOS, enzyme substrates (ie, oxygen, reduced nicotinamide adenine dinucleotide phosphate, and L-arginine), and cofactors (tetrahydrobiopterin and flavoproteins).⁴ All NOS isoforms are active in the lung.⁵⁻⁷ Types I and III NOS

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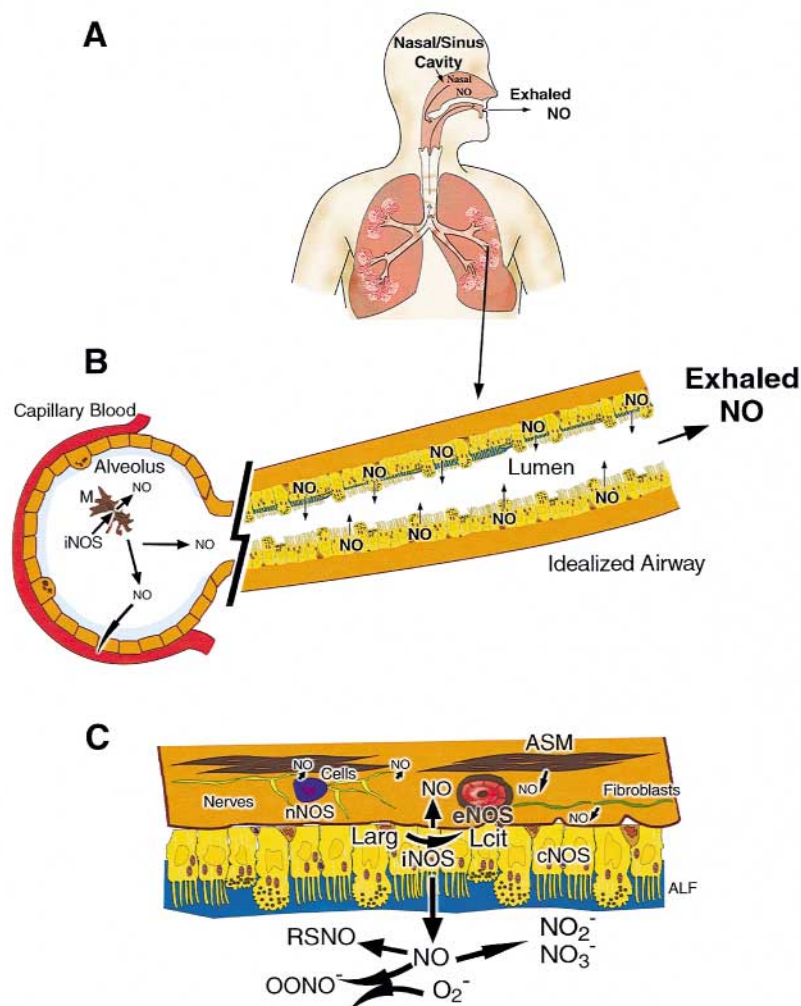


FIG 1. **A**, Exhaled NO derived from lower respiratory tract may contain NO derived from the nasal and oropharyngeal cavities. **B**, Exhaled NO derives from 2 compartments, namely, convection of alveolar NO and diffusion of airway NO, driven by a wall-to-lumen concentration difference. Alveolar region contains NO that is derived from that present in inhaled gas, NO diffusing into the inhaled gas stream from bronchial tree, and NO produced from alveolar cells such as macrophages (*M*). However, NO is avidly taken up by hemoglobin in pulmonary capillary blood; this explains the observation that alveolar levels are very low. **C**, Endogenous NO derives from conversion of L-arginine (*Larg*) to L-citrulline (*Lcit*) by cNOS and iNOS. In the airways NOS isoforms are found in many different cell types, including epithelium (iNOS and cNOS), nerve cells and processes (nNOS), endothelium (eNOS), airway smooth muscle (ASM), and fibroblasts. Epithelium is main site of iNOS induction in asthma, with production of large amounts of NO. This NO may be exhaled as NO or undergo conversion in airway wall or airway lining fluid (ALF), for example, by reaction with superoxide (O_2^-) to form diverse metabolites. These include nitrosothiols (*R-SNO*) such as glutathione-S-NO, which are bronchodilators, peroxynitrite ($ONOO^-$), which cause widespread oxidative damage, nitrite (NO_2^-), and nitrate (NO_3^-).

(Table I) are classically activated by mediator-signaled calcium fluxes leading to calmodulin binding and are present primarily in subepithelial neurons (type I) and vascular endothelium (type III),⁷ although expression elsewhere (eg, type III NOS expression at the epithelial cilia base) is important.⁸ In contrast, type II NOS is tightly bound to calmodulin after translation; its activity is primarily transcriptionally regulated.^{4,5} It is inducible in airway epithelial, vascular endothelial, and inflammatory cells and produces levels of NOx cytotoxic to organisms (eg, *Mycobacterium tuberculosis*).^{1,4,5}

Exhaled NO levels certainly reflect NOS activity because NOS inhibitors reduce FE_{NO} . This attenuation, although gradual and incomplete, is reproducible across species.^{1,9} Additionally, type I NOS knockout mice have only ~60% of the FE_{NO} of wild-type mice,¹⁰ although surprisingly type II NOS knockout mice do not differ from wild-type mice in airway responsiveness after ovalbumin challenge. Further, human studies have demonstrated that FE_{NO} falls after administration of aerosolized aminoguanidine, a relatively specific type II NOS inhibitor.⁹ These observations suggest that NOS plays a

TABLE I. NOS isoforms

NO synthases	Synonyms	Roles
Constitutive forms		
Type I NOS	Brain NOS (bNOS), neuronal NOS (nNOS)	Central and peripheral neurotransmission
Type III NOS	Endothelial NOS (eNOS), vascular NOS	Vasorelaxation
Inducible forms		
Type II NOS	Inducible NOS (iNOS)	Proinflammatory actions, anti-inflammatory actions, immunomodulation, antimicrobial action

critical role in producing FE_{NO} and that each NOS isoform has a different contribution to FE_{NO} in healthy and diseased lungs.

NO capacitors in the airway. Recent evidence suggests that there are a variety of biologically relevant NOx in human airways. For example, S-nitrosothiols, nitrate (NO_3^-), nitrite (NO_2^-), and peroxynitrite ($OONO^-$) have been identified in human BALF and condensed expirates,¹¹⁻¹³ and hydroxylamines are present in cystic fibrosis (CF) sputum (unpublished observations). Many of these compounds, particularly NO_2^- , S-nitrosothiols, and $OONO^-$ have complex metabolic pathways regulating their concentrations and effects.¹⁴ These effects range from bronchodilatation and augmented ciliary motility^{8,11} to modulation of apoptosis and mediation of host defense.¹⁵⁻¹⁸ Of note, pulmonary NOx may originate from or be transported to sites remote to the airway.

