J. Breath Res. **6** (2012) 047101 (7pp) doi:10.1088/1752-7155/6/4/047101

Review of exhaled nitric oxide in chronic obstructive pulmonary disease

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Received 8 November 2011
Accepted for publication 24 January 2012
Published 7 June 2012
Online at stacks.iop.org/JBR/6/047101

Abstract
The up-regulation of nitric oxide (NO) by inflammatory cytokines and mediators in central and peripheral airway sites can be easily monitored in exhaled air (F_ENO). It is now possible to estimate the predominant airway site of increased F_ENO i.e. large versus peripheral airway/alveoli, and its potential pathologic and physiologic role in obstructive lung disease. In asthma, six double-blind, randomized, controlled algorithm trials have reported only equivocal benefits of add-on measurements of F_ENO to usual clinical guideline management including spirometry. Significant design issues, as emphasized by Gibson, may exist. However, meta-analysis of these six studies (Petsky *et al* 2012 *Thorax* **67** 199–208) concluded that routine serial measurements of F_ENO for clinical asthma management does not appear warranted. In COPD including chronic bronchitis and emphysema, despite significant expiratory airflow limitation, when clinically stable as well as during exacerbation, F_ENO, j'_{awNO} and C_{ANO} may all be normal or increased. Furthermore, the role of add-on monitoring of exhaled NO to GOLD management guidelines is less clear because of the absence of conclusive doubleblind, randomized, control trial studies concerning potential clinical benefits in the management of COPD.

Abbreviations

C_{ANO}	peripheral/small airway/alveolar nitric oxide concentration
cNOS	constitutive nitric oxide synthase
eNOS	endothelial nitric oxide synthase (NOS3)
F_ENO	fraction exhaled nitric oxide
iNANC	inhibitory non-adrenergic non-cholinergic
iNOS	inducible nitric oxide synthase (NOS2)
j'_{awNO}	large/central airway maximal nitric oxide flux
nNOS	neuronal nitric oxide synthase (NOS1)

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Introduction

We have previously emphasized that nitric oxide (NO) is a gaseous signaling molecule produced by resident cells e.g. airway epithelial cells, airway and circulatory endothelial cells, and trafficking inflammatory cells in both large and peripheral airways/alveoli [1]. The fractional concentration of exhaled NO (F_ENO) can be easily measured and is well-established in clinical research. However, the incorporation of F_ENO into clinical practice is currently undergoing critical assessment. This paper generously builds upon our previous review on the role of F_ENO in COPD [1] with the goal to update the reader

with the potential diagnostic and therapeutic role of NO in COPD, evaluate new ways to model NO exchange in the large (j'_{awNO}) and small airways/alveoli (C_{ANO}) that may identify the anatomic location of NO production, and then to review published F_ENO measurement in COPD.

NO as a mediator of inflammatory airway disease

Endogenous nitric oxide (NO) plays an important role in regulating airway and vascular function and is generated by three isoenzymes of NO synthase (NOS) that are differentially regulated and expressed in the airways and appear to play different pathophysiologic roles [1, 2].

Barnes *et al* [1] have previously noted that all NOS isoenzymes convert L-arginine to L-citrulline with the generation of NO. Constitutive NOS (cNOS) isoenzymes include neuronal NOS (nNOS, NOS1) and endothelial NOS (eNOS, NOS3), both of which are presumed to play a local regulatory role, such as neurotransmission (NOS1) and regulation of local blood flow (NOS3). Inducible NOS (iNOS, NOS2) is not constitutively expressed but is induced by inflammatory and infectious stimuli and produces large amounts of NO which may have a proinflammatory effect [1]. However, the clear distinction between constitutive and inducible isoforms has been blurred by the recognition that cNOS may be inducible, whereas NOS2 may be constitutively expressed in some conditions [1]. Nevertheless, these distinct NOS isoenzymes are regulated by different genes and have different physiological and pathological functions. There are important species differences such that corticosteroids directly suppress NOS2 in rodent cells but do not directly inhibit NOS2 expression in human airway epithelial cells [3]. The increase in NO in exhaled breath in asthma is presumed to originate from increased NOS2 expression in the airway epithelial calls and macrophages, although cNOS isoforms may also contribute. Endothelial NOS3 is expressed in endothelial cells of the bronchial and pulmonary circulation and plays a role in regulating vascular flow [4]. It is also expressed in alveolar endothelial cells and airway epithelial cells throughout the respiratory tract. NOS3 expression is reduced in peripheral lung of patients with COPD, especially in severe disease due to alveolar wall destruction as a result of emphysema [5]. Neuronal NOS is localized to cholinergic nerves in the airways and mediates inhibitory non-adrenergic non-cholinergic (i-NANC) neural bronchodilatation, acting as a functional antagonist of its co-transmitter acetylcholine [6]. It is also expressed in airway epithelial cells and type I pneumocytes, and there is evidence that its expression and activity is increased in peripheral lungs of COPD patients as a result of oxidative stress [7]. NOS1 may contribute to the increase in peripheral NO in COPD and severe asthma that has been reported [8, 9].

