Eur Respir J, 1996, 9, 2671–2680
DOI: 10.1183/09031936.96.09122671
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European Respiratory Journal
ISSN 0903 - 1936

REVIEW

Nitric oxide in exhaled air

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ABSTRACT: Much interest is now being focused on measurements of nitric oxide (NO) in exhaled air. In healthy subjects exhaled NO seems to originate mainly in the nasal airways, whereas the contribution from the lower respiratory tract is low.

In certain inflammatory airway disorders, the excretion of NO into the airways is altered resulting in changes in the levels of NO in exhaled air. New techniques have been developed to measure NO release at different levels of the airways: asthmatics show increased orally-exhaled NO levels, whereas patients with cystic fibrosis or Kartagener's syndrome exhibit a marked reduction in nasal release of NO. It has been suggested that measurements of exhaled NO may be clinically useful in noninvasive diagnosis and monitoring of inflammatory airway diseases.

To further evaluate the potential clinical usefulness of measurement of exhaled NO, it is vital to explore how airway NO production is normally regulated and what factors influence airway NO excretion.

Eur Respir J., 1996, 9, 2671–2680.

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Keywords: Asthma, inflammation, inhaled nitric oxide, Kartegener's syndrome, nitric oxide synthase, paranasal sinuses.

Received: July 30 1996
Accepted after revision September 15 1996

Nitric oxide (NO) is not only a reactive free radical, it is also a highly diffusible gas. Direct measurements of NO in biological tissues are difficult to perform because the gas reacts rapidly with, *e.g.* haemoglobin or other Fe^{2+}-containing proteins [1]. Therefore, one must often rely on indirect measurements in order to detect NO synthesis *in vivo*. Such indirect methods include measurements of citrulline, the co-product when NO is synthesized enzymatically by a NO synthase, or of nitrate/nitrite, which are stable breakdown products of NO [1]. Unlike the situation in most biological tissues, where NO is rapidly destroyed, NO in gas phase is fairly stable at low concentrations [1]. Therefore, NO produced in superficial structures of hollow organs will diffuse to the lumen and, thus, be detectable in gas collected from such organs. Indeed, NO is excreted in the human airways and is detectable in exhaled air [2–4].

How to measure exhaled NO

Method of detection

The most widely-used technique for measurement of NO in exhaled air is the chemiluminescence method. The NO contained in a gas sample reacts with an excess of ozone (O_3) to produce NO_2 with an electron in an excited state (NO_2*). NO_2* changes back to the ground state (NO_2) while emitting electromagnetic radiation ranging 600–3,000 nm in wavelength. The chemiluminescence is detected by a photomultiplier tube that proportionally converts the intensity of luminescence into an electrical signal for display. The chemiluminescence technique is highly sensitive: NO can be detected down to a concentration of approximately 1 part per billion (ppb) [1]. This technique is easy to use and readily allows online registration of exhaled NO. Other techniques that have been used to further establish that NO is present in a human breath include mass spectrometry [2]; and gas chromatography-mass spectrometry (GC-MS) [5].

Because NO is highly diffusible and reactive, it is important to use inert nonabsorbing materials, such as glass, steel or Teflon, in all tubings contained in an NO measuring system. Furthermore, although most inhaled NO is rapidly absorbed by the lungs [6], it is nonetheless important to ensure that inhaled NO concentrations are kept low, in order not to interfere with exhaled levels, since NO in dead space is not absorbed to any large extent. In urban areas, ambient NO levels may sometimes exceed 100 ppb, and in these situations a closed system is preferable, in which NO-free air is inhaled from a reservoir [3].

Single-breath measurements

Rapid registration of exhaled NO levels during the course of a single exhalation allows fractioning of a breath into early and late phases. For single-breath measurements, a subject takes a deep breath of NO-free air and exhales into a system of appropriate tubings, from which an air fraction is continuously sampled, and NO is measured in the sample. Naturally, since most of the NO found in exhaled air is continuously formed within the upper airways and released into the dead space area [3, 4] (see below), the velocity of the exhalation will influence the concentration of NO in exhaled air. Thus, if exhalation is slow, more NO will be released in the lumen during the course of the exhalation, resulting

in higher concentrations (fig 1). This is particularly accentuated if a subject holds his breath prior to exhalation. In such settings, much NO accumulates in the airways and a large initial peak with a subsequent plateau is seen in the single-breath registration [4, 7] (fig. 2c).

Due to the flow-dependency of NO concentrations in exhaled air, it is vital to monitor the exhalation flow rate carefully when measuring NO, if one chooses to express the value obtained as a concentration. Airway NO release may also be expressed as the amount of NO excreted per time unit during exhalation.