There is interconversion among the different NOx in the lung.¹⁴ In this regard, more stable compounds may attenuate or accentuate both FE_{NO} and NO bioactivities, depending on the airway chemistry associated with each disease state. Four examples are provided. First, NO may only be the principal product of NOS in the presence of high concentrations of superoxide dismutase or, perhaps, catalase.¹⁸⁻²⁰ In this sense, FE_{NO} may reflect not only NOS activity but also the activity of antioxidant enzymes during airway inflammation. Second, NO reacts with superoxide to form $ONOO^-$; this may decrease FE_{NO} and increase sputum NO_3^- concentrations in patients with CF with airway neutrophil activation.²¹ Third, acute asthma exacerbations are associated with acidification of lung water (often to levels near the negative logarithm of the acid ionization constant of nitrous acid, which is 3.2). Under these conditions NO is evolved from inorganic NO_2^- , which is normally present in the airway lining fluid in micromolar concentrations.²² Indeed, acute asthmatic airway acidification is associated with a fall in lung water NO_2^- concentrations that parallel increased FE_{NO} (unpublished observations). Fourth, levels of bronchodilator nitrosothiols are profoundly low in acute asthmatic respiratory failure, despite a raised FE_{NO} .²³ In this regard tracheal aspirates from these patients show accelerated breakdown of nitrosothiols *ex vivo*, and a variety of enzymes have been described that may convert nitrosothiols to NO and other lower-mass NOx.²⁴ Taken together, these observations suggest that FE_{NO} measurements do not necessarily uniformly and directly reflect NOS activity but do reflect inflammation nonetheless.

Other biochemical parameters that may modulate

FE_{NO} . Prokaryotic "denitrifying" microorganisms in the airways grow well in certain diseases (eg, *Aspergillus* in asthma and *Pseudomonas* species in CF). These organisms have metabolic pathways that use NOx as electron acceptors in respiration. These pathways may decrease concentrations of airway NOx,²⁵ while increasing reduced forms such as hydroxylamines, ammonia, and urea. For example, therapy for *Pseudomonas aeruginosa* in CF is associated with increased FE_{NO} , the opposite of the observed effect of anti-inflammatory therapy in asthma, and decreased sputum levels of ammonia. These observations serve to warn against oversimplifying the interpretation of a given FE_{NO} measurement as simply reflecting NOS activity.

IN VITRO AND IN VIVO STUDIES OF NO IN THE LUNG

Richard Robbins, MD

Assessment of NO production. Gaseous NO in exhaled breath can be measured by diverse techniques, most commonly chemiluminescence (see last section). Similarly, NO gas can be detected in the headspace above lung epithelial cell cultures.²¹ However, NO is unstable in the presence of oxygen and rapidly auto-oxidizes to a variety of NOx. Therefore one strategy is to use NOx, including NO_2^- , NO_3^- , $OONO^-$, and nitrosothiols to indicate NO formation. NO and oxygen react to form NO_2^- ; NO_2^- accumulation in cell culture supernatant fluids is commonly used to estimate NO formation and has been detected in the culture supernatant fluids from a variety of cells present in the lung. However, NO_2^- is stable for several hours in water and plasma but is rapidly converted to NO_3^- in whole blood. Consistent with the high FE_{NO} in asthma, NO_2^- or NO_3^- are also elevated in sputum or BALF from asthmatic patients and the nasal lavage fluid in rhinitis.²⁶⁻²⁸ Other indirect measures of NO production include assessment of L-arginine/L-citrulline conversion and cGMP formation.

Peroxyntitrite. $OONO^-$, an important oxidant generated by the interaction between superoxide and NO, is a short-lived, highly reactive radical and rapidly reacts with proteins, DNA, and lipids. $OONO^-$ causes nitration of amino acids including cysteine, methionine, tryptophan, and tyrosine. Addition of a nitro group to the 3-position adjacent to the hydroxyl group of tyrosine residues by $OONO^-$ forms the stable product nitrotyrosine,²⁹ which may alter protein function.³⁰ Nitrotyrosine is found in the cell culture supernatant fluids and the cell

layers of lung epithelium when superoxide is present²¹ and also in the BALF of asthmatic subjects.²⁷

The interaction with NO with superoxide may affect FE_{NO} . Exhaled NO concentrations have been measured in various disease states and are elevated in inflammatory lung disorders such as asthma,³¹ perhaps directed by inflammatory cytokines (see below). On the basis of prior data showing increased amounts of inflammatory cytokines in CF,^{32,33} FE_{NO} would be expected to be increased in patients with CF. However, several studies found FE_{NO} levels to be comparable to or less than in healthy control subjects.^{34,35} One possible mechanism is that an increase in activated neutrophils in CF airways releases superoxide, which combines with NO to produce OONO⁻; the latter forms nitrotyrosine or decomposes to NO_3^- . This hypothesis was tested in vitro by incubating lung epithelial cells with stimulated polymorphonuclear cells to generate increased amounts of superoxide. NO in the headspace above the culture was decreased in the presence of polymorphonuclear cells²¹; however, NO_3^- and nitrotyrosine were increased in the culture supernatant fluids and cell layer. On the basis of this premise, increased levels of NO_3^- and increased nitrotyrosine would be expected if OONO⁻ formation accounted for the relatively reduced FE_{NO} in diseases such as CF. Recently, sputum NO_3^- and nitrotyrosine in patients with CF were found to be elevated compared with healthy subjects, consistent with the concept that OONO⁻ formation in the lower respiratory tract lowers FE_{NO} .³⁶

The formation of nitrotyrosine may have functional significance. Current concepts suggest that chemokines such as RANTES and IL-5 lead to eosinophil locomotion by binding to receptors; tyrosine residues may be critical in this binding. Chemotaxis of human eosinophils in response to RANTES and IL-5 incubated with OONO⁻ and other compounds was evaluated in vitro. OONO⁻ and 3-morpholiniosydnonimine, an OONO⁻ donor, significantly attenuated RANTES- and IL-5-induced eosinophil chemotactic activity, suggesting that OONO⁻ may play a role in reducing eosinophil recruitment and inflammation.³⁷ However, it is unknown whether the tyrosines of these chemokines are nitrated in vivo in disorders such as asthma.

Regulation of NO production. Although many cells may release NO, most studies have focused on bronchial epithelial cell production in humans and on the type II NOS, which appears to be constitutively expressed by bronchial epithelial cells but increased in disorders such as asthma.³⁸ Type II NOS expression is regulated in most cells by cytokines. An in vitro study examining BALF suggested that TNF- α and IL-1, in the presence of IFN- γ released by alveolar macrophages, were important in regulating type II NOS expression,³⁹ whereas another suggested that IL-4, in the presence of IFN- γ , played an important role.⁴⁰ All these cytokines are increased in BALF or cells obtained from asthma patients. Antigen challenge increases FE_{NO} .⁴¹ Recently, the lower respiratory tract origin of the increased FE_{NO} was confirmed in asthma with use of endobronchial sampling and, addi-

tionally, increases in NO_3^- and nitrotyrosine were observed, consistent with increases in NO and OONO⁻ formation in response to antigen.²⁷

EXHALED NO: A TOOL FOR THE MANAGEMENT OF ALLERGIC INFLAMMATION

R. T. Townley

Proinflammatory effects of NO in allergic inflammation. Eosinophilia is a hallmark of asthma, and the infiltration of eosinophils correlates with airway hyperresponsiveness. Treatment with corticosteroids modestly decreases airway hyperresponsiveness as well as eosinophilia; the latter correlates with FE_{NO} levels.⁴² In addition, NO is chemotactic to eosinophils, neutrophils, and monocytes.^{43,44} Direct chemotaxis assays performed with use of a modified Boyden's chamber showed a significant decrease in eosinophil chemotaxis after administration of NOS inhibitors.⁴⁵ Inhibition of NOS also inhibits the late cutaneous reaction, which is mediated by the influx of inflammatory cells.⁴⁶ High concentrations of NO may have effects on the immune system and the inflammatory response. NO inhibits T_H1 lymphocytes in mice and thus favors the development of a T_H2 response with eosinophilia.^{47,48} Epithelial expression of type II NOS is increased in asthma.³⁸ In mice given NOS inhibitors, allergic inflammation after ovalbumin sensitization and challenge was attenuated, demonstrating an essential role for NO in the allergic process.⁴⁹