Increased inducible NOS2 expression is found in airway epithelial cells of patients with asthma and is reduced by inhaled corticosteroids (ICS) [10]. Increased NOS2 expression is also found in peripheral lung and small airways in patients with COPD [5, 11]; however, there was no effect of high-dose ICS on exhaled nitric oxide [9]. Furthermore, it is unlikely that the inflammation that induces NOS2 is steroid resistant in patients with COPD [9]. Oxidative stress generates superoxide anions and in combination with NO may result in the formation of the highly reactive species peroxynitrite, which is increased in exhaled breath condensate of COPD patients [12] and may account for the increased tyrosine nitration found in peripheral lungs by immunocytochemistry [11]. The formation of peroxynitrite removes NO from the gaseous phase so that its concentration in the airways is reduced when there is a high level of oxidative stress, as in COPD patients. Selective inhibitors of NOS2 reduce F_ENO in asthmatic patients and even in normal subjects [13–15], but have less effect in COPD patients, indicating that the increased peripheral NO may derive from NOS1 as well as NOS2 in these patients.

Modeling of NO excretion in the lungs (see figures 1 and 2)

George *et al* [1] have emphasized the principles that formed the foundation of the two-compartment model (i.e. an airway compartment and an alveolar compartment) of NO exchange, first reported in 1998 [16] and later confirmed by four additional independent research groups [17–20].

In the simplest form, the two-compartment model can be characterized by two parameters: a maximum flux of NO from the large airway compartment (j'_{awNO}, nl s^{-1}, airway generations 1–16) and a steady state mean distal airway/alveolar concentration of NO (C_{ANO}, ppb) (see figure 1). A series of experimental algorithms characterized by measuring F_ENO at different constant exhalation flows have been presented and reviewed [21]. While the simplicity of the initial two-compartment model is a tremendous strength, recent work has demonstrated that axial back-diffusion of NO in the gas phase (i.e. NO back-diffusing from airways toward the alveolar region against the direction of exhalation) cannot be neglected [22–25]. Incorporating axial gas phase back-diffusion of NO produces a two-compartment model with more complex governing equations, and modified algorithms to characterize j'_{awNO} and C_{ANO} [26, 27]. For example, a simple method used widely to determine j'_{awNO} and C_{ANO} is to regress a line through a plot of the elimination rate of NO versus the exhalation flow, and the intercept and slope are estimates of j'_{awNO} and C_{ANO}, respectively ('slope–intercept' method) (see figure 2) [16]. When axial back-diffusion of NO in the gas phase is considered, NO from the airway tree diffuses back (back-diffusion) into the alveolar region where it can falsely elevate the estimate of C_{ANO} and depress the estimate of j'_{awNO}. Thus, the modified algorithm using the slope–intercept method still uses the slope to estimate C_{ANO}, but subtracts a term proportional to the airway flux ($j'_{awNO}/0.53$) to account for axial diffusion; similarly, the estimate for j'_{awNO} remains the intercept but is multiplied by a factor (1.7) to account for the loss to the alveolar region [26].

Clinical interest in modeling NO exchange dynamics, which has the ability to discriminate between large airway NO flux (j'_{awNO}) and the distal alveolated airways and alveolar region (C_{ANO}), remains strong due to the potential clinical utility of measuring and potentially determining the

