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TOPICAL REVIEW

Extended NO analysis in asthma

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Abstract
The discovery of the flow dependence of exhaled NO made it possible to model NO production in the lung. The linear model provides information about the maximal flux of NO from the airways and the alveolar concentrations of NO. Nonlinear models give additional flow-independent parameters such as airway diffusing capacity and airway wall concentrations of NO. When these models are applied to patients with asthma, a clear-cut increase in NO flux is found, and this is caused by an increase in both airway diffusing capacity and airway wall concentrations of NO. There is no difference in alveolar concentrations of NO compared to healthy subjects, except in severe asthma where an increase has been found. Inhaled corticosteroids are able to reduce the airway wall concentrations but not diffusing capacity or alveolar concentrations. Oral prednisone affects the alveolar concentration, suggesting that in severe asthma there is a systemic component. Steroids distributed by any route do not affect the airway diffusing capacity. Therefore, the airway diffusing capacity should be in focus in testing new drugs or in combination treatment for asthma. Exhaled NO analysis is a promising tool in characterizing asthma in both adults and children. However, there is a strong need to agree on the models and to standardize the flow rates to be used for the modelling in order to perform a systematic and robust analysis of NO production in the lung.

Background

The first reports of exhaled NO in humans were published more than 15 years ago [1]. Since NO analysers were already on the market for measuring air pollution, it was easy to utilize them for human research. Reports of peak NO concentrations during tidal breathing showed a 2- to 3-fold increase in asthmatics [2], and after a vital capacity manoeuvre the peak was about 300 ppb [3]. The discovery of the flow dependence of exhaled NO by two research groups independently shed light on the different results published in healthy controls as in disease [4, 5]. This also pointed out the need for standardization, and so far three recommendations have been published with the latest ERS/ATS publication 2005. The recommended flow rate is 50 mL s^{-1} without the need for a vital capacity manoeuvre. As a rule, an $F_ENO_{0.05}$ value above 30–35 ppb was considered to be increased, and in a random sample population the upper limit for healthy individuals was 29 ppb [6].

However, using just one flow rate is not enough to reveal the origin of the NO and its release mechanism to the exhaled air. From a system theory perspective, the conductive airway tree can be modelled as a black box adding NO to the input flow coming from the lungs. The experimentally obtained mutual relationships between NO output and NO input for a set of exhalation flow rates can be used to construct a transfer function able to predict the system NO output for any other flow. However, instead of a purely mathematical approach it is important to be able to link all the parameters defining the system to physical/physiological properties of the human airway parameters.

Flow-independent parameters—theory

A simplification of the lung can be made based on a two-compartment model (2CM), consisting of one rigid

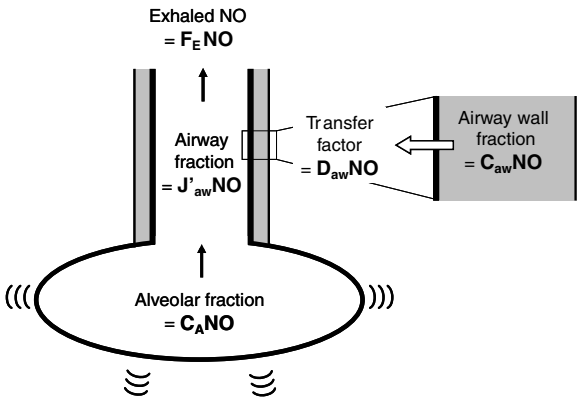

Figure 1. A simplified model of NO production in the lung. The expansile compartment of the lung represents the fraction of NO in the gas phase of the alveolar region (ppb) = C_ANO. Total maximum flux of NO in the airway compartment (pL s^{-1}) = J'_{aw}NO, which contains the airway tissue concentration of NO (wall concentration, ppb) = C_{aw}NO and the total airway compartment diffusing capacity, transfer factor or conductance for radial mass transfer of NO from the airway wall to the gas stream (mL s^{-1}) = D_{aw}NO. Hence, J'_{aw}NO is the maximum flux of NO from the airways, simply the product D_{aw}NO · C_{aw}NO if C_ANO is zero. If C_ANO is above zero, it has to be taken into the calculations.

compartment representing the airways and one expansile compartment representing the gas exchange areas [7]. See figure 1. The mechanism controlling the NO transfer to the airway lumen is Fick's first law of diffusion [8]. Measurements of exhaled NO at three or more flow rates give the possibility of determining three different parameters defining the NO production. The expansile compartment of the lung produces the fraction of NO in the gas phase of the alveolar region (C_ANO in ppb). The rigid conducting airway system lumped into a single tube is characterized by the airway tissue concentration of NO (wall concentration, C_{aw}NO in ppb) and the total airway compartment diffusing capacity, transfer factor or conductance for radial mass transfer of NO from the airway wall to the gas stream (D_{aw}NO in mL s^{-1}). The flux of NO from the wall to the lumen depends on the distance z along the airway and it has a total value of $J_{aw}NO = D_{aw}NO(C_{aw}NO - C_ANO)$ at flows high enough to keep the local lumen NO value close to C_ANO all the way along the airway. The highest potential total flux is then $J'_{aw}NO = D_{aw}NO \cdot C_{aw}NO$, and is obtained when C_ANO is zero. The definitions and physical meanings of the two NO fluxes, J_{aw}NO and J'_{aw}NO, have been inadequately described and understood in some of the exhaled NO publications. This relates to the attempts to solve the three independent parameters from measurements at only two flows, which is only possible assuming C_ANO to be zero, making J'_{aw}NO equal to J_{aw}NO.