NO metabolites (eg, OONO⁻) play a role in the defense against infectious organisms, but they are also cytotoxic.⁵⁰ OONO⁻ causes airway hyperresponsiveness and airway epithelial damage, enhances inflammatory cell recruitment, and inhibits pulmonary surfactant. Asthma is associated with increased OONO⁻ formation in the airways, which may contribute to airway obstruction, hyperresponsiveness, and epithelial damage in asthma; OONO⁻ levels fall after budesonide treatment.⁵¹ Inhibition of bronchoprotective effects of β_2 -adrenoceptor agonists by OONO⁻ in guinea pig airways has been reported by Kanazawa et al.⁵² This action of OONO⁻ is directed either at adenylate cyclase activity or at effects downstream of such activity.⁵²

Asthmatic inflammation, type II NOS, and corticosteroids. Increased expression of type II NOS has been shown in asthmatic bronchial epithelium.^{38,51} This increased expression in asthma, where proinflammatory cytokines are present, is consistent with in vitro studies showing that these same cytokines induce type II NOS in human bronchial epithelial cells.³⁸ Exhaled NO is increased severalfold in patients with asthma, particularly during exacerbations.³¹ Other conditions with a raised FE_{NO} include viral respiratory infections,⁵³ acute allograft lung rejection,⁵⁴ and collagen diseases with lung involvement.⁵⁵ In asthma, inhaled or oral corticosteroids result in a rapid fall in FE_{NO} to levels not significantly different from those in nonasthmatic subjects.^{51,56-58} The mechanism of reduction of FE_{NO} by corticosteroids is by

inhibition of transcription of type II NOS through blockade of nuclear factor- κ B and indirectly by reduced synthesis of proinflammatory cytokines. The observation that FE_{NO} increases with the late allergic reaction is also consistent with increased expression of type II NOS in response to proinflammatory cytokines.⁴¹ A similar increase in FE_{NO} occurs with exacerbations of asthma^{59,60} or when the dose of oral and inhaled corticosteroids is reduced.⁵⁷ Kharitonov et al⁵⁷ demonstrated that FE_{NO} levels return to pathologic levels within 2 weeks of discontinuing inhaled steroid therapy, whereas lung function tests results were unchanged. Serial measurements of FE_{NO} have shown that FE_{NO} increased when the dose of steroid was reduced and fell when the dose was increased.⁶⁰ Because airway inflammation is a corollary to increased asthma symptoms and airway hyperresponsiveness, it may be plausible to use FE_{NO} levels to titrate and monitor the dose of inhaled steroid therapy or other anti-inflammatory treatment. The rapid change of this marker with therapy may also enable adherence to therapy to be assessed. As a diagnostic tool, in a recent study on chronic cough, FE_{NO} discriminated asthmatic from nonasthmatic subjects with a high sensitivity and specificity.⁶¹

All the above observations together suggest that airway inflammation in asthma may be monitored by measuring FE_{NO} .

Utility of exhaled NO to monitor inflammation in asthma. The measurement of FE_{NO} is noninvasive and can be performed repeatedly, even in patients with severe asthma or respiratory infections, where it is not possible to perform more invasive procedures. Asthma symptoms, pulmonary function tests, and bronchodilator use are the current mainstream assessment tools. It is recommended that the dose of inhaled steroids be titrated on the basis of these parameters; however, these may not be sensitive indicators of airway inflammation. Sont et al⁶² found that a treatment strategy aimed at reducing airway hyperresponsiveness in addition to recommendations in the existing guidelines (reference strategy) improved asthma control and reduced chronic airway inflammation. This implies a role for monitoring of airway hyperresponsiveness or other surrogate markers of inflammation such as FE_{NO} that correlates with methacholine responsiveness and with sputum eosinophilia in nonsteroid-treated asthma.⁶³

Future studies should be directed to determine the correlation of FE_{NO} with more direct measurements of inflammation such as bronchial biopsy and bronchoalveolar lavage and induced sputum. We need to know whether measurement of FE_{NO} will be useful in evaluating the anti-inflammatory effects of asthma drugs such as leukotriene synthesis inhibitors, phosphodiesterase inhibitors, and novel immunomodulators and the dose-response effects of these agents. This is exemplified by the ability of FE_{NO} to discriminate the effects of budesonide 100 mg daily from budesonide 400 mg daily,⁶⁴ not achievable with other parameters such as methacholine reactivity.

NO IN THE UPPER AIRWAYS

J. O. N. Lundberg, MD, PhD

The presence of NO in human exhalate was first demonstrated by Gustafsson et al¹ in 1991, and subsequently Alving et al⁶⁵ showed that nasal air contained considerable amounts of NO. Later studies clearly showed that in freely exhaling healthy control subjects, a major proportion of NO found in exhaled air originated from the upper airways with only a minor contribution from the lower respiratory tract.^{2,66}

The exact origin of the NO found in nasal air and the relative contribution from different sources within the nasal airways are not fully known. High production of NO takes place in the epithelium of the paranasal sinuses; this NO enters the nose through the sinus ostia and may be the main source of nasal NO.⁶⁷

Immunohistochemical and in situ hybridization studies indicate that healthy sinus epithelial NOS⁶⁷ is identical or very closely related to type II NOS as cloned from activated human hepatocytes.⁶⁸ Moreover, this sinus NOS activity is predominantly calcium independent, an attribute usually associated with type II NOS.⁶⁹ However, regulation of the expression and activity of sinus NOS differs from what has been described for type II NOS. This sinus NOS is constitutively expressed and is resistant to steroids,^{67,69} properties normally associated with the low NO output cNOS isoforms. Immunostaining has shown only weak staining for type II NOS in nasal mucosa.⁶⁷ Moreover, Ramis et al⁷⁰ found only calcium-dependent NOS activity in nasal mucosa from healthy subjects, whereas type II NOS was expressed in nasal polyp epithelium.

Factors that influence nasal NO

Measurement technique. A standardized nasal NO measurement technique is crucial for reproducible measurements (see final section of this review).

Physiologic factors. Nasal NO was measured in healthy subjects at different ages ranging from 0 to 70 years.⁶⁷ Interestingly, nasal NO was present already at birth and subsequently Schedin et al⁷¹ found significant nasal NO levels in neonates, including those delivered by cesarean section. There is no published evidence for sex differences in nasal NO; nasal NO has not been studied in relation to the menstrual cycle. NO output in the nasal airways is acutely decreased by heavy physical exercise.⁷² This cannot be explained merely by dilution of nasal air as a result of changes in nasal cavity volume or increased ventilation but has been attributed to a reduction in the blood flow in the mucosa of the nasal airways with a concomitant decrease in substrate supply to the high-output NOS in the paranasal sinuses.