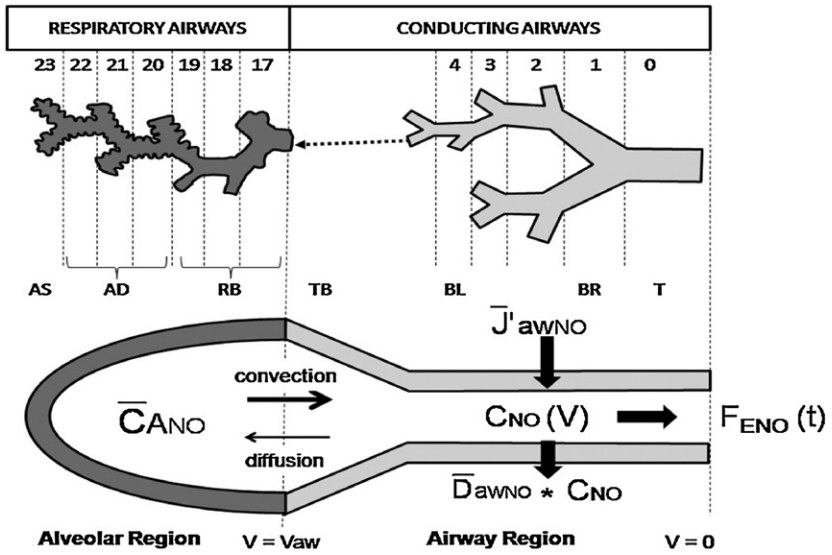

Figure 1. Schematic of the two-compartment model. During exhalation, a steady state mean alveolar or peripheral concentration ($\bar{C}_{ANO}$) enters the airway compartment (net transfer is convection minus diffusion) where upon additional NO is transferred from the airway walls. The alveolar or peripheral concentration represents the acinar region of the lungs (Weibel generations 17–23). The airway compartment represents the oropharynx and Weibel generations 1–16, and considers the increasing surface area per unit volume of the airway tree (i.e. trumpet shape). The contribution from the airways depends on the exhalation flow and is the sum of two terms: $\bar{J}'_{awNO} = \bar{J}_{awNO} - \bar{D}_{awNO} * C_{NO}$. $\bar{J}'_{awNO}$ is the mean maximum airway flux (picoliters/second). $\bar{D}_{awNO}$ is the mean airway diffusing capacity (or conductance). C_{NO} is the airway compartment gas phase NO concentration which depends on axial volumetric position (V), and the airway compartment volume is V_{aw} (T: trachea; BR: bronchus; BL: bronchiole; TB: terminal bronchiole; RB: respiratory bronchiole; AD alveolar duct; AS: alveolar sac). The axial diffusion component takes into account the diffusion of NO in the gas phase from the airways (high concentration) to the alveolar region (low concentration) in accordance with Fick's laws of diffusion. The gradient for diffusion is in the opposite direction of the exhaled flow. Thus, by taking into consideration the axial diffusion of NO, one can correct for the 'back-diffusion' of airway NO and resultant contamination of the alveolar region. Reprinted from [78] with permission from Elsevier.

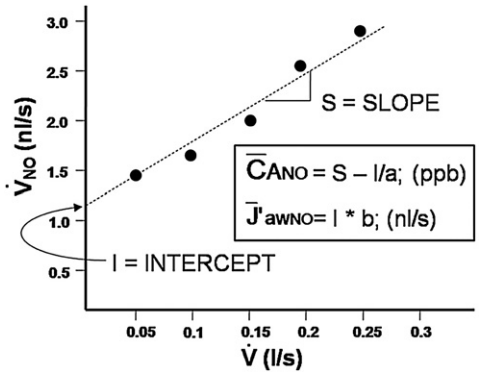

Figure 2. Illustrative data demonstrating the elimination rate versus flow technique to estimate the mean alveolar concentration and maximum airway flux. The steady or plateau nitric oxide concentration can be measured at a series of constant exhalation flows, and then the elimination rate (pL s^{-1}, product of concentration and flow) can be plotted as a function of exhalation flow (mL s^{-1}). For flows greater than ~50–100 mL s^{-1} in adults, this relationship is approximately linear (solid circles), as predicted by the two-compartment model with axial diffusion. The slope minus a term proportional to the airway flux is an estimate of the mean alveolar concentration ($\bar{C}_{ANO}$ (pL s^{-1})/(mL s^{-1}) = ppb), while the intercept is proportional to the mean maximum airway flux of NO ($\bar{J}'_{awNO}$, pL s^{-1}). Specific values for the coefficients 'a' and 'b' are described in Condorelli *et al* [26] and depend on the flow range utilized. Reprinted from [78] with permission from Elsevier.

predominant site and source of inflammation [1]. From the practical viewpoint, F_ENO obtained at 50 ml s^{-1} has been presumed to be a surrogate of large central airway NO flux (j'_{awNO}). However, we have recently reported that the increase in F_ENO at 50 ml s^{-1} in normals, age > 60 yr, was related to an increase in C_{ANO} whereas j'_{awNO} remains relatively constant with ageing [28]. There is no simple surrogate for determining j'_{awNO} versus peripheral NO production (C_{ANO}); therefore F_ENO needs to be obtained at multiple expiratory flow rates and using the aforementioned algorithm [25–27] to determine j'_{awNO} and C_{ANO}.

Exhaled NO as marker of airway inflammation in asthma

F_ENO measurements have been considered a surrogate for eosinophilic airway inflammation, especially in asthma. However, correlations with sputum eosinophils are modest. In the largest population studied, the r^2 value was 0.26, $p = 0.001$; the sensitivities and specificities for clinically significant eosinophilia were around 70–75% [29] and $r^2 = 0.29$, $p = 0.007$ with tissue eosinophilia in treated asthmatics with severe refractory asthma [30].

However, in most mild asthmatics, high F_ENO at 50 ml s^{-1} (>45 ppb) has been regarded as a marker for steroid responsiveness [31, 32] including improvement in spirometry and airway hyper-responsiveness [33]. Alternatively, we have noted that exacerbations in chronic moderate-to-severe asthmatics maintained on ICS and LABA but not oral corticosteroids had similar spirometric improvement to add-on oral corticosteroid irregardless of the baseline F_ENO level including normal value at 50 ml s^{-1} [34]. Increased F_ENO

may also occur with eosinophilic bronchitis [35]. The ATS clinical practice guideline for exhaled nitric oxide in asthma concluded that add-on F_ENO monitoring provides potential easier detection of eosinophilic airway inflammation, and likelihood of corticosteroid responsiveness [36].

Using asthma treatment guidelines *including spirometry* [37] several investigators [38–43] have addressed the clinical role of add-on monitoring of exhaled NO at a single expiratory airflow rate (F_ENO), usually at 50 ml s^{-1}, to help guide inhaled and/or systemic corticosteroid dosing in clinically stable and unstable children [38, 39, 41, 43] and adults [40, 42, 43] with asthma. Results of these six double-blind, randomized, control trials [38–43] were equivocal. Gibson [44] has raised multiple issues related to study design that challenge the validity of the conclusions. However, after analyses of the trials [38–43] Petsky *et al* [45] concluded that routine serial measurements of F_ENO for clinical asthma management does not appear warranted.