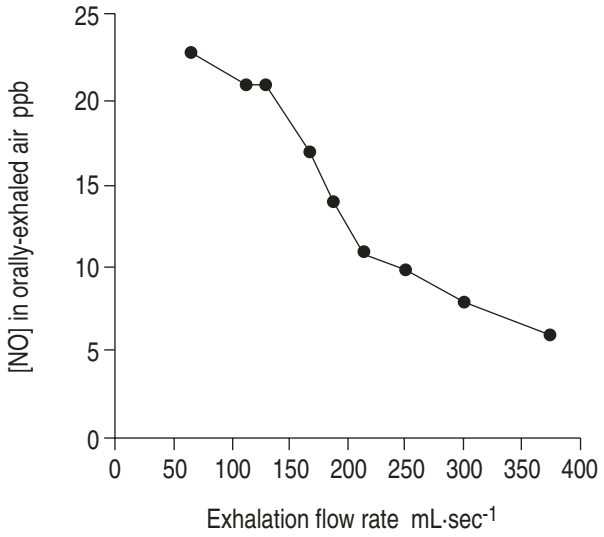

Fig. 1. – Graph illustrating the relationship between exhalation flow rate and nitric oxide concentration [NO] in orally-exhaled air of a healthy subject.

Continuous measurements

NO may also be measured during normal tidal breathing. This method is suitable for continuous measurements of NO over longer periods of time, for example to study the effect of physical exercise on exhaled NO levels. One problem with this method is that even the normal exhalation rate will dilute NO to concentrations close to the detection limit of most analysers. As with the single-breath measurements, one must also bear in mind the influence of flow rate on NO concentrations when using this method. For measurements of NO in orally-exhaled air, the subject breathes through a mouthpiece whilst wearing a noseclip. NO-free air is inhaled either directly from ambient air or from a reservoir, and the exhaled fraction is led into tubings *via* a nonrebreathing valve [3]. Air is continuously sampled from the exhalation limb of the system and NO levels are registered.

Orally-exhaled NO levels have been used to estimate alterations in NO excretion in lower parts of the airways, for example in asthma (see below). However, NO derived from the upper airways may severely contaminate the air exhaled through the mouth. Thus, subjects with a permanent tracheostomy exhale only low levels when breathing through the tracheostomy, while the