Nasal NO in pathologic states. In children with Kartagener's syndrome, a triad consisting of sinusitis, bronchiectasis, and situs inversus, nasal NO levels are extremely low compared with those in healthy age-matched control subjects (Fig 2).² Similarly, nasal NO is lower than controls in subjects with CF.³⁵ Baraldi et al⁷³ found low nasal NO in a group of children with acute

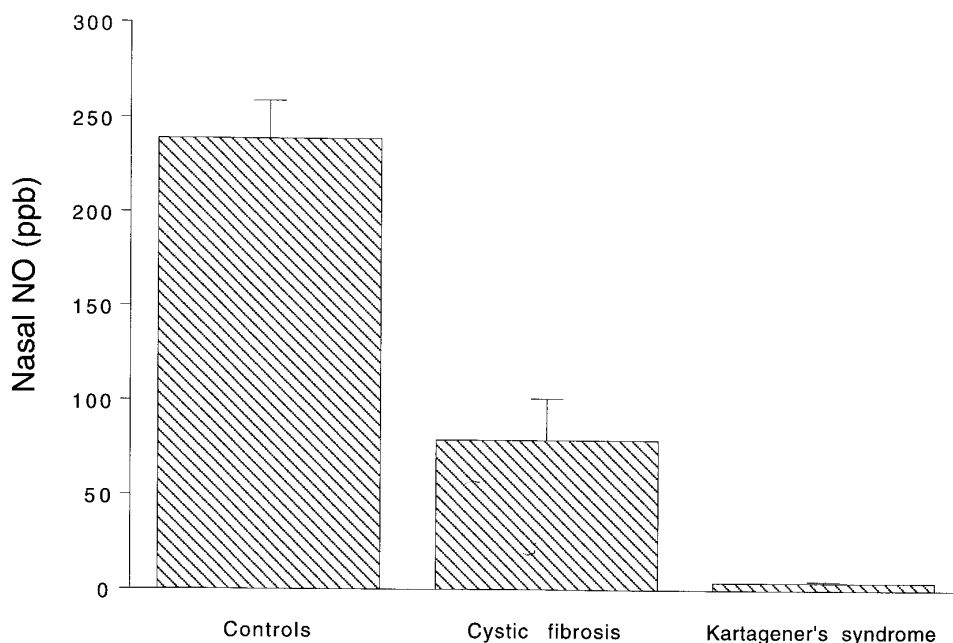


FIG 2. Nasal NO in different disease states.

sinusitis; nasal NO increased after treatment with antibiotics. Thus nasal NO is lower in sinus disorders, but it remains to be determined whether this is a result of reduced passage of sinus-derived NO to the nasal cavity caused by sinus ostia blockage or whether sinus NO production is decreased.

Consistent alteration of nasal NO in patients with rhinitis of different etiologies has not been fully established. Some investigators report higher nasal NO in patients with allergic rhinitis.^{74,75} In one study nasal NO decreased after treatment with nasal topical steroids.⁷⁴ In contrast, Lundberg et al³⁵ found no alterations in nasal NO in children with perennial rhinitis. The reasons underlying these discrepancies are unknown. Perhaps type II NOS expression is up-regulated in the nose during rhinitis as in asthmatic lower airways.³⁸ This would explain the higher levels of nasal NO in some studies. On the other hand, swelling of the nasal mucosa in rhinitis might lead to partial blockage of sinus ostia with less passage of sinus NO to the nasal cavity.

Effects of medications. Early studies on topical nasal corticosteroids reported no effect on nasal NO.² In fact, not even high-dose systemic steroids altered nasal NO.⁶⁹ Gerlach et al⁶⁶ suggested that bacteria in the nasopharynx may induce nasal NO release. However, Lundberg et al² found no effects of systemic antibiotic treatment on nasal NO. Moreover, newborn babies delivered by cesarean section, with sterile nasal passages, had measurable nasal NO levels as mentioned above.⁷¹ Therefore nasal NO release seems to be independent of the presence of bacteria.

Topical nasal decongestants such as oxymetazoline decrease nasal NO.⁷⁶ The reason for this is not clear, but it has been suggested that the decrease in nasal-sinus

blood flow induced by these drugs leads to a reduction of substrate supply to the high-output type II NOS in the sinuses. Indeed, substrate supply seems to be of importance for nasal-sinus NO release because intravenous administration of L-arginine increases nasal NO levels.⁶⁹

Rinder et al⁷⁶ found no effects of histamine and capsaicin on nasal NO. The NOS inhibitor (*N*^ω-nitro-L-arginine methyl ester) has only minor effects on nasal NO when it is administered topically.^{67,76} In contrast, Albert et al⁷⁷ found a decrease in nasal NO after intravenous administration of N(G)-monomethyl-L-arginine, another NOS inhibitor. This fits well with the theory that the paranasal sinuses are the main source of nasal NO because NOS inhibitors probably penetrate poorly to the sinuses if administered topically but gain access to the sinus mucosa if given intravenously.

Role of nasal NO. The exact roles of nasal NO are unknown, but it is reasonable to believe that this pluripotent biologic messenger is involved in many physiologic as well as pathophysiologic events in the airways.

Host-defense. NO produced by white blood cells is important in the killing of microorganisms; some bacteria are sensitive to NO concentrations as low as 100 ppb.⁷⁸ The presence of paranasal sinus NO concentrations as high as 30 ppm⁶⁷ supports the notion that NO is involved in local host defense in the upper airways; Lundberg et al have suggested that this NO keeps the sinuses sterile under normal conditions. Accordingly, the very low nasal NO in patients with Kartagener's syndrome or CF⁷⁹ may increase susceptibility to airway infection. If correct, stimulation of endogenous NO production could increase the resistance to airway infection in patients with low nasal NO.² It is not clear whether NO acts directly on micro-organisms or by combination

with other components to yield toxic reactive nitrogen intermediates. NO may also contribute to local host defense by stimulating ciliary motility because application of an NO donor in the nasal mucosa in humans increased ciliary beat frequency.⁸⁰

Proinflammatory and anti-inflammatory effects. NO synthesis is clearly enhanced locally at sites of inflammation, not only in asthma⁶⁵ but also in other inflammatory conditions such as inflammatory bowel disease⁸¹ and cystitis.⁸² The role of NO in inflammation is far from clear, with some studies indicating a proinflammatory and others an anti-inflammatory role. Proinflammatory actions of NO may include activation of enzymes such as cyclo-oxygenase or metalloproteinases. Moreover, OONO⁻, formed from the reaction of NO with superoxide, can exert widespread toxic effects on tissues.⁸³ The harmful effects of NO have been attributed to the large amounts of this gas produced by type II NOS during inflammation. However, the recent finding of constitutively expressed type II NOS in the nasal airways complicates this picture because it clearly demonstrates that the sole expression of type II NOS and the subsequent large production of NO is not associated with tissue damage but may serve important protective functions as discussed above. Furthermore, in an animal model of colon inflammation, McCafferty et al⁸⁴ have shown that type II NOS knockout mice develop worse inflammation compared with wild-type mice.

Pulmonary effects of nasal NO. The therapeutic effects of inhaled exogenous NO are being investigated in pulmonary hypertension and adult respiratory distress syndrome. Clear beneficial effects of inhaled NO on oxygenation and pulmonary artery pressure with NO concentrations as low as 10 to 100 ppb have been found.⁸⁵ During normal breathing, endogenous NO is inhaled at concentrations known to have vasodilatory effects in the pulmonary circulation.^{2,66} Lundberg et al⁸⁶ have shown that nasal breathing reduces pulmonary vascular resistance and improves oxygenation compared with oral breathing in healthy subjects. Intubated patients are deprived of the natural inhalation of nasal NO; supplementation of nasal air to intubated patients treated with a ventilator improved arterial oxygenation and reduced pulmonary vascular resistance.