Exhaled NO as marker of airway inflammation in COPD

COPD is an inflammatory disease of both large and small airways and alveoli that are predominantly mediated by cytokines and interleukins via neutrophilic cellular pathways [46]. Papi *et al* [47] have noted that in COPD patients with severe exacerbation, airway neutrophilia is increased irregardless of bacterial versus viral etiology, whereas increased eosinophilia usually reflects a viral source. Rutgers *et al* [48] also described increased airway neutrophils and eosinophils in clinically stable COPD patients who underwent bronchoalveolar lavage and large airway bipopsy. However, Rutgers *et al* [49] noted normal values for exhaled NO and only a modest correlation ($r^2 = 0.4$, $p = 0.009$) between sputum eosinophils and exhaled NO.

In stable COPD, F_ENO measurements need to be obtained in non-smokers to avoid misleading reduction in F_ENO. When measured at a *single* expiratory flow rate, F_ENO has been *elevated* [50–52] or *normal* [2] and *increased with exacerbations* [51–53]. Papi *et al* [54], Kunisaki *et al* [55], Dummer *et al* [56], de Laurentis *et al* [57] and Antus *et al* [58] reported that an elevated F_ENO in COPD may also be a variable signal for increased spirometric response to ICS, although the correlation was poor ($r^2 = 0.19$, $p < 0.001$) [58]. In a randomized trial, Siva *et al* [59] successfully used sputum eosinophils compared to standard care as a tool to reduce severe COPD exacerbations. In that study sputum eosinophils and F_ENO were not associated, possibly due to the interaction of ICS and concurrent smoking, both of which independently suppress F_ENO. Despite these conflicting findings, measurement of large airway NO flux (j'_{awNO}) and distal airway/peripheral lung NO (C_{ANO}) via modeling [16, 26, 27] may potentially detect increased large airway NO flux, and allow selection of individual COPD patients with severe expiratory airflow limitation who may benefit from ICS. Using the two-compartment NO model as previously described [16] and with correction for NO axial back-diffusion [26, 27] we [34, 60, 61], Berry *et al* [62], van Veen *et al* [63], Brindicci *et al* [8], Kerckx *et al* [27], Mahut *et al* [64], Paraskakis *et al* [65], Lehtimaki *et al* [66] and Shin *et al* [67] have noted increased j'_{awNO} and increased or normal C_{ANO} in mild and moderate-to-severe clinically stable asthmatics [34]. Using similar NO modeling [16] with and without correction for NO axial back-diffusion [26, 27] Hogman *et al* [68], Lehtimaki *et al* [69], Lehouk *et al* [70] and Brindicci *et al* [9] reported normal [70] and increased j'_{awNO} as well as normal [69, 70] and increased C_{ANO} [9, 68] in clinically stable COPD patients compared to controls. However, in the study by Brindicci *et al* [9] their healthy non-smoking controls had a mean age of 45 yr compared to their COPD cohort with a mean age of 62 yr. This may have confounded their findings, since we have noted that normal younger subjects, age <60 yr, have significantly lower values for C_{ANO} compared to older normals, age > 60 yr [28]. However, in a subsequent study, Brindicci *et al* [5] reported increased mRNA expression and activity of NO synthase isoenzyme nNOS, and not iNOS, in COPD peripheral lung tissue that reflected the severity of the disease. While Hogman *et al* [68] used age-matched controls, neither Hogman *et al* or Brindicci *et al* [9] corrected for axial back-diffusion of NO from large airways to peripheral lung [26, 27]. This underestimates large airway NO flux (j'_{awNO}) and overestimates C_{ANO}. After correction, j'_{awNO} will increase and C_{ANO} will decrease [26, 27]. Alternatively, Verbanck *et al* [71] have suggested that peripheral airway constriction would block axial back-diffusion leading to overestimation of j'_{awNO} and underestimation of C_{ANO}. We [72] recently compared clinically stable COPD patients to age-matched controls using the two-compartment model [16] and after correcting for NO axial back-diffusion [26] found normal values for j'_{awNO} and C_{ANO}. Furthermore, we [72] noted that moderate dose but not low-dose ICS could suppress normal values for j'_{awNO}. Previously, Lehouk *et al* [70] and Roy *et al* [73] also noted normal j'_{awNO} and C_{ANO} in COPD patients compared to age-matched healthy, older, non-smoking subjects, but without correcting for NO axial back-diffusion [73].