Different approaches are used to calculate the flow-independent parameters and most of the models were presented during 1998 to 2000. During the past few years, the role of axial back diffusion of NO at low flows in the airway generations close to the alveolar compartment has been investigated [9]. A trumpet-shaped geometry for the airways is utilized instead of a constant diameter tube, as used in earlier models. It remains to be confirmed if the single trumpet model is adequate enough to assess the magnitude of axial diffusion. In the real fractal airway tree, the ratio between the local airway wall area and the airway cross-sectional area is much different than in a trumpet with wall area calculated from the lumped cross section of each generation.

NO modelling

The *Silkoff technique* (ST) utilizes a nonlinear regression technique using equation (1), which is the basic equation of the 2CM for presenting the dependence of exhaled NO values as a function of flow [10]. Silkoff *et al* used nine flow rates between 4 and 1550 mL s^{-1} to estimate the three flow-independent parameters:

$$F_ENO = C_{aw}NO + (C_ANO - C_{aw}NO)\exp(-D_{aw}NO/\dot{V}_E). \quad (1)$$

Consequently the NO output is obtained from (1) by multiplying both sides by $\dot{V}_E$:

$$\dot{V}_{NO} = \dot{V}_E \cdot F_ENO = \dot{V}_E(C_{aw}NO + (C_ANO - C_{aw}NO) \times \exp(-D_{aw}NO/\dot{V}_E)). \quad (2)$$

For all the flows $\dot{V}_E \gg D_{aw}$NO, the exponential in equation (2) can be linearized and replaced by $(1 - D_{aw}NO/\dot{V}_E)$, then equation (2) becomes

$$\dot{V}_{NO} = \dot{V}_E \cdot F_ENO = C_ANO \cdot \dot{V}_E + (C_{aw}NO - C_ANO)D_{aw}NO = C_ANO \cdot \dot{V}_E + J_{aw}NO \cdot D_{aw}NO. \quad (3)$$

Silkoff also suggested that D_{aw}NO and J'_{aw}NO can be estimated from two low flow rates (<50 mL s^{-1}) by using the slope ($-D_{aw}$NO) and the intercept ($J'_{aw}NO/D_{aw}NO$) of a plot of $\dot{V}_{NO}$ versus F_ENO. However, this is only a semi-quantitative approximation which can be shown by solving $\dot{V}_E$ as a function of F_ENO from equation (1) and substituting the result in equation (2).

Tsoukias and George (2CM) had earlier described a two-parameter model, where NO values at multiple constant exhalation flow rates between 100 and 500 mL s^{-1} were obtained and $\dot{V}_{NO}$ was plotted versus the flow rates [7]. The slope and the intercept of the resulting linear regression line provided an estimate of C_ANO and J'_{aw}NO according to equation (4) and the r-value of the line gave the quality of the fit (see figure 2):

$$\dot{V}_{NO} = C_ANO\dot{V}_E + J'_{aw}NO. \quad (4)$$

However, comparing equations (4) and (1) reveals a controversy that equation (4) must, by definition of J'_{aw}NO, be based on the assumption C_ANO = 0, but on the other hand C_ANO is available as a slope from the data of $\dot{V}_{NO}$ versus $\dot{V}_E$ registered at high flow rates.

The *Pietropaoli technique* (PT) includes six constant-exhalation vital capacity manoeuvres with flow rates of 6–1355 mL s^{-1} [11]. The theory of this technique was first published by Hyde *et al* [12]. C_ANO and J'_{aw}NO are estimated together with the estimation of D_{aw}NO. A plot of F_ENO versus $1/\dot{V}_E$ for $\dot{V}_E$ > 200 mL s^{-1} uses the intercept as the estimation of C_ANO according to

$$F_ENO = C_ANO + J'_{aw}NO \cdot 1/\dot{V}_E. \quad (5)$$

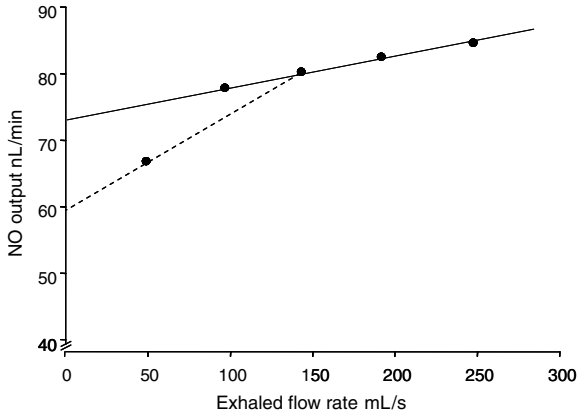

Figure 2. The linear model. Even though they are called flow-independent parameters of NO, the flow rates that are used for the calculations are very important. Results from the solid line give one r-value of 0.99 (very good fit), C_ANO of 1 ppb and J'_{aw}NO of 73 nL min^{-1}. The same subject gets C_ANO of 3 ppb and J'_{aw}NO of 59 nL min^{-1}, when other flow rates are chosen (dashed line). Since the line is drawn between two points, there is no indication how good the fit is.