Clinical value of nasal NO measurements. Measurements of nasal NO are noninvasive and easily performed even in small children. The low nasal NO in patients with chronic sinus disorders is of great interest.⁷⁹ It is tempting to speculate that nasal NO measurements may be useful in the early diagnosis of sinus disorders. However, the utility of nasal NO measurements in the diagnosis and therapy monitoring of allergic rhinitis needs further evaluation. If nasal NO were a reliable marker of nasal inflammation, this simple test could be used to monitor patients with rhinitis of different etiologies. To fully evaluate the potential of nasal NO measurements in the clinical setting, a standardized and reliable measurement technique is essential. We also need to know more about the different factors that influence nasal NO.

THE STANDARDIZED MEASUREMENT OF EXHALED AND NASAL NO

P. E. Silkoff

Exhaled and nasal NO measurement has been hampered by the variety of techniques used to measure FE_{NO}, resulting in widely varying values. A European Respiratory Society task force published guidelines in 1997,⁸⁷ and an American Thoracic Society workshop, held in Toronto in 1998, has reached consensus on preferred methods to be used in the measurement of exhaled and nasal NO in adults and children.⁸⁸

NO measurement. The most widely used method of measurement uses the principle of chemiluminescence. Here NO in the sample is drawn into a reaction cell where it is reacted with ozone, emitting light that is then detected by a photomultiplier tube. Modern instruments can measure fractional concentrations in the parts-per-billion and even parts-per-trillion range.

Exhaled NO. Several studies have demonstrated that lower respiratory FE_{NO} arises in the airways by diffusion from the airway wall to lumen.^{89,90} For reproducible measurements it is essential to exclude the large amounts of NO that have been described in the supravocal airway. This can be achieved by elevation of the velum during expiration, which partitions the upper and lower airways.⁹¹ Although there are several methods to achieve velum closure (eg, voluntary elevation in trained individuals), the most commonly used method is exhalation against expiratory resistance while maintaining a positive mouth pressure that is displayed to the subject. Velum closure has been verified by demonstrating that nasal carbon dioxide remains low during the exhalations.⁹¹

Exhaled NO levels vary markedly and inversely with the exhalation flow rate.⁹¹ Alveolar NO levels appear to be very low (<5 ppb) because of avid binding by hemoglobin in the alveolar capillary bed.⁸⁹ As alveolar gas ascends the airway, it is conditioned with NO. Faster exhalations result in reduced transit times in the airway and therefore lower FE_{NO}. Exhalation flow rate must therefore be held constant for reproducible measurements. Additionally, low flow rates (<100 mL/s) increase FE_{NO} and are felt to amplify differences between health and disease.

Online measurement refers to exhalations performed with real-time display of FE_{NO} and mouth pressure profiles. In offline measurement, exhalation proceeds into a collection receptacle; the sample is then taken to the laboratory for analysis. Offline NO measurements allow measurements to be performed at sites remote from the analyzer (eg, in the emergency department, in the workplace/school, or at home).

Online exhaled NO. The subject inhales to total lung capacity from a source of air containing levels of NO <5 ppb because ambient air NO levels can be much higher than endogenous levels. Exhalation then proceeds through an expiratory resistance while the subject targets a fixed expiratory mouth pressure.⁹¹ The combination of fixed pressure and resistance creates a constant expirato-

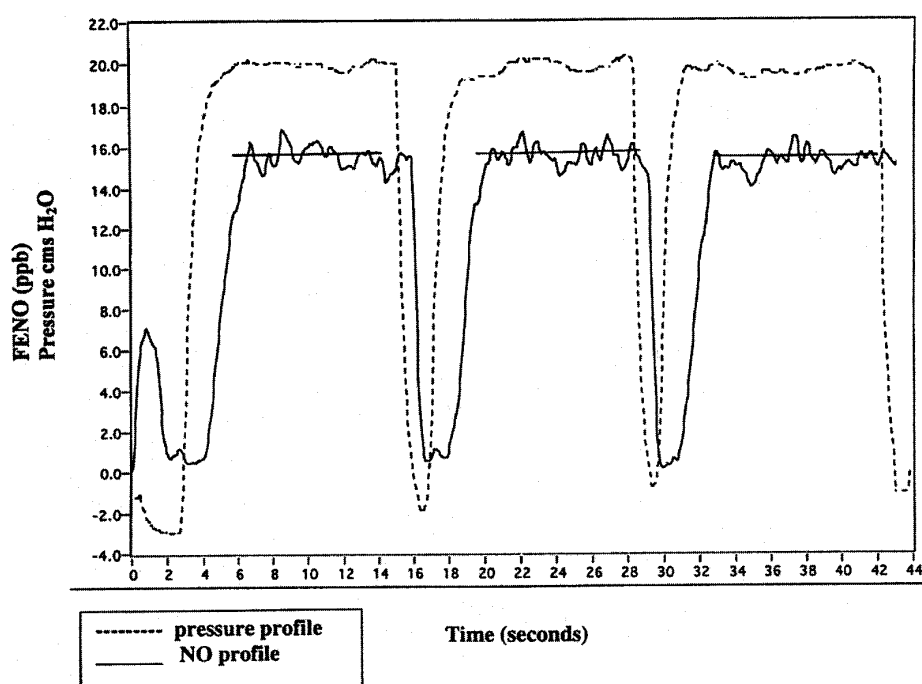


FIG 3. Three single breath profiles of exhaled NO and mouth pressure showing reproducible tracings. The NO profile shows a washout phase followed by a steady plateau.

ry flow. The exhalation proceeds until a clear 3-second plateau is seen on a NO profile versus time display (Fig 3). One acceptable configuration for performing the exhalations is shown in Fig 4.

Offline exhaled NO. Subjects inhale to total lung capacity and then exhale the entire vital capacity into an apparatus that incorporates feedback of mouth pressure, expiratory resistance, and a constant exhalation flow rate. The exhalate is collected into a receptacle (eg, Mylar balloons, Tedlar bags, or any other storage container that must guarantee acceptable stability of the NO concentration until analysis).⁹²

Pediatric exhaled NO. Pediatric online and offline measurements may be made with use of the same techniques as those used for adults with the caveats that only a 2-second plateau and at least 4 seconds of expiration time are required for children less than 12 years old. Of note, there is evidence that online and offline measurements are strongly correlated and that it may be difficult for many young children reproducibly to sustain a flow of >50 mL/s.⁹³ For younger subjects and those unable to perform the maneuvers required for either conventional online or offline collection, Baraldi et al⁹⁴ have published extensively on a tidal collection technique involving (1) oral breathing of NO-scrubbed air through 1-way valves and (2) expiratory velum closure caused by end expiratory pressure. This technique allows both for continuous online sampling and for offline sample collection in an inert gas sampling bag. The results are highly reproducible and demonstrate excellent sensitivity and specificity in identifying asthmatic infants and children.