With respect to therapeutic intervention, in the TORCH 3 yr double-blind, placebo controlled trial in moderate–severe COPD patients, Jenkins *et al* [74] noted that inhaled salmeterol/fluticasone propionate reduced moderate-to-severe exacerbations and improved health status and FEV_1 (L) across GOLD [75] Stage 2 and 3 and reduced mortality in Stage 2. Meta-analysis by Drummond *et al* [76] in 11 eligible randomized controlled trials (14 426 participants) reported that ICS therapy did not affect 1 yr all-cause mortality but was associated with higher incidence of pneumonia. In a similar meta-analysis including 16 996 COPD patients since 2008, Singh *et al* reached similar conclusions that use of ICS ⩾24 weeks was associated with a significant increase in serious pneumonia without affecting an increase in death [77]. In conclusion, in COPD, the role of add-on F_ENO monitoring to therapeutic intervention [74–77] is less clear with respect to clinical benefits, especially in the absence of conclusive double-blind, randomized, control studies. Therefore, routine monitoring of F_ENO in COPD [75] is less-established than in asthma as noted in the recent ATS Clinical Guidelines [36].

Summary

The up-regulation of NO by inflammatory cytokines and mediators in central and peripheral airway sites can be easily monitored in exhaled air (F_ENO). It is now possible to estimate the predominant airway site of increased F_ENO i.e. large versus peripheral airway/alveoli, and its potential pathologic and physiologic role in obstructive lung disease. In asthma, six double-blind, randomized, controlled, algorithm trials [38–43] have reported only equivocal benefits of add-on measurements of F_ENO to usual clinical guideline [37] management including spirometry. Significant design issues as emphasized by Gibson [44] may exist. However, meta-analysis of these six studies [38–43] by Petsky *et al* [45] concluded that routine serial measurements of F_ENO for clinical asthma management does not appear warranted. In COPD including chronic bronchitis and emphysema, despite significant expiratory airflow limitation, when clinically stable as well as during exacerbation, F_ENO, j'_{awNO} and C_{ANO} may all be normal or increased. Furthermore, the role of add-on monitoring of exhaled NO to GOLD management guidelines [75] is less clear because of the absence of conclusive double-blind, randomized, control trial studies concerning potential clinical benefits in the management of COPD. Empirical use of ICS and long acting beta$_2$-agonist and long acting anti-muscarinic agent appear justified in severe COPD $\geqslant$ Stage 3 to reduce exacerbation and possibly mortality [75], despite higher incidence of pneumonia but not mortality with use of ICS [74–77].

Potential conflicts of interest

Steven C George MD PhD has patents issued and pending that have been licensed in the past by Aerocrine, Ltd, Sweden, and have resulted in royalties. His employer, the University of California, Irvine, has received NO analyzers as gifts from Aerocrine, Ltd, and currently holds the rights to the patents. Noe Zamel MD has received royalties ($2000 per year) from patents that were licensed to Aerocrine, Ltd, and Aperion Inc., manufacturers of exhaled NO monitors. No other contributing authors have potential conflicts of interest to report.

References

[1] Barnes P J *et al* 2010 Exhaled nitric oxide in pulmonary diseases: a comprehensive review *Chest* **138** 682–92

[2] Ricciardolo F L, Sterk P J, Gaston B and Folkerts G 2004 Nitric oxide in health and disease of the respiratory system *Physiol. Rev.* **84** 731–65

[3] Donnelly L E and Barnes P J 2002 Expression and regulation of inducible nitric oxide synthase from human primary airway epithelial cells *Am. J. Respir. Cell Mol. Biol.* **26** 144–51

[4] Shaul P W 2002 Regulation of endothelial nitric oxide synthase: location, location, location *Annu. Rev. Physiol.* **64** 749–74

[5] Brindicci C, Kharitonov S, Ito M, Elloit M W, Hogg J C, Barnes P J and Ito K 2010 Nitric oxide synthase isoenzyme expression and activity in peripheral lungs of COPD patients *Am. J. Respir. Crit. Care Med.* **181** 21–30

[6] Ward J K, Belvisi M G, Fox A J, Miura M, Tadjkarimi S, Yacoub M H and Barnes P J 1993 Modulation of cholinergic neural bronchoconstriction by endogenous nitric oxide and vasoactive intestinal peptide in human airways *in vitro J. Clin.* **92** 736–42

[7] Maarsingh H, Leusink J, Bos I S, Zaagsma J and Meurs H 2006 Arginase strongly impairs neuronal nitric oxide-mediated airway smooth muscle relaxation in allergic asthma *Respir. Res.* **7** 6

[8] Brindicci C, Ito K, Barnes P J and Kharitonov S A 2007 Differential flow analysis of exhaled nitric oxide in patients with asthma of differing severity *Chest* **131** 1353–62

[9] Brindicci C, Ito K, Resta O, Pride N B, Barnes P J and Kharitonov S A 2005 Exhaled nitric oxide from lung periphery is increased in COPD *Eur. Respir. J.* **26** 52–9

[10] Saleh D, Ernst P, Lim S, Barnes P J and Giaid A 1998 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.* **12** 929–37

[11] Ricciardolo F L *et al* 2005 Nitrosative stress in the bronchial mucosa of severe chronic obstructive pulmonary disease *J. Allergy Clin. Immunol.* **116** 1028–35

[12] Osoata G O, Hanazawa T, Brindicci C, Ito M, Barnes P J, Kharitonov S and Ito K 2009 Peroxynitrite elevation in exhaled breath condensate of COPD and its inhibition by fudosteine *Chest* **135** 1513–20