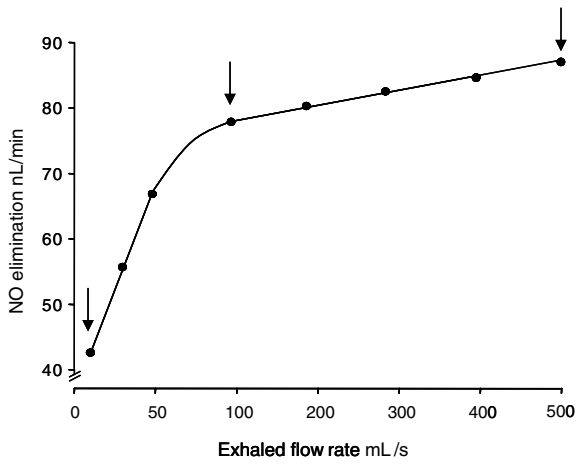

Figure 3. The nonlinear model. Note that the curve has a biphasic pattern. There is an equation that fits this curve and the iteration algorithm search to find the solution for the airway tissue concentration of NO (C_{aw}NO), the airway transfer factor for NO (D_{aw}NO) and the alveolar concentration of NO (C_ANO) using this equation. The arrows mark the flow rate used in the Högman–Meriläinen algorithm [14].

By comparing equation (5) to equation (3), one finds them identical if J'_{aw}NO is replaced by J_{aw}NO, which is the accurate expression for non-zero C_ANO values.

C_ANO and J_{aw}NO can be solved from two data points if flow values high enough to allow linear approximation of the exponential are used. With more data points, any nonlinear curve-fitting algorithm can be used, allowing also separation of C_{aw}NO and D_{aw}NO.

The *Högman and Meriläinen algorithm* (HMA) was first described in 1999 and is a nonlinear model [13, 14] with a set of three exhalation flow rates with typical values of officially 10 mL s^{-1} (low), 100 mL s^{-1} (medium) and 300–500 mL s^{-1} (high), as shown in figure 3. The $\dot{V}_{NO}$ versus flow rate is plotted and a line set through the medium and high flow rates gives estimates of C_ANO and J'_{aw}NO. D_{aw}NO and C_{aw}NO are then separated from J_{aw}NO by using all three flow rates employing an iterative algorithm with second-order correction term added to the first-order linear approximation of the exponential in equations (1) and (2).

A special added feature of this approach is to use an algorithm to test if the measured set of data points is mathematically consistent with the model. This is to avoid the potential problem of standard curve-fitting algorithms if applied on three data points only, which usually find a perfect fitting to equation (1), even if some of the data points are corrupted by measurement errors of technical origin. The condition required for data allowing reliable separation of D_{aw}NO and C_{aw}NO can be written in general form as

$$\frac{F_ENO_{low} - F_ENO_{medium}}{F_ENO_{medium} - F_ENO_{high}} < \frac{\dot{V}_{E,high}}{\dot{V}_{E,low}} \cdot \frac{\dot{V}_{E,medium} - \dot{V}_{E,low}}{\dot{V}_{E,high} - \dot{V}_{E,medium}}. \quad (6)$$

If this is not the case, the data set suffers from errors in measuring either the flow or NO concentration, or both. Additionally it should naturally be required for the indication of C_ANO to be positive.

Trumpet model axial diffusion (TMAD) is a model combining 2CM with the consideration of axial diffusion and the trumpet shape of the airway tree [15]. Four different flow rates between 100 and 250 mL s^{-1} are used to estimate J'_{aw}NO and C_ANO. The model predicts that a plot of $\dot{V}_{NO}$ versus $\dot{V}$ produces a linear relationship in which the slope is equal to C_ANO + J'_{aw}NO $\times$ 0.000 78 and the intercept is equal to J'_{aw}NO/1.7:

$$\dot{V}_{NO} = (C_ANO + J'_{aw}NO \times 0.000\,78)\dot{V} + J'_{aw}NO/1.7. \quad (7)$$

Comparisons of the models

In order to see how similar the different models are, we obtained NO values from subjects without classification of disease: low (n = 7), middle (n = 17) and high (n = 8) values of $F_ENO_{0.05}$ using the CLD 88sp NO analyser (ECO Medics AG, Switzerland). A comparison was made of the models that estimate C_ANO and J'_{aw}NO, i.e. 2CM, TMAD and HMA. C_ANO was lower in subjects with middle $F_ENO_{0.05}$ with the methods 2CM and TMAD ($p < 0.01$, ANOVA). J'_{aw}NO is significantly different from TMAD, where the TMAD consistently gives about a 70% increase. This is simply because the adjustment for axial diffusion on J'_{aw}NO is directly indicated by equation (7), see table 1.

In the comparison of the models that estimate C_{aw}NO and D_{aw}NO, i.e. ST, PT and HMA, NO values from the same subjects were used. In table 2, the differences between the models are presented. The ST gives higher C_{aw}NO for the groups with middle and high $F_ENO_{0.05}$ ($p < 0.001$). For the same $F_ENO_{0.05}$ groups, D_{aw}NO was significantly lower with the ST and PT ($p < 0.001$).

Table 1. Comparisons of three methods of modelling NO to estimate J'_{aw}NO and C_ANO. The TMAD model gives about a 70% increase in J'_{aw}NO. 2CM = two-compartment model by Tsokias and George [7], TMAD = trumpet model axial diffusion by Condorelli *et al* [15] and HMA = Högman–Meriläinen algorithm [14].