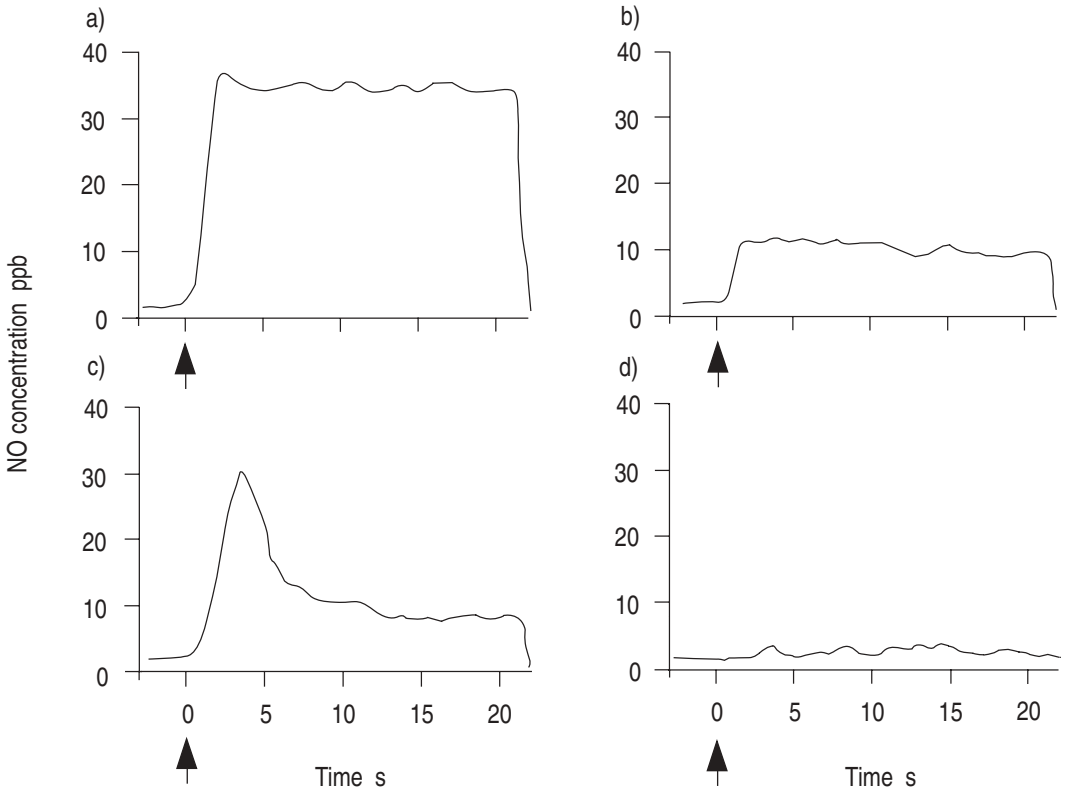

Fig. 2. – Original recordings from single-breath exhalations from total lung capacity of a conscious subject with a permanent tracheostomy. The arrow indicates start of exhalation. NO measurements were made over an exhalation time of 20 s during: a) exhalation through the nose without breathholding; b) exhalation through the mouth without breathholding; c) exhalation through the mouth after 15 s of breathholding; and d) exhalation through the tracheostomy after 15 s of breathholding. (From [4] with permission).

same individuals show considerably higher exhaled NO levels when breathing through the mouth or nose [4] (fig. 2d).

The contribution of NO from the upper airways to orally-exhaled levels may be further accentuated by using continuous oral breathing using a noseclip. When a noseclip is used, large amounts of NO will accumulate in the nasal airways and this NO may be added to orally-exhaled air when air from the nose passes to the pharynx following changes in the position of the soft palate. Thus, the large NO production in the upper airways may easily mask relatively small changes in NO excretion in the lower airways. Indeed, KIMBERLY *et al.* [8] have shown that approximately 50% of exhaled NO comes from the nose during mouth breathing with an open posterior nasopharynx.

Direct nasal sampling

In order to measure NO excretion into the nasal airways, a direct sampling technique has been developed [4]. The subjects are asked either to hold their breath with the mouth closed, or to breathe normal tidal volumes through the mouth during the measurements. An occlusive catheter is introduced into one nostril and air is aspirated continuously at a constant flow rate from the nasal cavity. Aspirated air is led directly to the NO analyser. In this procedure, ambient air (only used if free of NO) is continuously drawn through the contralateral nostril and around the septum *via* the nasopharynx. Using a fixed rather high flow rate (0.7–0.8 $L \cdot min^{-1}$) during these continuous measurements ensures that the resulting NO concentrations will be representative of the total amount of NO excreted into the nasal airways per unit of time [9].

Alternatively, to measure the actual NO concentration in the nasal cavity at a given time, a small volume, not exceeding total nasal cavity volume, can be sampled in a syringe directly from one nostril and subsequently injected into the NO analyser [9, 10]. To further separate the nasal airways from the rest of the respiratory tract during nasal NO measurements, one might use a technique where the soft palate is closed voluntarily [8]. Measurements of carbon dioxide may be performed simultaneously in the nose to ensure that no contamination with air from the lower airways is occurring [8].

Origin of exhaled NO in healthy individuals

Anatomical origin

There has been considerable confusion in the literature concerning the origin of NO found in the exhaled air of humans. Some authors have suggested that this NO is produced in the lung [11], whilst others have claimed that exhaled NO is produced in the terminal region of the bronchial tree [7]. However, several studies have now clearly shown that, in healthy subjects at rest, virtually all exhaled NO is produced within the upper airways, with only a minor contribution from the lower respiratory tract and the lungs [3, 4, 12]. Hence, conscious tracheostomized subjects exhaled only low levels when breathing through the tracheostomy, while these subjects showed much higher levels during oral or nasal breathing [4] (fig. 2). In almost all healthy subjects, NO values are higher during nasal than during oral breathing, indicating large NO excretion in the nasal airways [4].

We recently demonstrated that there is a large production of NO in the paranasal sinuses of humans [13]. Sinus-derived NO reaches the nasal cavity through the ducts connecting the sinuses with the nose, and makes a large contribution to the levels of NO found in the nasal cavity [14]. The contribution from the nasal cavity mucosa to NO levels found in nasally-exhaled air seems to be of less importance, since local instillation of a NO synthase (NOS) inhibitor into the nasal cavity only slightly reduced NO levels in nasal cavity air [13]. As mentioned above, only very low levels of NO (usually below the levels in ambient air) are normally found in air derived from the lower airways. In certain conditions, however, it is possible that there is also a substantial NO excretion into the lumen in the lower airways (see below).

Cellular origin

It is likely that most of the NO excreted into the airway lumen is produced superficially in the mucosa, since NO produced in deeper mucosal structures would probably be trapped, *e.g.* by reaction with haemoglobin, and, therefore, would not reach the lumen. Sinus NO presumably originates from the epithelial cells lining the sinuses, since strong NOS immunoreactivity has been found in those cells [13]. Weak NOS immunoreactivity has also been found in epithelial cells in the nasal cavity [13]. GERLACH *et al.* [12] suggested the presence of a substantial NO release in the nasopharynx and pharynx, but this has not yet been established.