Nasal NO. The measurement of nasal NO requires the exclusion of lower respiratory tract NO and ambient NO and a constant transnasal flow rate because nasal NO is also flow dependent.⁹⁵

The velum must be closed to exclude lower respiratory tract NO. This can be achieved in several ways, but one good method is to use the same exhalation against resistance as described in the online FE_{NO} method above. Nasal gas is aspirated from one naris while NO free gas is entrained via the other, at a constant flow rate. An NO sample line entrains air from the exiting gas. The NO profile shows a rise to a NO plateau, which is the nasal NO value recorded.

Factors that can affect exhaled NO. A number of factors that influence FE_{NO} values need to be considered. Ambient NO levels may be higher than endogenous levels being measured. Ideally, subjects should inhale air that contains low levels of NO (<5 ppb). This is particularly important when collecting exhalate for offline measurements. Age and sex show no apparent relationship to the level of FE_{NO} in adults, but FE_{NO} increases with age in children.⁹⁶ Exhaled NO should be measured before spirometry, which transiently reduces FE_{NO}.⁹⁷ Exhaled NO rises after bronchodilator administration⁹⁷ and falls after bronchoconstriction.⁹⁸ Therefore these factors need to be controlled for; the time of last bronchodilator administration should be recorded, as well as airway caliber. The influence of food and beverages on FE_{NO} needs to be clarified because an increase in FE_{NO} has been found after ingestion of foods containing NO₃⁻, such as vegetables.⁹⁹ It is best to refrain from eating or drinking

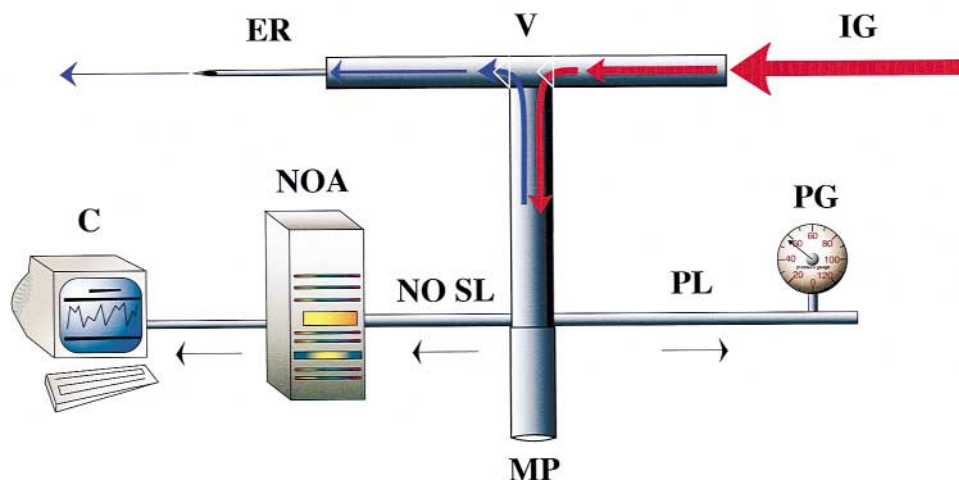


FIG 4. One configuration for measurement of FE_{NO} with restricted breath technique.

for 1 hour before testing. NO is a major constituent in cigarette smoke. Long-term smoking reduced FE_{NO} in cigarette smokers in addition to acute effects immediately after cigarette smoking.¹⁰⁰ Infection may also lead to increased levels of FE_{NO} ⁵³; measurements may be deferred until recovery or the presence of infection should be recorded. During exercise FE_{NO} and nasal NO fall while NO output increases. It is therefore prudent to avoid strenuous exercise for 1 hour before measurement of NO. Finally, all current medication at the time of FE_{NO} measurement should be recorded.

REFERENCES

- Gustafsson LE, Leone AM, Persson MG, et al. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochem Biophys Res Commun* 1991;181:852-7.
- Lundberg JO, Weitzberg E, Nordvall SL, et al. Primarily nasal origin of exhaled nitric oxide and absence in Kartagener's syndrome. *Eur Respir J* 1994;7:1501.
- Silkoff PE, McClean P, Slutsky AS, et al. Exhaled nitric oxide and bronchial reactivity during and after inhaled beclomethasone in mild asthma. *J Asthma* 1998;35:473.
- Geller DA, Billiar TR. Molecular biology of nitric oxide synthases. *Cancer Metastasis Rev* 1998;17:7.
- Asano K, Chee CB, Gaston B, et al. Constitutive and inducible nitric oxide synthase gene expression, regulation, and activity in human lung epithelial cells. *Proc Natl Acad Sci U S A* 1994;91:10089.
- Gaston B, Kobzik L, Stamler JS. Distribution of nitric oxide synthase in the lung. In: Zapol W, Bloch K, editors. *Nitric oxide and the lung*. New York: Marcel Dekker; 1997. p 75-86.
- Kobzik L, Bredt DS, Lowenstein CJ, et al. Nitric oxide synthase in human and rat lung: immunocytochemical and histochemical localization. *Am J Respir Cell Mol Biol* 1993;9:371.
- Xue C, Botkin SJ, Johns RA. Localization of endothelial NOS at the basal microtubule membrane in ciliated epithelium of rat lung. *J Histochem Cytochem* 1996;44:463.
- Yates DH, Kharitonov SA, Thomas PS, et al. Endogenous nitric oxide is decreased in asthmatic patients by an inhibitor of inducible nitric oxide synthase. *Am J Respir Crit Care Med* 1996;154:247.
- De Sanctis GT, MacLean JA, Hamada K, et al. Contribution of nitric oxide synthases 1, 2, and 3 to airway hyperresponsiveness and inflammation in a murine model of asthma. *J Exp Med* 1999;189:1621.
- Gaston B, Reilly J, Drazen JM, et al. Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways. *Proc Natl Acad Sci U S A* 1993;90:10957.
- Haddad IY, Pataki G, Hu P, et al. Quantitation of nitrotyrosine levels in lung sections of patients and animals with acute lung injury. *J Clin Invest* 1994;94:2407.
- Hunt J, Byrns RE, Ignarro LJ, et al. Condensed expirate nitrite as a home marker for acute asthma [letter]. *Lancet* 1995;346:1235.
- Gaston B, Stamler JS. Nitric oxides and lung function. In: Crystal R, West J, Weibel E, Barnes P, editors. *The lung: scientific foundations*. Philadelphia: Lippincott-Raven; 1997. p 239-53.
- De Groote MA, Testerman T, Xu Y, et al. Homocysteine antagonism of nitric oxide-related cytostasis in *Salmonella typhimurium*. *Science* 1996;19:272:414.
- Mannick JB, Hausladen A, Liu L, et al. Fas-induced caspase denitrosylation. *Science* 1999;284:651.
- Saura M, Zaragoza C, McMillan A, et al. An antiviral mechanism of nitric oxide: inhibition of a viral protease. *Immunity* 1999;10:21.
- Soderman C, Leone A, Furst V, et al. Endogenous nitric oxide in exhaled air from patients with liver cirrhosis. *Scand J Gastroenterol* 1997;32:591.
- Mian KB, Martin W. The inhibitory effect of 3-amino-1,2,4-triazole on relaxation induced by hydroxylamine and sodium azide but not hydrogen peroxide or glyceryl trinitrate in rat aorta. *Br J Pharmacol* 1995;116:3302.
- Schmidt HH, Hofmann H, Schindler U, et al. NO from NO synthase. *Proc Natl Acad Sci U S A* 1996;93:14492.
- Jones KL, Bryan TW, Jinkins PA, et al. Superoxide released from neutrophils causes a reduction in nitric oxide gas. *Am J Physiol* 1998;275:L1120.
- Hunt J, Fang K, Platts-Mills TAE, et al. Nitrogen oxide redox balance in asthma [abstract]. *Am J Respir Crit Care Med* 1999;159:A860.
- Gaston B, Sears S, Woods J, et al. Bronchodilator S-nitrosothiol deficiency in asthmatic respiratory failure. *Lancet* 1998;351:1317.
- Gaston B. Nitric oxide and thiol groups. *Biochim Biophys Acta* 1999;1411:1317.
- Gaston B, Canady R, Hunt J, et al. High ammonia and low nitric oxide levels in the cystic fibrosis airway: adverse effects of *Pseudomonas*? [abstract] *Am J Respir Crit Care Med* 1999;159:A306.
- Kanazawa H, Shoji S, Yamada M, et al. Increased levels of nitric oxide derivatives in induced sputum in patients with asthma. *J Allergy Clin Immunol* 1997;99:624-9.
- Robbins RA, Boggs PJ, Simpson KL, et al. Endobronchial challenge is associated with increased lower respiratory tract nitric oxide [abstract]. *Am J Respir Crit Care Med* 1999;159:A862.
- Sato M, Fukuyama N, Sakai M, et al. Increased nitric oxide in nasal lavage fluid and nitrotyrosine formation in nasal mucosa—indices for severe perennial nasal allergy. *Clin Exp Allergy* 1998;28:597.
- Ischiropoulos H, Zhu L, Chen J, et al. Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. *Arch Biochem Biophys* 1992;298:431.
- Haddad IY, Crow JP, Hu P, et al. Concurrent generation of nitric oxide and superoxide damages surfactant protein A. *Am J Physiol* 1994;267:L242.