[13] Brindicci C, Ito K, Barnes P J and Kharitonov S A 2007 Effect of an inducible nitric oxide synthase inhibitor on differential flow-exhaled nitric oxide in asthmatic patients and healthy volunteers *Chest* **132** 581–8

[14] Hansel T T, Kharitonov S A, Donnelly L E, Erin E M, Currie M G, Moore W M, Manning P T, Recker D P and Barnes P J 2003 A selective inhibitor of inducible nitric oxide synthase inhibits exhaled breath nitric oxide in healthy volunteers and asthmatics *Faseb J.* **17** 1298–300

[15] Singh D, Richards D, Knowles R G, Schwartz S, Woodcock A, Langley S and O'Connor B J 2007 Selective inducible nitric oxide synthase inhibition has no effect on allergen challenge in asthma *Am. J. Respir. Crit. Care Med.* **176** 988–93

[16] Tsoukias N M and George S C 1998 A two-compartment model of pulmonary nitric oxide exchange dynamics *J. Appl. Physiol.* **85** 653–66

[17] Hogman M, Drca N, Ehrstedt C and Meriläinen P 2000 Exhaled nitric oxide partitioned into alveolar, lower airways and nasal contributions *Respir. Med.* **94** 985–91

[18] Jorres R A 2000 Modelling the production of nitric oxide within the human airways *Eur. Respir. J.* **16** 555–60

[19] Pietropaoli A P, Perillo I B, Torres A, Perkins P T, Frasier L M, Utell M J, Frampton M W and Hyde R W 1999 Simultaneous measurement of nitric oxide production by conducting and alveolar airways of humans *J. Appl. Physiol.* **87** 1532–42

[20] Silkoff P E, Sylvester J T, Zamel N and Permutt S 2000 Airway nitric oxide diffusion in asthma: role in pulmonary function and bronchial responsiveness *Am. J. Respir. Crit. Care Med.* **161** 1218–28

[21] George S C, Hogman M, Permutt S and Silkoff P E 2004 Modeling pulmonary nitric oxide exchange *J. Appl. Physiol.* **96** 831–9

[22] Shin H W, Condorelli P and George S C 2006 Examining axial diffusion of nitric oxide in the lungs using heliox and breathhold *J. Appl. Physiol.* **100** 623–30

[23] Shin H W, Condorelli P, Rose-Gottron C M, Cooper D M and George S C 2004 Probing the impact of axial diffusion on nitric oxide exchange dynamics with heliox *J. Appl. Physiol.* **97** 874–82