NOS isoforms in the airways

Conceivably, most of the NO found in exhaled air is endogenously produced by "NOSs" present in airway mucosal cells. In a number of studies, inhaled NOS inhibitors have markedly reduced airway excretion of NO [2, 13, 15]. For example, intrasinus instillation of N^G-nitro-L-arginine methyl ester (L-NAME) resulted in an almost complete inhibition of sinus NO release [13]. However, a bacterial contribution to luminal NO cannot be entirely excluded, since some strains of bacteria are known to produce NO from nitrite [16]. Furthermore, a recent study indicated that NO may be formed in the oral cavity by reduction of nitrite at the surface of the tongue [17].

All three known isoforms of NOS (neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS)) have been identified in human airway mucosa. Endothelial NOS is found in mucosal blood vessels and also in cultured human bronchial epithelium [18]. Neuronal NOS has been identified in bronchial epithelial cells [19], as well as in certain mucosal nerves. Inducible NOS may be expressed in bronchial epithelial cells, for example during inflammation [20] or *in vitro* after stimulation

with certain cytokines [19]. However, there has been confusion in the literature as to whether this NOS isoform is also present in healthy bronchial epithelium. HAMID *et al.* [20] and ASANO *et al.* [19] found no evidence of iNOS expression in healthy bronchial epithelium, whereas GUO *et al.* [21] reported iNOS expression in bronchial mucosa of healthy subjects [21].

Nevertheless, it seems quite clear that in certain areas of the upper airways iNOS is constantly expressed [13]. The NOS found in healthy sinus epithelium [13] is identical or very closely related to the iNOS that has been cloned in activated human hepatocytes [22], as shown by messenger ribonucleic acid (mRNA) *in situ* hybridization as well as immunohistochemical studies. Furthermore, like iNOS, sinus NOS is predominantly Ca^{2+}-independent in its nature, as revealed by a citrulline bioassay [23]. However, the regulation of expression and the activity of sinus iNOS seems to differ from what has been described previously for this isoform. Thus, sinus NOS is constitutively expressed and seems resistant to steroids [13, 23, 24], properties that are normally associated with nNOS and eNOSs, which have low productivity levels [25]. Recent studies indicate that there may be multiple iNOS-like sequences in the human genome that encode iNOS-like products [26]; this may help to explain these marked differences in regulation of expression.

The finding of a highly productive NOS in the upper airways and the absence of this enzyme in the lower respiratory tract in normal individuals fits well with the levels of NO normally excreted into the airway lumen in the upper and lower airways, respectively. The much larger NO excretion in the upper compared to the lower airways is even more clearly apparent if one compares the actual amounts of NO released into different parts of the airways per time unit. Total NO release in the entire normal lower airways as measured in exhaled air of adults is less than 5 $nmol \cdot min^{-1}$ at rest [13, 27]. This is less than the excretion from the paranasal sinuses [13]. Hence, NO excretion in one maxillary sinus may well exceed 20 $nmol \cdot min^{-1}$. Although NO levels in air derived from the lower airways are very low, it cannot be excluded that a substantial NO production also takes place in these parts of the airways; this NO may be trapped in the lower airways and the lungs following reaction with, *e.g.* haemoglobin, or other compounds that react rapidly with NO.

Factors that influence exhaled NO

There is increasing knowledge about the various physiological and pathological factors that influence levels of NO in exhaled air, although much further study is still required. Various diseases that are known to influence airway excretion of NO are summarized in table l.

Physiological factors

Body posture has not been shown to have any influence on exhaled NO levels. However, the amount of NO released into the nasal airways from the paranasal sinuses could, theoretically, decrease when a subject is lying down, due to alterations in sinus ostial patency. Ostial patency is known to decrease when a subject lies down [35]; hence, NO passage from the sinuses to the nasal cavity may decrease.

Nasal NO levels (measured by continuous sampling from the nose) are lower in newborns and increase with age, reaching levels similar to those in adults at the age of approximately 10 yrs [13]. In this study, the same sampling rate (0.8 $L \cdot min^{-1}$) was used in all subjects. If one calculates airway NO excretion in relation to body surface area and compares these relative nasal NO levels in children at the age of 10 yrs and in adults, one finds that these children excrete approximately twice as much NO into the nasal airways compared to adults [13]. The reason for this is not clear, but it may be due to the fact that total paranasal sinus volume does not parallel the increase in body surface area during growth. Instead, the sinuses develop primarily during the first 10–12 yrs in humans [36]. Another possible reason could be that the regional distribution of NO-producing cells in the nasal airways differs between adults and children. Thus, a larger proportion of the excreted NO might be released from the nasal mucosa in children.