F_ENO	2CM	TMAD	HMA	p-value
F_ENO < 12 ppb, $n = 7$				
C_ANO (ppb)	0.4 ± 0.2	0.4 ± 0.2	0.7 ± 0.2	ns
J'_{aw}NO (pL s^{-1})	453 ± 69	737 ± 117	375 ± 48	$P < 0.001$
F_ENO 12–27 (ppb), $n = 17$				
C_ANO (ppb)	0.4 ± 0.2	0.3 ± 0.2	0.8 ± 0.1	$p < 0.01$
J'_{aw}NO (pL s^{-1})	971 ± 75	1651 ± 128	965 ± 78	$p < 0.001$
F_ENO > 27 ppb, $n = 8$				
C_ANO (ppb)	0.6 ± 0.6	0.5 ± 0.6	0.8 ± 0.3	ns
J'_{aw}NO (pL s^{-1})	2313 ± 349	3932 ± 594	2418 ± 422	$p < 0.001$

Data are given as median ± $CI_{68\%}$, ANOVA.

Table 2. Comparisons of three methods of modelling NO to estimate C_{aw}NO and D_{aw}NO. ST = Silkoff technique [10], PT = Pietropaoli technique [11] and HMA = Högman–Meriläinen algorithm [14].

	ST	PT	HMA	p-value
F_ENO < 12 ppb, $n = 7$				
C_{aw}NO ppb	54 ± 16	35 ± 20	39 ± 6	ns
D_{aw}NO (mL s^{-1})	10 ± 2	13 ± 2	10 ± 2	ns
F_ENO 12–27 ppb, $n = 17$				
C_{aw}NO (ppb)	111 ± 19	74 ± 12	62 ± 18	$p < 0.001$
D_{aw}NO (mL s^{-1})	10 ± 1	11 ± 1	15 ± 1	$p < 0.001$
F_ENO > 27 ppb, $n = 8$				
C_{aw}NO (ppb)	272 ± 34	187 ± 22	174 ± 20	$p < 0.001$
D_{aw}NO (mL s^{-1})	9 ± 1	13 ± 1	15 ± 2	$p < 0.001$

Data are given as median ± $CI_{68\%}$, ANOVA.

Limitations

Understanding the limitations of the different models is fundamental, but it is also extremely important to know the limitations of the equipment. Some analysers are built for environmental research and are too slow to be used for modelling while other analysers are built for clinical use and high NO concentrations. Extended NO analysis demands an analyser with an accuracy of 0.1 ppb to adequately measure low NO concentration present at high flow rates. For the flow measurements, an accuracy of 0.1 mL s^{-1} for low flow rates and ±1% for high flow rates is preferably required. It should be easy to calibrate and verify both NO signal and flow rate and check the overall performance of the system. Presenting the r-value for fitness when using linear regression will assure the reader that the data are sound. It is not acceptable to use flow rates not appropriate for the chosen model. How the flow rates used to estimate the flow-independent parameters affect these is shown in figure 2.

Flow-independent parameters—practice

There is a lot more information about the NO production in the lung to be gained when applying extended NO analysis. A drawback of the model based on Fick's first law of diffusion is that the airways cannot be divided into small and large airways. However, the knowledge achieved by this non-invasive measurement may possibly justify the application of the model. A sample of what is published regarding asthma and atopy can be seen in table 3 for adults and in table 4 for children. An example of how an extended NO analysis can give important information can be seen in a study of asthmatic children [16]. In this study, the F_ENO$_{0.05}$ was equally increased in asthmatics on inhaled corticosteroids (ICS) and in steroid naïve asthmatics. However, the C_{aw}NO was increased by 244% in the group with ICS and 354% in the steroid naïve group compared to healthy children. This means that ICS is effective in downregulating the NO production. D_{aw}NO was increased by 115% in the group with ICS and 69% in the steroid naïve group. An interpretation of this can be that the children on ICS have a more severe asthma, although the two groups had identical F_ENO$_{0.05}$. An attempt to discuss what the flow-independent parameters represent and how they are altered in asthma is presented below.

Alveolar NO

C_ANO represents the NO production in the expansile compartment, the alveoli and respiratory bronchioles. The alveolar macrophages produce NO, and NO syntase has been found in the alveolar epithelium [17]. Theoretically, it would be unlikely that NO is released from the capillary network since blood is a sink for NO. Rather an increase in C_ANO

Table 3. Flow-independent NO parameters in health, atopy and asthma in adults. 2CM = two-compartment model [7], ST = Silkoff technique [10], SB = single breath [44], HMA = Högman–Meriläinen algorithm [14], TMAD = trumpet model axial diffusion [15].