[24] Shin H W and George S C 2002 Impact of axial diffusion on nitric oxide exchange in the lungs *J. Appl. Physiol.* **93** 2070–80
[25] Van Muylem A, Noel C and Paiva M 2003 Modeling of impact of gas molecular diffusion on nitric oxide expired profile *J. Appl. Physiol.* **94** 119–27
[26] Condorelli P, Shin H W, Aledia A S, Silkoff P E and George S C 2007 A simple technique to characterize proximal and peripheral nitric oxide exchange using constant flow exhalations and an axial diffusion model *J. Appl. Physiol.* **102** 417–25
[27] Kerckx Y, Michils A and Van Muylem A 2008 Airway contribution to alveolar nitric oxide in healthy subjects and stable asthma patients *J. Appl. Physiol.* **104** 918–24
[28] Gelb A F, George S C, Camacho F, Fraser C, Flynn Taylor C and Shakkottai S 2011 Increased nitric oxide concentrations in the small airway of normal subjects *Chest* **139** 368–75
[29] Berry M A, Shaw D E, Green R H, Brightling C E, Wardlaw A J and Pavord I D 2005 The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma *Clin. Exp. Allergy* **35** 1175–9
[30] Silkoff P E, Lent A M, Busacker A A, Katial R K, Balzar S, Strand M and Wenzel S E 2005 Exhaled nitric oxide identifies persistent eosinophilic phenotype in severe refractory asthma *J. Allergy Clin. Immunol.* **116** 1249–55
[31] Pijnenburg M W, Hofhuis W, Hop W C and de Jongste J C 2005 Exhaled nitric oxide predicts asthma relapse in children with clinical asthma remission *Thorax* **60** 215–8
[32] Zacharasiewicz A, Wilson N, Lex C, Erin E M, Li A M, Hansel T, Khan M and Bush A 2005 Clinical use of noninvasive measurements of airway inflammation in steroid reduction in children *Am. J. Respir. Crit. Care Med.* **171** 1077–82
[33] Smith A D, Cowan J O, Brassett K P, Filsell S, McLachlan C, Monti-Sheehan G, Herbison G P and Taylor D R 2005 Exhaled nitric oxide: a predictor of steroid response *Am. J. Respir. Crit. Care Med.* **172** 453–9
[34] Gelb A F, George S C, Silkoff P E, Krishnan A, Fraser C, Taylor C F, Shinar C M and Maginot T 2010 Central and peripheral airway/alveolar site of exhaled nitric oxide in acute asthma *Thorax* **65** 619–25
[35] Brightling C E, Symon F A, Birring S S, Bradding P, Wardlaw A J and Pavord I D 2003 Comparison of airway immunopathology of eosinophilic bronchitis and asthma *Thorax* **58** 528–32
[36] Dweik R A, Boggs P B, Erzurum S C, Irvin C G, Leigh M W, Lundberg J O, Olin A C, Plummer A L and Taylor D R 2011 ATS documents. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide level (F_ENO) for clinical application *Am. J. Respir. Crit. Care Med.* **184** 602–15
[37] Global strategy for asthma management and prevention www.ginasthma.org
[38] Szefler S J *et al* 2008 Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults: a randomised controlled trial *Lancet* **372** 1065–72
[39] de Jongste J C *et al* 2009 Daily telemonitoring of exhaled nitric oxide and symptoms in the treatment of childhood asthma *Am. J. Respir. Crit. Care Med.* **179** 93–7
[40] Shaw D E, Berry M A, Thomas M, Green R H, Brightling C E, Wardlaw A J and Pavord I D 2007 The use of exhaled nitric oxide to guide asthma management: a randomized controlled trial *Am. J. Respir. Crit. Care Med.* **176** 231–7
[41] Pijnenburg M W, Bakker E M, Lever S, Hop W C and De Jongste J C 2005 High fractional concentration of nitric oxide in exhaled air despite steroid treatment in asthmatic children *Clin. Exp. Allergy* **35** 920–5
[42] Smith A D, Cowan J O, Brassett K P, Herbison G P and Taylor D R 2005 Use of exhaled nitric oxide measurements to guide treatment in chronic asthma *N. Engl. J. Med.* **352** 2163–73
[43] Mahut B *et al* 2010 Multicentre trial evaluating alveolar NO fraction as a marker of asthma control and severity *Allergy* **65** 636–44
[44] Gibson P G 2009 Using fractional exhaled nitric oxide to guide asthma therapy: design and methodological issues for ASthma TReatment ALgorithm studies *Clin. Exp. Allergy* **39** 478–90
[45] Petsky H L, Cates C J, Lasserson T J, Li A M, Turner C, Kynaston J A and Chang A B 2012 A systemic review and meta-analysis tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils) *Thorax* **67** 199–208
[46] Barnes P J, Chowdhury B, Kharitonov S A, Magnussen H, Page C P, Postma D and Saetta M 2006 Pulmonary biomarkers in COPD *Am. J. Respir. Crit. Care Med.* **174** 6–14
[47] Papi A, Bellettato C M and Braccioni F 2006 Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations *Am. J. Respir. Crit. Care Med.* **173** 1114–21
[48] Rutgers S R, Postma D S, ten Hacken N H, Kauffman H F, van Der Mark T W, Koëter G H and Timens W 2000 Ongoing airway inflammation in patients with COPD who do not currently smoke *Thorax* **55** 12–8
[49] Rutgers S R, van der Mark T W, Coers W, Moshage H, Timens W, Kauffman H F, Koëter G H and Postma D S 1999 Markers of nitric oxide metabolism in sputum and exhaled air are not increased in chronic obstructive pulmonary disease *Thorax* **54** 576–80
[50] Corradi M, Majori M, Cacciani G C, Consigli G F, de'Munari E and Pesci A 1999 Increased exhaled nitric oxide in patients with stable chronic obstructive pulmonary disease *Thorax* **54** 572–5
[51] Ansarin K, Chatkin J M, Ferreira I M, Gutierrez C A, Zamel N and Chapman K R 2001 Exhaled nitric oxide in chronic obstructive pulmonary disease: relationship to pulmonary function *Eur. Respir. J.* **17** 934–8
[52] Maziak W, Loukides S, Culpitt S, Sullivan P, Kharitonov S A and Barnes P J 1998 Exhaled nitric oxide in chronic obstructive pulmonary disease *Am. J. Respir. Crit. Care Med.* **157** 998–1002
[53] Bhowmik A, Seemungal T A, Donaldson G C and Wedzicha J A 2005 Effects of exacerbations and seasonality on exhaled nitric oxide in COPD *Eur. Respir. J.* **26** 1009–15
[54] Papi A, Romagnoli M, Baraldo S, Braccioni F, Guzzinati I, Saetta M, Ciaccia A and Fabbri L M 2000 Partial reversibility of airflow limitation and increased exhaled NO and sputum eosinophilia in chronic obstructive pulmonary disease *Am. J. Respir. Crit. Care Med.* **162** 1773–7
[55] Kunisaki K M, Rice K L, Janoff E N, Rector T S and Niewoehner D E 2008 Exhaled nitric oxide, systemic inflammation, and the spirometric response to inhaled fluticasone propionate in severe chronic obstructive pulmonary disease: a prospective study *Ther. Adv. Respir. Dis.* **2** 55–64
[56] Dummer J F, Epton M J, Cowan J O, Cook J M, Condliffe R, Landhuis C E, Smith A D and Taylor D R 2009 Predicting corticosteroid response in chronic obstructive pulmonary disease using exhaled nitric oxide *Am. J. Respir. Crit. Care Med.* **180** 846–52
[57] de Laurentis G, Maniscalco M, Cianciulli F, Stanziola A, Marsico S, Lundberg J O, Weitzberg E and Sofia M 2008 Exhaled nitric oxide monitoring in COPD using a portable oxygen analyzer *Pulm. Pharmacol. Ther.* **21** 689–93