In adult males and children, intraindividual changes in orally- or nasally-exhaled NO over time seem to be

Table 1. – Influence of respiratory tract disease on airway excretion of nitric oxide (NO)

Disease	Age	Orally-exhaled NO	Nasal NO	[Ref.]
Asthma	Adults	Increased	Unchanged	[3]
	Adults	Increased	NR	[15, 28, 29]
	Children, 5–15 yrs	Increased	Unchanged	[30]
Rhinitis	Children, 5–15 yrs	NR	Unchanged	[30]
	Adults	Increased	Increased	[31]
Cystic fibrosis	Children, 5–15 yrs	Unchanged*	Decreased	[30]
Kartagener's syndrome	Children, 2.5–12 yrs	Unchanged‡	Decreased	[4]
Bronchiectasis	Adults	Increased	NR	[32]
Chronic bronchitis	Adults	Increased#	NR	[33]
Sarcoidosis	Adults	Unchanged	NR	[33]
URTI	Adults	Increased	NR	[34]
LRTI	Adults	Increased	NR	[3]

*: none of the patients had ongoing severe respiratory infection at the time of the measurements; ‡: unpublished observations; #: during acute exacerbation. URTI: upper respiratory tract infection; LRTI: lower respiratory tract infection; NR: not reported.

minor, provided that the measurements are performed in the same way [4]. However, in menstruating females great variations of exhaled NO in synchrony with the menstrual cycle have been reported [37]. Thus, exhaled NO peaked at midcycle during ovulation, whereas it dropped during menstruation. It is known that circulating nitrate/nitrite levels increase with follicular development [38], and that oestradiol may increase the expression of eNOS. However, it is not clear whether the cyclic alterations in exhaled NO reflects changes in excretion of NO into the upper or lower airways. Exhaled NO levels have been reported to remain fairly constant during the course of a normal pregnancy [39].

Several groups have investigated whether orally-exhaled NO levels are affected by physical exercise [7, 27, 40]. The results are similar: absolute concentrations of exhaled NO decrease during exercise but, since the ventilation increases greatly, the calculated amounts of excreted NO are increased (table 2). In contrast, we recently showed that NO excretion in the nasal airways is acutely reduced by heavy physical exercise [10], possibly due to a reduced mucosal blood flow in the upper airways (including the paranasal sinuses) during exercise [46], which in turn might reduce the availability of substrate for NOS. This is supported by the fact that *i.v.* infusion of L-arginine increases upper airway NO excretion [23], indicating that substrate availability is a rate-limiting factor for the Ca^{2+}-independent NOS in the human paranasal sinus mucosa.

The amount of NO exhaled by cigarette smokers has been observed to be lower than normal [12, 28] (table 2). The reason for this is not clear but it could be due to a downregulation of endogenous NO synthesis because of the high NO content in cigarette smoke [47], or damage to NO producing cells by toxic agents in smoke. The presence of NO in cigarette smoke also explains its vasodilatory effects on the pulmonary and bronchial circulation [48]. Since basal NO excretion from the lower airways is minimal, as discussed above, it is likely that this downregulation occurs in the upper parts of the airways. Indeed, GERLACH *et al.* [12] reported lower nasopharyngeal NO levels in smokers than in nonsmokers.

Interestingly, large NO production in the upper airways has only been observed in humans and other primates [49]. In pigs, rats and rabbits, for example, we have found only low nasal NO levels (unpublished observations). On the other hand, some commonly used experimental animals, such as guinea-pigs and rabbits, show a high basal NO release in the lower airways [2].

Asthma and exhaled NO

Inflammation in general has been reported to be associated with enhanced production of NO, and NO has been implicated in the pathogenesis of certain inflammatory diseases [50]. In 1993, ALVING *et al.* [3] reported increased NO levels in orally-exhaled air of asthmatics. These findings have since been confirmed by several research groups [15, 28, 29]. Nasal NO excretion does not differ between asthmatics and controls in children [30] or adults [3], indicating that the increase in exhaled NO involves the lower airways in asthmatics. Indeed, MASSARO *et al.* [51] reported increased NO concentrations in isolated lower airways of asthmatic subjects [51]. Furthermore, HAMID *et al.* [20] reported expression of high producing iNOS in asthmatic bronchial epithelium, but not in control bronchial tissue from healthy subjects. This may be one explanation for the elevation of the orally-exhaled NO levels in asthmatic patients.