	$F_{E}NO_{0.05}$ (ppb)	$J'_{aw}NO$ (nL s^{-1})	$C_{A}NO$ (ppb)	$C_{aw}NO$ (ppb)	$D_{aw}NO$ (mL s^{-1})	Flow rates (mL s^{-1})	n F/M	Technique	Equipment	Reference
Adults										
Health	18.2 (11–29)[a]	0.9 (0.5–1.5)	1.5 (0.0–3.2)	120 (65–221)	7.6 (4–13)	5, 100, 500	52 (0/52)	HMA	NOA 280	[6]
		0.7 ± 0.1	1.0 ± 0.1			100, 175, 370	40	2CM	NOA 280	[23]
			5.0 ± 0.3	137 ± 34	5.6 ± 1.0	4–1550	10 (2/8)	ST	NOA 270	[10]
		1.0 ± 0.2		149 ± 32	6.8 ± 1.2	17 and 38	10 (2/8)	ST	NOA 270	[10]
	12.3 ± 1.3	0.5 ± 0.1	3.1 ± 0.5	220 ± 36	3.1± 0.3	Descending	24	SB	NOA 280	[45]
		0.85 ± 0.1	3.2 ± 0.3			100–200	34 (21/13)	2CM	NOA 280	[21]
			1.9 ± 0.2	98 ± 9	7.7 ± 0.5	5, 100, 500	40	HMA	NOA 280	[14]
		0.8 ± 0.2	1.0 ± 0.3			100–250	8 (5/3)	TMAD	NOA 280	[15]
	Not given	0.4 ± 0.0	1.8 ± 0.2			50–320	10 (8/2)	2CM	NOA 280	[30]
Asthma		2.6 ± 0.4	0.8 ± 0.3			100, 175, 370	21	2CM	NOA 280	[23]
		2.5 ± 0.3	1.1 ± 0.2			100, 175, 370	40	2CM	NOA 280	[25]
		6.5 ± 0.9		255 ± 46	25.5 ± 3.8	17 and 38	15	ST	NOA 270	[10]
SN[b]	56.3 ± 13.4	2.7 ± 0.6	5.7 ± 1.1	438 ± 110	8.7 ± 2.0	Descending	8	SB	NOA 280	[45]
	28.8 ± 4.5	1.2 ± 0.2	3.3 ± 0.8	143 ± 19	11.8 ± 3.4	Descending	12	SB	NOA 280	[44]
SN		2.0 ± 0.4	7.8 ± 2,2			100–200	13 (7/6)	2CM	NOA 280	[21]
		2.4 ± 0.4	7.0 ± 1.0			100–200	53 (29/24)	2CM	NOA 280	[21]
		2.8 ± 0.3	9.4 ± 1.8			100–200	25	2CM	NOA 280	[22]
			1.7 ± 0.1	144 ± 21	11.9 ± 1.0	5, 100, 500	15	HMA	NOA 280	[14]
	Not given	3.1 ± 0.5	2.3 ± 0.5			50–320	10 (8/2)	2CM	NOA 280	[30]
Atopy			1.8 ± 0.1	98 ± 10	11.8 ± 1.3	5, 100, 500	15	HMA	NOA 280	[14]
	24.9 (22–28)[c]		1.4 (1–2)	124 (111–139)	10.5 (9–12)	5, 50, 100, 500	111	HMA	NOA 280	[46]
AHR	Not given	2.9 (1.4–5.4)[d]	8.7 (6–12)			50, 250	23	2CM	EVA 4000	[47]

[a] Range in $CI_{68\%}$.
[b] SN = steroid naïve.
[c] Range in $CI_{95\%}$.
[d] 25–75th percentile, AHR = airway hyper-responsiveness.

Table 4. Flow-independent NO parameters in health, atopy and asthma in children. 2CM = two-compartment model [7], ST = Silkoff technique [10], HMA = Högman–Meriläinen algorithm [14].

	$F_{E}NO_{0.05}$ (ppb)	$J'_{aw}NO$ (nL s^{-1})	$C_{A}NO$ (ppb)	$C_{aw}NO$ (ppb)	$D_{aw}NO$ (mL s^{-1})	Flow rates (mL s^{-1})	n	Technique	Equipment	Reference
Children										
Health	10.4 ± 0.8	0.3 ± 0.1	4.1 ± 0.6			60–100, 100–150	15	2CM	EVA 4000	[24]
		0.7 ± 0.1	1.2 ± 0.1	55 ± 8	13.2 ± 1.7	10, 50, 100, 500	15	HMA	CLD 77	[16]
	24			109	13	15, 25, 50	5	ST	NOA 280	[48]
	10.3 (3–29)[a]	0.5 (0–2)	1.6 (0–3)			50, 100, 200, 260	20	2CM	NIOX	[49]
Asthma	Not given	1.3 ± 0.2	5.8 ± 0.5			25, 39	25	ST	EVA 4000	[24]
	52 ± 24	2.2 ± 0.2	7.2 ± 0.5			60–100, 100–150	15	2CM	EVA 4000	[24]
	28.1 (4–190) [a]	1.2 (0–9)	2.2 (0–7)			50, 100, 200, 260	52	2CM	NIOX	[49]
		3.5 ± 0.4	1.5 ± 0.2	154 ± 23	26.3 ± 3.2		15	HMA	CLD 77	[16]
Atopy	21.8 (9–69) [a]	1.2 (0–4)	1.2 (0–3)			50, 100, 200, 260	20	2CM	NIOX	[49]

[a] Range.

could be interpreted as systemic circulation mediators causing induction of the inducible NOS (iNOS) in the alveolar region [18] or a reduced uptake due to ventilation/perfusion mismatch seen in COPD [14]. A healthy subject should therefore have $C_{A}NO$ levels close to zero. In a healthy population, a median value was around 2 ppb for both men and women with an upper $CI_{68\%}$ limit of 4 ppb [6]. Table 3 shows that there is a range from 0 ppb to above 9 ppb in adult asthmatics. For example, the $C_{A}NO$ levels range from values between 1 and 2 ppb [6–19] to 6 ppb [20] and even 8 and 9 ppb [21, 22].