31. Kharitonov SA, Yates D, Robbins RA, et al. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994;343:133.
32. Balough K, McCubbin M, Weinberger M, et al. The relationship between infection and inflammation in the early stages of lung disease from cystic fibrosis. *Pediatr Pulmonol* 1995;20:63.
33. Greally P, Hussein MJ, Cook AJ, et al. Sputum tumour necrosis factor- α and leukotriene concentrations in cystic fibrosis. *Arch Dis Child* 1993;68:389.
34. Dotsch J, Demirakca S, Terbrack HG, et al. Airway nitric oxide in asthmatic children and patients with cystic fibrosis. *Eur Respir J* 1996;9:2537.
35. Lundberg JO, Nordvall SL, Weitzberg E, et al. Exhaled nitric oxide in paediatric asthma and cystic fibrosis. *Arch Dis Child* 1996;75:323.
36. Jones KL, Hegab AH, Hillman BC. Elevation of nitrate and nitrotyrosine in cystic fibrosis sputum. *Pediatr Pulmonol* 1999. In press.
37. Sato E, Simpson KL, Grisham MB, et al. Effects of reactive oxygen and nitrogen metabolites on RANTES and IL-5 induced eosinophil chemotactic activity in vitro. *Am J Pathol* 1999;155:591.
38. Hamid Q, Springall DR, Riveros-Moreno V, et al. Induction of nitric oxide synthase in asthma. *Lancet* 1993;342:1510.
39. Robbins RA, Sisson JH, Springall DR, et al. Human lung mononuclear cells induce nitric oxide synthase in murine airway epithelial cells in vitro: role of TNF α and IL-1 β . *Am J Respir Crit Care Med* 1997;155:268.
40. Guo FH, Uetani K, Haque SJ, et al. Interferon gamma and interleukin 4 stimulate prolonged expression of inducible nitric oxide synthase in human airway epithelium through synthesis of soluble mediators [published erratum appears in *J Clin Invest* 1997;100:1322]. *J Clin Invest* 1997;100:829.
41. Kharitonov SA, O'Connor BJ, Evans DJ, et al. Allergen-induced late asthmatic reactions are associated with elevation of exhaled nitric oxide. *Am J Respir Crit Care Med* 1995;151:1894.
42. Jatakanon A, Lim S, Kharitonov SA, et al. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998;53:91.
43. Belenky SN, Robbins RA, Rennard SI, et al. Inhibitors of nitric oxide synthase attenuate human neutrophil chemotaxis in vitro. *J Lab Clin Med* 1993;122:388.
44. Belenky SN, Robbins RA, Rubinstein I. Nitric oxide synthase inhibitors attenuate human monocyte chemotaxis in vitro. *J Leukoc Biol* 1993;53:498.
45. Robbins RA, Romero FA, Nelson KJ, et al. Inhibitors of nitric oxide synthase attenuate human neutrophil chemotaxis in vitro [abstract]. *Am J Respir Crit Care Med* 1995;151:A238.
46. Townley RG, Romero FA, Robbins RA. The effect of nitric oxide synthase on the early and late allergic skin response. *J Allergy Clin Immunol* 1995;95:358.
47. Barnes PJ, Liew FY. Nitric oxide and asthmatic inflammation. *Immunol Today* 1995;16:128.
48. Taylor-Robinson AW, Philipos RS, Severin A, et al. The role of Th1 and Th2 cells in a rodent malaria infection. *Science* 1993;260:1931.
49. Feder LS, Stelts D, Chapman RW, et al. Role of nitric oxide on eosinophilic lung inflammation in allergic mice. *Am J Respir Cell Mol Biol* 1997;17:436.
50. Radi R, Beckman JS, Bush KM, et al. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch Biochem Biophys* 1991;288:481.
51. Saleh D, Ernst P, Lim S, et al. Increased formation of the potent oxidant peroxynitrite in the airways of asthmatic patients is associated with induction of nitric oxide synthase: effect of inhaled glucocorticoid. *FASEB J* 1998;12:929.
52. Kanazawa H, Satoshi S, Okamoto T, et al. Inhibition of bronchoprotective effects of beta-2 adrenoceptor agonists by peroxynitrite in guinea pig airways. *Am J Respir Crit Care Med* 1999;159:1272.
53. Kharitonov SA, Yates D, Barnes PJ. Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. *Eur Respir J* 1995;8:295.
54. Silkoff PE, Caramori M, Tremblay L, et al. Exhaled nitric oxide in human lung transplantation: a noninvasive marker of acute rejection. *Am J Respir Crit Care Med* 1998;157:1822.
55. Rolla G, Brussino L, Bertero MT, et al. Increased nitric oxide in exhaled air of patients with systemic lupus erythematosus. *J Rheumatol* 1997;24:1066.
56. Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. *Am J Respir Crit Care Med* 1996;153:454.
57. Kharitonov SA, Yates DH, Chung KF, et al. Changes in the dose of inhaled steroid affect exhaled nitric oxide levels in asthmatic patients. *Eur Respir J* 1996;9:196.
58. Robbins RA, Sisson JH. Nitric oxide. In: Townley RG, Agrawal DK, editors. *Immunopharmacology of allergic diseases*. New York: Marcel Dekker; 1996.
59. Kharitonov SA, Yates D, Springall DR, et al. Exhaled nitric oxide is increased in asthma. *Chest* 1995;107:156S.
60. Massaro AF, Gaston B, Kita D, et al. Expired nitric oxide levels during treatment of acute asthma. *Am J Respir Crit Care Med* 1995;152:800.
61. Chatkin JM, Ansarin K, Silkoff PE, et al. Exhaled nitric oxide as a non-invasive assessment of chronic cough. *Am J Respir Crit Care Med* 1999;159:1810.
62. Sont JK, Willems LN, Bel EH, et al. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment: the AMPUL Study Group. *Am J Respir Crit Care Med* 1999;159:1043.
63. Dupont LJ, Rochette F, Demedts MG, et al. Exhaled nitric oxide correlates with airway hyperresponsiveness in steroid-naïve patients with mild asthma. *Am J Respir Crit Care Med* 1998;157:894.
64. Jatakanon A, Kharitonov S, Lim S, et al. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. *Thorax* 1999;54:108.
65. Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J* 1993;6:1368.
66. Gerlach H, Rossaint R, Pappert D, et al. Autoinhalation of nitric oxide after endogenous synthesis in nasopharynx. *Lancet* 1994;343:518.
67. Lundberg JO, Farkas-Szallasi T, Weitzberg E, et al. High nitric oxide production in human paranasal sinuses. *Nat Med* 1995;1:370.
68. Geller DA, Lowenstein CJ, Shapiro RA, et al. Molecular cloning and expression of inducible nitric oxide synthase from human hepatocytes. *Proc Natl Acad Sci U S A* 1993;90:3491.
69. Lundberg JO, Weitzberg E, Rinder J, et al. Calcium-independent and steroid-resistant nitric oxide synthase activity in human paranasal sinus mucosa. *Eur Respir J* 1996;9:1344.
70. Ramis I, Lorente J, Rosello-Catafau J, et al. Differential activity of nitric oxide synthase in human nasal mucosa and polyps. *Eur Respir J* 1996;9:202.
71. Schedin U, Norman M, Gustafsson LE, et al. Endogenous nitric oxide in the upper airways of healthy newborn infants. *Pediatr Res* 1996;40:148.
72. Lundberg JO, Rinder J, Weitzberg F, et al. Heavy physical exercise decreases nitric oxide levels in the nasal airways in humans. *Acta Physiol Scand* 1997;159:51.
73. Baraldi E, Azzolin NM, Biban P, et al. Effect of antibiotic therapy on nasal nitric oxide concentration in children with acute sinusitis. *Am J Respir Crit Care Med* 1997;155:1680.
74. Kharitonov SA, Rajakulasingam K, O'Connor B, et al. Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *J Allergy Clin Immunol* 1997;99:58.
75. Martin U, Bryden K, Devoy M, et al. Increased levels of exhaled nitric oxide during nasal and oral breathing in subjects with seasonal rhinitis. *J Allergy Clin Immunol* 1996;97:768.
76. Rinder J, Lundberg JON, Anggard A, et al. Effects of topical nasal decongestants, L-arginine and nitric oxide synthase inhibition on nasal cavity nitric oxide levels and nasal cavity volume in man. *Am J Rhinol* 1996;10:399.
77. Albert J, Schedin U, Lindqvist M, et al. Blockade of endogenous nitric oxide production results in moderate hypertension, reducing sympathetic activity and shortening bleeding time in healthy volunteers. *Acta Anaesthesiol Scand* 1997;41:1104.
78. Mancinelli RL, McKay CP. Effects of nitric oxide and nitrogen dioxide on bacterial growth. *Appl Environ Microbiol* 1983;46:198.
79. Lundberg JO. Airborne nitric oxide: inflammatory marker and aerocrine messenger in man. *Acta Physiol Scand Suppl* 1996;633:1.
80. Runer T, Cervin A, Lindberg S, et al. Nitric oxide is a regulator of mucociliary activity in the upper respiratory tract. *Otolaryngol Head Neck Surg* 1998;119:278.
81. Lundberg JON, Hellstrom PM, Lundberg JM, et al. Greatly increased luminal nitric oxide in ulcerative colitis. *Lancet* 1994;344:1673.