However, other sources, such as macrophages, mast cells or other NO-generating cells found in great quantities in inflamed mucosa, cannot be excluded. Glucocorticoids are known to generally inhibit the expression of iNOS [52]. Therefore, one would expect that exhaled NO levels would decrease in patients on treatment with such drugs. Indeed, exhaled NO levels in adult asthmatic patients on regular treatment with inhaled steroids are similar to controls [15, 29, 30], indicating that the expression of a steroid-sensitive iNOS is responsible for the elevated NO levels seen in asthmatics. Furthermore, both systemic [29] and local [41] steroid treatment reduces exhaled NO over a period of days to weeks in asthmatics.

Table 2. – Effects of drugs, smoking habits and exercise on airway excretion of nitric oxide (NO)

Agent	Administration	Orally-exhaled NO	Nasal NO	[Ref.]
Glucocorticoids				
Healthy subjects	Nasal instillation	Unchanged	Unchanged	[4]
	Systemic	Unchanged	Unchanged	[23]
Asthmatics	Oral inhalation	Decreased	NR	[15, 41]
	Oral/nasal inhalation	Decreased	Unchanged	[24]
L-arginine	Systemic	Increased	NR	[42]
	Systemic	NR	Increased	[23]
	Nasal spray	NR	Unchanged	[9]
NO synthase inhibitors	Nasal instillation	Unchanged	Minor decrease	[13]
	Orally inhaled	Decreased		[43]
Ethanol	Ingestion	Decreased	NR	[44]
Histamine, capsaicin	Nasal spray	NR	Unchanged	[9]
α_2-agonists	Nasal spray		Decreased	[9]
Nitroglycerine	Intravenous	Increased	NR	[45]
Antibiotics	Systemic	Unchanged	Unchanged	[4]
Cigarette smoke		Decreased	NR	[28, 33]
			Decreased	[12]
Physical exercise		Increased	NR	[7, 27, 40]
		NR	Decreased	[10]

NR: not reported.

In asthmatic children a dose-dependency has been observed; those treated with no or low-to-moderate doses of topical steroids showed increased exhaled NO, whereas those treated with the highest doses of steroids showed exhaled NO levels that did not differ from controls [30].

Recently, KHARITONOV *et al.* [53] reported further elevation of exhaled NO during the late phase reaction of asthmatics. Measurements were made after antigen challenge and only dual responders showed elevated exhaled NO levels. This indicates that the late phase reaction increase in NO levels is due to an inflammation-driven increase in iNOS expression.

Many studies on exhaled NO in asthmatics have now been performed, and it is likely that the expression of an iNOS in asthmatic airways is responsible for the elevation in exhaled NO levels seen in these patients. Asthma therapy today is very much focused on treatment of the underlying inflammatory disease and there is a great need for an objective marker of airway inflammation [54]. It is tempting to speculate on the possible usefulness of NO in exhaled air as a marker of airway inflammation; these measurements are easy to perform, noninvasive, objective and rapidly provide information about a local process.

However, further studies are needed to investigate the potential of this method in the diagnosis and monitoring of airway inflammation. Such studies include longitudinal trials to explore how well exhaled NO correlates with severity of disease, medication and to other known markers of inflammation, such as eosinophil proteins. Furthermore, it is important to thoroughly examine what other factors (some discussed in this article) influence levels of exhaled NO. Finally, much remains to be improved in the methodology of NO measurements. NO measuring systems should be designed so as to obtain a maximal contribution of NO from the parts of the airways that are of interest, and to minimize contamination due to normal NO production elsewhere in the respiratory tract. For example, asthmatic subjects show elevated orally-exhaled NO levels, whereas NO levels in nasally-exhaled air of asthmatics do not differ from controls [3]. Therefore, it is vital to minimize nasal contribution in these patients during measurements. In this aspect, oral single-breath measurements may be superior to continuous sampling, as discussed above.

There has been a great variation in reported absolute values for NO in exhaled air, probably because of the different measurement techniques used. A European Respiratory Society taskforce is presently working on recommendations for a standard technique in measuring exhaled NO to monitor pulmonary inflammation.

Other diseases of the respiratory tract

Asthma is not the only airway disease associated with alterations in exhaled NO. ALVING *et al.* [3] reported transient elevations of orally-exhaled NO in subjects with lower respiratory tract infection, and KHARITONOV *et al.* [34] reported increased NO levels during the symptomatic period in subjects with upper airway infection. Increased NO levels have also been reported in patients with bronchiectasis [32]. Hence, both inflammation and infection in the airways may be associated with enhanced airway production of NO. This is not surprising, since the factors known to induce expression of iNOS (*e.g.* certain proinflammatory cytokines and bacterial products) may be present in increased amounts in inflamed/infected tissues. SCHILLING *et al.* [55] reported reduced exhaled NO in patients with hypertension.