Increased $C_{A}NO$ has been found in symptomatic asthmatics [23, 24] and although treatment with ICS did not affect this parameter, oral prednisone did [21]. $C_{A}NO$ has been found to correlate positively with blood eosinophil count [25] and BAL with no correlation with bronchial wash or sputum eosinophil count [26], but negatively to small airway function [25]. The interpretation of these findings is that in severe and uncontrolled asthma, there is a peripheral inflammation that can be seen with simple modelling of NO. Other interpretations may also be valid since $C_{A}NO$ has been found to increase with alveolar volume [27] and alveolar–arterial oxygen difference [20]. However, the increase in $C_{A}NO$ in severe asthma could hypothetically reflect a systemic inflammation since oral prednisone affects this parameter.

NO flux from the airways

$J'_{aw}NO$ is the maximum flux of NO from all the rigid airways lumped together (bronchioles, bronchus and trachea) and the upper airway. Therefore, $J'_{aw}NO$ is simply the product of $D_{aw}NO$ and $C_{aw}NO$ if $C_{A}NO$ is zero. If $C_{A}NO$ is above zero it has to be taken into the calculations,

as indicated by equation (3), or if the exhalation flow rates are <50 mL s^{-1}, then $J'_{aw}NO$ differs from the $J_{aw}NO$ (total flux).

There is clear evidence for the increase of $J'_{aw}NO$ in asthma regardless of the model used, as can be seen in tables 3 and 4. ICS is most effective in reducing $J'_{aw}NO$ [10]. In steroid naïve patients, $J'_{aw}NO$ is not elevated, see table 3, but that could simply be due to less severity of their asthma, as discussed earlier.

The relaxant effect of inhaled NO in animals is blocked after allergen exposure [28] and after hypertonic provocation [29], a known stimulus for the asthmatic airway. It has been shown that exhaled NO decreases due to hypertonic challenge [30] and that the decrease is situated in the airway compartment [31]. Hence, the airway epithelium and its production of NO are an important modulator in the defence system of the airways.

Airway tissue NO

$C_{aw}NO$ gives the concentration of NO in the airway tissue. It is known that epithelial NO is important for normal airway homeostasis. Both constitutive (eNOS, nNOS) and iNOS are present in the lung. There is also an expression of constitutively iNOS in the airway epithelium [32]. In asthma, $C_{aw}NO$ is increased and ICS can effectively decrease this parameter [10]. $C_{aw}NO$ is increased in allergic asthma but not in allergic rhinitis [14]. Asthmatics with increased exhaled NO have increased iNOS mRNA and protein due to transcriptional regulation through activation of Stat1. iNOS mRNA expression decreases in asthmatics receiving ICS [33]. The L-arginine transporter is not affected by ICS. Interestingly, asthmatics have increased levels of L-arginine ready to be used and if taken off ICS, the F_ENO increases rapidly [33].

NO transfer factor

$D_{aw}NO$ gives airway compartment diffusing capacity, transfer factor or conductance for radial mass transfer of NO from the airway wall to the gas stream. The increase in $D_{aw}NO$ found in allergic asthmatics [14] is not affected by ICS [10]. This is important to have in mind if $F_ENO_{0.05}$ is used to target the treatment for asthmatics [34]. It cannot be expected that normal levels, i.e. <30 ppb, could be reached just with ICS. The same is valid for patients with allergic rhinitis who also have an increase in $D_{aw}NO$, hence an increase in $F_ENO_{0.05}$. These patients may not benefit from ICS since their $C_{aw}NO$ is normal [14]. It is therefore important to determine $D_{aw}NO$ in asthmatic patients in order to make a treatment plan with best possible ICS use. The development of new drugs and the combination of drugs should perhaps be targeting $D_{aw}NO$ if this parameter plays a role in airway hyper-reactivity seen in allergic asthma and rhinitis. Nishio *et al* have shown that F_ENO correlates with PC_{20} for acetylcholine in patients off ICS and that this correlation was absent in patients treated with ICS [35]. This suggests that $D_{aw}NO$, which is not affected by ICS, could most likely correlate with AHR.

Extended NO analysis in other diseases

In cystic fibrosis (CF), there are reports of both increased and decreased F_ENO values. Shin *et al* showed that there was no difference in the calculated values of $F_ENO_{0.05}$ (derived from the flow-independent parameters) between children with CF and healthy children [36]. In CF, however, the $D_{aw}NO$ was increased and C_ANO and $C_{aw}NO$ were decreased compared to healthy children.

Smoking is associated with low levels of NO and specially low $C_{aw}NO$ [14]. Interestingly, in a disease strongly associated with smoking, i.e. chronic obstructive pulmonary disease (COPD), extended NO analysis was able to distinguish two groups of COPD patients [14]. One group had high F_ENO values, which were due to an increase in $D_{aw}NO$, which is similar to allergic asthma. Both groups had increased C_ANO, an observation also made by Brindicci *et al* [37]. $C_{aw}NO$ has been found to be decreased in COPD patients currently smoking [38]. Another disease associated with smoking is lung cancer where there is strong evidence for up-regulations of iNOS [39, 40]. One could expect that when applying NO extended analysis, one would find increased C_ANO levels because there was an increase in NO production in the alveolar macrophages [40]. Since the increased NO production was not specific to the tumour side, it might be attributed to the tumour-associated non-specific immunological and inflammatory processes of the host. This is probably the reason why there was an increase in all flow-independent parameters in a patient with Hodgkin's disease [41].