[58] Antus B, Barta I, Horvath I and Csiszer E 2010 Relationship between exhaled nitric oxide and treatment responses in COPD patients with exacerbations *Respirology* **15** 472–7
[59] Siva R *et al* 2007 Eosinophilic airway inflammation and exacerbations of COPD: a randomised controlled trial *Eur. Respir. J.* **29** 906–13
[60] Gelb A F, Taylor C F, Shinar C M, Gutierrez C and Zamel N 2006 Role of spirometry and exhaled nitric oxide to predict exacerbations in treated asthmatics *Chest* **129** 1492–9
[61] Gelb A F, Taylor C, Nussbaum E, Gutierrez C, Schein A, Shinar C M, Schein M J, Epstein J D and Zamel N 2004 Alveolar and airway sites of nitric oxide inflammation in treated asthma *Am. J. Respir. Crit. Care Med.* **170** 737–41
[62] Berry M, Hargadon B, Morgan A, Shelley M, Richter J, Shaw D, Green R H, Brightling C, Wardlaw A J and Pavord I D 2005 Alveolar nitric oxide in adults with asthma: evidence of distal lung inflammation in refractory asthma *Eur. Respir. J.* **25** 986–91
[63] van Veen I H, Sterk P J, Schot R, Gauw S A, Rabe K F and Bel E H 2006 Alveolar nitric oxide versus measures of peripheral airway dysfunction in severe asthma *Eur. Respir. J.* **27** 951–6
[64] Mahut B, Delacourt C, Zerah-Lancner F, De Blic J, Harf A and Delclaux C 2004 Increase in alveolar nitric oxide in the presence of symptoms in childhood asthma *Chest* **125** 1012–8
[65] Paraskakis E, Brindicci C, Fleming L, Krol R, Kharitonov S A, Wilson N M, Barnes P J and Bush A 2006 Measurement of bronchial and alveolar nitric oxide production in normal and asthmatic children *Am. J. Respir. Crit. Care Med.* **174** 260–7
[66] Lehtimaki L, Kankaanranta H, Saarelainen S, Turjanmaa V and Moilanen E 2001 Inhaled fluticasone decreases bronchial but not alveolar nitric oxide output in asthma *Eur. Respir. J.* **18** 635–9
[67] Shin H W, Rose-Gottron C M, Cooper D M, Newcomb R L and George S C 2004 Airway diffusing capacity of nitric oxide and steroid therapy in asthma *J. Appl. Physiol.* **96** 65–75
[68] Hogman M, Holmkvist T, Wegener T, Emtner M, Andersson M, Hedenström H and Meriläinen P 2002 Extended NO analysis applied to patients with COPD, allergic asthma and allergic rhinitis *Respir. Med.* **96** 24–30
[69] Lehtimaki L, Kankaanranta H, Saarelainen S, Annila I, Aine T, Nieminen R and Moilanen E 2010 Bronchial nitric oxide is related to symptom relief during fluticasone treatment in chronic obstructive lung disease *Eur. Respir. J.* **35** 72–8
[70] Lehouk A, Carremans C, DeBent K, Decramer M and Janssens W 2010 Alveolar nitric oxide in chronic obstructive pulmonary disease *Respir. Med.* **104** 1020–6
[71] Verbanck S, Kerckx Y, Schuermans D, Vincken W, Paiva M and Van Muylem A 2008 Effect of airways constriction on exhaled nitric oxide *J. Appl. Physiol.* **104** 909–11
[72] Gelb A, Taylor C F, Krishnan A, Fraser C, Shinar C M, Schein M J and Osann K 2010 Central and peripheral airway sites of nitric oxide gas exchange in COPD *Chest* **137** 575–84
[73] Roy K, Borrill Z L, Starkey C, Hazel A L, Morris J, Vestbo J and Singh D 2007 Use of different exhaled nitric oxide multiple flow rate models in COPD *Eur. Respir. J.* **29** 651–9
[74] Jenkins C R, Jones P W, Calverley P M, Celli B, Anderson J A, Ferguson G T, Yates J C, Willits L R and Vestbo J 2009 Efficacy of salmeterol/fluticasone propionate GOLD stage of chronic obstructive pulmonary disease: analysis from the randomized placebo-controlled TORCH study *Respir. Res.* **30** 10–59
[75] Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease 2011 http://www.goldcopd.org
[76] Drummond M B, Dasenbrook E C, Pitz M W, Murphy D J and Fan E 2008 Inhaled corticosteroids in patients with stable chronic obstructive pulmonary disease: a systematic review and meta-analysis *J. Am. Med. Assoc.* **300** 2407–16
[77] Singh S, Amin A V and Loke Y K 2009 Long-term use of inhaled corticosteroids and the risk of pneumonia in chronic obstructive pulmonary disease. A meta-analysis *Arch. Intern. Med.* **169** 219–29
[78] Puckett J L and George S C 2008 *Respir. Physiol. Neurobiol.* **163** 166–77