NO excretion in the nasal airways does not seem to be altered in asthmatics, as mentioned above. However, in children with Kartagener's syndrome (a triad consisting of bronchiectasis, sinusitis, and *situs inversus*) nasal NO levels are extremely low [4]. Whether this finding reflects reduced diffusion of NO from the airway mucosa or a genuine reduction in mucosal NO synthesis, is not clear. We have also found decreased nasal NO levels in children with another chronic airway disease, namely cystic fibrosis [30]. In a group of children with perennial allergic rhinitis, we found no alterations in nasal excretion of NO as compared to controls [30], whereas MARTIN *et al*. [31] reported increased NO levels both in orally- and nasally-exhaled air in adults with seasonal rhinitis.

Since a large part of airway NO production normally takes place in the paranasal sinuses [13, 14], it will, of course, be of great interest to investigate whether patients with sinus disorders exhibit altered airway NO levels. The finding of low nasal NO levels in patients with chronic sinus disorders, such as Kartagener's syndrome, or with cystic fibrosis may indeed indicate that sinus NO release can be altered in these patients.

Drugs

Various drugs that may interact with NO synthesis and release are summarized in table 2.

Glucocorticoids have been mentioned above; these agents depress already increased exhaled NO levels in, for example, asthmatics. However, steroids do not influence basal exhaled NO levels in healthy individuals [4]. Interestingly, the "inducible-like" NOS found in the upper airways appears not to be steroid sensitive, since intranasal steroids do not affect nasal NO levels [4], and sinus NO excretion seems to be unaffected even by high doses of systemic steroids [13, 23]. Competitive NOS inhibitors can decrease airway NO release in the nasal airways when given locally [13, 24]. YATES *et al.* [43] reported decreased orally-exhaled NO levels in controls and in asthmatics following oral inhalation of an NOS inhibitor. Antibiotics do not alter excretion of NO in the upper airways [4]. PERSSON and co-workers [44] reported decreased orally-exhaled NO levels following ingestion of ethanol.

Nitrovasodilators, such as nitroglycerine, act through the release of NO, and increased exhaled NO levels have been observed in experimental animals following *i.v.* administration of this drug [45].

L-arginine, the substrate for enzymatic NO synthesis, has been shown to increase exhaled NO in a dose-dependent manner when taken orally [42], or infused intravenously [23]. This increase seems to take place primarily in the upper airways [23]. Conversely, locally administered α-adrenergic agonists reduce nasal NO levels acutely [9], possibly due to reduced substrate supply, as discussed above.

Measurements of luminal NO in the gastrointestinal and urinary tracts

The concept of collecting air or gases from hollow organs and measuring NO content may also be applied in other luminal structures, for example the gastrointestinal tract. In fact, during early studies, we noted that if a subject swallowed during measurements of NO in exhaled air, there was a marked peak in exhaled NO levels.

Stomach

High levels of NO have been found in expelled air from the stomach [56]. Intragastric NO production is nonenzymatic and requires an acidic environment. Stomach NO is originally derived from the nitrate present in saliva. Nitrate is concentrated in human saliva and is partly reduced to nitrite by bacteria in the oral cavity. Nitrite-containing saliva is regularly swallowed, and NO is formed in the stomach following acidification of the nitrite. When acid secretion is inhibited by omeprazole, stomach NO synthesis is almost abolished [56]. This was the first observation of nonenzymatic NO formation in humans. It has been suggested that intragastric NO could act as a defence mechanism against ingested pathogens [56, 57], or take part in regulation of superficial mucosal blood flow [56]. NO derived from the stomach may accidentally interfere with measurements of NO in exhaled air following regurgitation of stomach air (unpublished observations). However, gastric NO does not seem to contribute continuously to exhaled NO levels either in controls or asthmatics [56].

Large intestine

Low levels of NO have been found in gas aspirated from the colon of controls during colonoscopy [58]. In contrast, patients with active ulcerative colitis [58] or Crohn's disease [24, 59] showed greatly increased luminal NO levels. The source of this NO is probably the mucosa, since increased NOS activity has been detected there by indirect methods in ulcerative colitis patients [60].

Urinary bladder

Increased luminal NO levels have also been observed in the urinary bladder during cystitis [61]. NO-free air was introduced into the urinary bladder in controls and in patients with cystitis of bacterial or chemical genesis. After an incubation period, the air was removed and immediately injected into an NO analyser. NO levels were greatly increased in patients with cystitis of different genesis, compared to controls.

Direct measurement of airborne NO in the gastrointestinal and urinary tracts seems to be an attractive diagnostic tool to reveal inflammation in these hollow organs.

Role of airway-derived NO

One may speculate over the biological significance of the NO that is released into the airways. Is it just a spillover product, or can it play a role in the regulation of certain airway or lung functions?