Patients with Sjögren's syndrome are known to have increased levels of exhaled NO [42] and when an extended NO analysis was performed, it was seen that the C_ANO and $C_{aw}NO$ were elevated but not $D_{aw}NO$ [18]. Sjögren's syndrome is a systemic disease and circulating cytokines might be the cause of an up-regulation of iNOS in alveolar and airway tissue.

In interstitial lung disease, such as scleroderma, the C_ANO levels were increased while the $J'_{aw}NO$ were decreased [43]. An interpretation, by the authors, was that the diffusing capacity of NO was decreased or that the production of NO in the alveolar site was increased. The low $J'_{aw}NO$ might possibly be due to a low $D_{aw}NO$ pointing towards some intrinsic epithelial abnormality, which makes the hypothesis of a compromised diffusion of NO most possible.

Future directions

The discovery of exhaled NO in humans led to a rapid progress in physiological science. This is yet to be continued, but with a pathophysiology focus, due the increased interest in NO modelling and its flow-independent parameters as a diagnostic tool in different lung diseases. Just as there was a need to agree to one flow rate at which to measure F_ENO, there is a need to find a consensus about which model to use for extended NO analysis. Condorelli *et al* have presented a model with higher flow rates, which put demands on the NO analysers [15]. The recommended flow rate of 50 mL s^{-1} was chosen because it gave a high NO signal, and a clinical tool for just that flow rate was built. Today research groups have various NO analysing

stations of their own design, but we need stations that are designed and optimized for specific clinical use, since there is a lot of information in extended NO analysis to be gained and for the benefit of the patients.

Which patient groups could potentially benefit most? There is already clinical evidence that asthmatics can gain control and target their treatment [44]. If asthma is a systemic disease, as has been put forward, then it is necessary to be able to determine alveolar levels of NO. If the diffusing capacity correlates to airway hyper-reactivity, then new drugs for asthma or any other hyper-reactive respiratory disease will gain information with the use of extended NO analysis. An obvious need is to find an acceptable model for extended NO analysis in asthmatic children. The extended NO analysis can possibly be used to distinguish between atopic and non-atopic asthma. There is also a need to strengthen the classification of asthma and extended NO analysis is a promising tool in that work.

Summary

Extended NO analysis is a promising diagnostic tool and a tool for creating treatment plans in asthma. There are a handful of models, but most frequently the model by Tsoukias and George is in use. The drawback of this linear model is that only two flow rates are used and a line between them gives only two flow-independent parameters. This has been taken into consideration with the model proposed recently by the same research group, in collaboration with the research group of Silkoff, where also a quality control of the model is presented. The nonlinear models have been found to be more accurate. The Högman–Meriläinen algorithm is a robust model with a quality control, which can give all flow-independent parameters. Although extended NO analysis is a promising tool, the direct biological validation is lacking for all models and the clinical significance is not yet proven.

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References

[1] Gustafsson L E, Leone A, Persson M, Wiklund N and Moncada S 1991 Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans *Biochem. Biophys. Res. Commun.* **181** 852–7

[2] Alving K, Weitzberg E and Lundberg J M 1993 Increased amount of nitric oxide in exhaled air of asthmatics *Eur. Respir. J.* **6** 1368–70

[3] Kharitonov S A, Yates D, Robbins R A, Logan-Sinclair R, Shinebourne E A and Barnes P J 1994 Increased nitric oxide in exhaled air of asthmatic patients *Lancet* **343** 133–5

[4] Silkoff P E *et al* 1997 Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide *Am. J. Respir. Crit. Care. Med.* **155** 260–7

[5] Högman M, Strömberg S, Schedin U, Frostell C, Hedenstierna G and Gustafsson L E 1997 Nitric oxide from the human respiratory tract efficiently quantified by standardized single breath measurements *Acta. Physiol. Scand.* **159** 345–6

[6] Högman M *et al* 2006 Extended NO analysis in a healthy subgroup in a random sample population *Eur. Respir. J.* **28** 653s

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

[8] Fick A 1856 *Die Medizinische Physik* (Braunschweig: Vieweg)

[9] 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

[10] 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

[11] Pietropaoli A P *et al* 1999 Simultaneous measurements of nitric oxide production by conduction and alveolar airways of humans *J. Appl. Physiol.* **87** 1532–42

[12] Hyde R W *et al* 1997 Determination of production of nitric oxide by lower airways of humans—theory *J. Appl. Physiol.* **82** 1290–6

[13] Högman 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

[14] Högman M *et al* 2002 Extended NO analysis applied to patients with COPD, allergic asthma and allergic rhinitis *Respir. Med.* **96** 24–30

[15] 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

[16] Pedroletti C, Hogman M, Merilainen P, Nordvall L S, Hedlin G and Alving K 2003 Nitric oxide airway-diffusing capacity and mucosal concentration in asthmatic schoolchildren *Pediatr. Res.* **54** 496–501

[17] Warner R L *et al* 1995 Lung sources and cytokine requirements for in vivo expression of inducible nitric oxide synthase *Am. J. Respir. Cell. Mol. Biol.* **12** 649–61

[18] Högman M 2002 Extended NO analysis applied to patients with known altered values of exhaled NO *Disease Markers in Exhaled Breath. Basic Mechanism and Clinical Application* ed N Marczin and M H Yacoub (Amsterdam: IOS Press) pp 187–90