82. Lundberg JO, Ehren I, Jansson O, et al. Elevated nitric oxide in the urinary bladder in infectious and noninfectious cystitis. *Urology* 1996;48:700.
83. Beckman JS, Beckman TW, Chen J, et al. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A* 1990;87:1620.
84. McCafferty DM, Mudgett JS, Swain MG, et al. Inducible nitric oxide synthase plays a critical role in resolving intestinal inflammation. *Gastroenterology* 1997;112:1022.
85. Puybasset L, Rouby JJ, Mourgeon E, et al. Inhaled nitric oxide in acute respiratory failure: dose-response curves. *Intensive Care Med* 1994;20:319.
86. Lundberg JO, Lundberg JM, Settergren G, et al. Nitric oxide, produced in the upper airways, may act in an 'aerocrine' fashion to enhance pulmonary oxygen uptake in humans. *Acta Physiol Scand* 1995;155:467.
87. Kharitonov S, Alving K, Barnes PJ. Exhaled and nasal nitric oxide measurements: recommendations: the European Respiratory Society Task Force. *Eur Respir J* 1997;10:1683.
88. Silkoff P/E, Drazen JM, Gaston B, et al. American Thoracic Society/American Lung Association: recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children. *Am J Respir Crit Care Med* 1999. In press.
89. Dweik RA, Laskowski D, Abu-Soud HM, et al. Nitric oxide synthesis in the lung: regulation by oxygen through a kinetic mechanism. *J Clin Invest* 1998;101:660.
90. Silkoff PE, McClean P, Caramori M, et al. A significant proportion of exhaled nitric oxide arises in large airways in normal subjects. *Respir Physiol* 1998;113:33.
91. Silkoff PE, McClean PA, Slutsky AS, et al. Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. *Am J Respir Crit Care Med* 1997;155:260.
92. Silkoff PE, Stevens A, Pak J, et al. A method for the standardized offline collection of exhaled nitric oxide. *Chest* 1999;116:754.
93. Canady RG, Platts-Mills T, Murphy A, et al. Vital capacity reservoir and online measurement of childhood nitrosopnea are linearly related: clinical implications. *Am J Respir Crit Care Med* 1999;159:311.
94. Baraldi E, Dario C, Ongaro R, et al. Exhaled nitric oxide concentrations during treatment of wheezing exacerbation in infants and young children. *Am J Respir Crit Care Med* 1999;159:1284.
95. Silkoff PE, Chatkin J, Qian W, et al. Nasal nitric oxide: a comparison of measurement techniques. *Am J Rhinol* 1999;13:169.
96. Franklin PJ, Taplin R, Stick SM. A community study of exhaled nitric oxide in healthy children. *Am J Respir Crit Care Med* 1999;159:69.
97. Silkoff PE, Chatkin J, McClean P, et al. Exhaled nitric oxide after beta-2 agonist inhalation and spirometry in bronchial asthma. *Am J Respir Crit Care Med* 1999;159:940.
98. de Gouw HWFM, Hendriks J, Woltman AM, et al. Exhaled nitric oxide (NO) is reduced shortly after bronchoconstriction to direct and indirect stimuli in asthma. *Am J Respir Crit Care Med* 1998;158:315.
99. Zetterquist W, Pedroletti C, Lundberg JO, et al. Salivary contribution to exhaled nitric oxide. *Eur Respir J* 1999;13:327.
100. Kharitonov SA, Robbins RA, Yates D, et al. Acute and chronic effects of cigarette smoking on exhaled nitric oxide. *Am J Respir Crit Care Med* 1995;152:609.