Host defence

The fact that NO can be produced in large amounts by an iNOS [25] suggests that NO plays a role in primary host defence. Indeed, NO is involved in mouse macrophage killing of a variety of pathogens, including virus [25]. The concentration of NO in normal paranasal sinuses and even in the nasal cavity, greatly exceeds the NO concentrations that are bacteriostatic to, for example, *Staphylococcus aureus* [62], thereby indicating a role for NO in upper airway host defence. Interestingly, children with Kartagener's syndrome or cystic fibrosis, who have very low nasal NO levels [4, 30] also have severe problems with recurrent airway infections. NO has been shown to increase ciliary beat frequency in airway epithelium both *in vitro* [63] and *in vivo* [64], suggesting another possible role of NO in host defence.

Inflammation

NO produced by an iNOS has also been implicated in the pathogenesis of inflammation [50]. Indeed, NO synthesis is clearly enhanced locally at sites of inflammation, and inhibition of NO synthesis, by *e.g.* L-arginine analogues, may attenuate the tissue damage caused by the inflammation in certain experimental settings [50]. Possible proinflammatory actions of NO may be due to activation of enzymes, such as cyclo-oxygenase [65] or metalloproteases [66]. Furthermore, NO may react with free oxygen radicals present in inflamed tissue to form the highly oxidizing agent peroxynitrite [67]. Peroxynitrite, in turn, may directly cause tissue damage or further decompose to even more oxidizing compounds [67].

NO has also been implicated in the pathogenesis of pertussis, since NOS inhibitors dramatically attenuated the epithelial damaging effects of a cytotoxin that is released by *Bordetella pertussis* [68]. However, the role of NO in inflammation is far from settled, and the recent finding of a constantly-expressed iNOS in the upper airways of humans [13] further complicates this picture, since it clearly demonstrates that the sole expression of iNOS and the subsequent large production of NO is not necessarily associated with tissue damage. On the contrary, iNOS in the upper airways may serve important protective functions in the airways. It has been suggested that upregulation of NO synthesis in asthma serves to counterbalance bronchoconstrictor stimuli [69], although NO is a rather weak bronchodilator compared to its vasodilator properties.

NO as an aerocrine messenger

In most tissues, NO is thought to exert its biological effects in a paracrine manner: this free radical gas is too short-lived to act at greater distances. However, the situation in the airways is strikingly different, in that NO in gas phase is fairly stable. Theoretically, NO produced at one site may, therefore, be transported in luminal air to a distal site of action. NO produced in the upper airways will follow the airstream to the lower airways and the lungs with every inhalation. Hence, a continuous low-dose flushing of the lower airways with NO takes place normally. It has not been clear if this NO

has any biological effects; however, inhaled exogenous NO at concentrations as low as 100 ppb significantly decreases pulmonary vascular resistance (PVR) and improves arterial oxygenation in subjects with severe pulmonary disease [70]. Normally, inhaled endogenous NO levels are in the same concentration range [4, 12]. This supports the notion that NO derived from the upper airways could have physiological effects in the lung and, thereby, act in an aerocrine fashion.

We tested this hypothesis in intubated patients, who are deprived of the possibility of inhaling the endogenous NO produced in their upper airways. We aspirated NO-containing nasal air from the patient's nose and introduced this air into the inhalation limb of the ventilator. A significant fall in PVR and an increase in arterial oxygen tension (Pa,o_2) was seen when nasal air was added to the inhaled air [24, 71]. This finding supports the hypothesis that inhaled NO, produced in the upper airways and then inhaled, may function as an aerocrine messenger involved in the regulation of pulmonary function in man.

Summary and future research

Measurements of exhaled NO may be used to estimate local mucosal NO production in the airways. Such measurements may be helpful in further understanding the physiological and pathophysiological roles of NO in the airways. Recent studies clearly show that almost all NO found in exhaled air of healthy humans is produced in the upper airways, with only a minor contribution from the lower respiratory tract. The largest source of NO in the upper airways seems to be located in the paranasal sinuses; an "inducible-like" NO synthase is constitutively expressed in sinus epithelium and is able to produce bacteriostatic concentrations of NO in the sinuses.

Since airway excretion of NO is altered in certain disorders, measurements of exhaled NO may be helpful in noninvasive diagnosis and monitoring of such diseases. However, before any broader conclusions can be drawn over the usefulness of exhaled NO as a marker of airway disease, it will be important to further characterize normal airway NO production and the factors that influence NO levels in exhaled air.

***Acknowledgements*:** Many thanks to Captain H. Björne and the rest of the crew at Bertha for excellent co-operation during the preparation of this manuscript.

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