[19] Lehtimäki L *et al* 2001 Extended exhaled NO measurement differentiates between alveolar and bronchial inflammation *Am. J. Respir. Crit. Care Med.* **163** 1557–61

[20] Delclaux C *et al* 2002 Increased nitric oxide output from alveolar origin during liver cirrhosis versus bronchial source during asthma *Am. J. Respir. Crit. Care Med.* **165** 332–7

[21] Gelb A F *et al* 2004 Alveolar and airway sites of nitric oxide inflammation in treated asthma *Am. J. Respir. Crit. Care Med.* **170** 737–41

[22] Gelb A F, Flynn T C, 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

[23] Lehtimäki L, Kankaanranta H, Saarelainen S, Turjanmaa V and Moilanen E 2002 Increased alveolar nitric oxide concentration in asthmatic patients with nocturnal symptoms *Eur. Respir. J.* **20** 841–5

[24] 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

[25] Lehtimäki L, Kankaanranta H, Saarelainen S, Turjanmaa V and Moilanen E 2005 Peripheral inflammation in patients with asthmatic symptoms but normal lung function *J. Asthma* **42** 605–9
[26] Berry M *et al* 2005 Alveolar nitric oxide in adults with asthma: evidence of distal lung inflammation in refractory asthma *Eur. Respir. J.* **25** 989–91
[27] Tsoukias N M and George S C 2001 Impact of volume-dependent alveolar diffusing capacity on exhaled nitric oxide concentration *Ann. Biomed. Eng.* **29** 731–9
[28] Miura M *et al* 1997 Impairment of neural nitric oxide-mediated relaxation after antigen exposure in guinea pig airways *in vitro Am. J. Respir. Crit. Care Med.* **156** 217–22
[29] Högman M, Hjoberg J and Hedenstierna G 1998 Increased airway osmolarity inhibits the action of nitric oxide in the rabbit *Eur. Respir. J.* **12** 1313–7
[30] Högman M *et al* 2001 Inhaled mannitol shifts exhaled nitric oxide in opposite directions in asthmatics and healthy subjects *Respir. Physiol.* **124** 141–50
[31] Shin H W, Shelley D A, Henderson E M, Fitzpatrick A, Gaston B and George S C 2007 Airway nitric oxide release is reduced after PBS inhalation in asthma *J. Appl. Physiol.* **102** 1028–33
[32] Guo F H, De Raeve H R, Rice T W, Stuehr D J, Thunnissen F B and Erzurum S C 1995 Continuous nitric oxide synthesis by inducible nitric oxide synthase in normal human airway epithelium *in vivo Proc. Natl Acad. Sci. USA* **92** 7809–13
[33] Guo F H *et al* 2000 Molecular mechanisms of increased nitric oxide (NO) in asthma: evidence for transcriptional and post-translational regulation of NO synthesis *J. Immunol.* **164** 5970–80
[34] 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
[35] Nishio K, Odajima H, Motomura C, Nakao F and Nishima S 2006 Effect of inhaled steroid therapy on exhaled nitric oxide and bronchial responsiveness in children with asthma *J. Asthma* **43** 739–43
[36] Shin H W *et al* 2002 Flow-independent nitric oxide exchange parameters in cystic fibrosis *Am. J. Respir. Crit. Care Med.* **165** 349–57
[37] 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
[38] Roy K *et al* 2007 Use of different exhaled nitric oxide multiple flow rate models in COPD *Eur. Respir. J.* **29** 651–9
[39] Masri F A *et al* 2005 Abnormalities in nitric oxide and its derivatives in lung cancer *Am. J. Respir. Crit. Care Med.* **172** 597–605
[40] Liu C Y, Wang C H, Chen T C, Lin H C, Yu C T and Kuo H P 1998 Increased levels of exhaled nitric oxide and up-regulation of inducible nitric oxide synthase in patients with primary lung cancer *Br. J. Cancer* **78** 534–41
[41] Holmkvist T, Erlanson M, Meriläinen P and Högman M 2003 Exhaled nitric oxide is highly increased in a case of Hodgkin's disease *Acta. Oncol.* **42** 788–9
[42] Ludviksdottir D *et al* 1999 Increased nitric oxide in exhaled air in patients with Sjögren's syndrome *Eur. Respir. J.* **13** 739–43
[43] Girgis R E, Gugnani M K, Abrams J and Mayes M D 2002 Partitioning of alveolar and conducting airway nitric oxide in scleroderma lung disease *Am. J. Respir. Crit. Care Med.* **165** 1587–91
[44] Smith A D, Cowan J O, Brasett 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
[45] 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
[46] Malinovschi A, Janson C, Holmkvist T, Norback D, Merilainen P and Hogman M 2006 IgE sensitisation in relation to flow-independent nitric oxide exchange parameters *Respir. Res.* **7** 92
[47] Delclaux C *et al* 2005 Alveolar nitric oxide and effect of deep inspiration during methacholine challenge *Chest* **127** 1696–702
[48] Martinez T, Weist A, Williams T, Clem C, Silkoff P and Tepper R S 2003 Assessment of exhaled nitric oxide kinetics in healthy infants *J. Appl. Physiol.* **94** 2384–90
[49] Paraskakis E *et al* 2006 Measurement of bronchial and alveolar nitric oxide production in normal children and children with asthma *Am. J. Respir. Crit. Care Med.* **174** 260–7