Use of different exhaled nitric oxide multiple flow rate models in COPD.

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Running Head: Modelling of nitric oxide in COPD
Abstract

Multiple flow rates FeNO data can be modelled to estimate NO airway wall concentration (CawNO) and diffusing capacity (DawNO), alveolar concentration (CalvNO) and maximal flux (J’awNO). FeNO at 10, 30, 50, 100 and 200ml/s from 50 COPD patients and 35 healthy controls (smokers and non-smokers) modelled by five different methods was compared and the effect of the number of flow rates was investigated. All methods showed that current smoking reduced CawNO in COPD patients, with some methods showing that smoking reduced J’awNO. Smoking did not affect CalvNO and DawNO. For CawNO, the methods gave similar results, but there was variability between methods for J’awNO, CalvNO and DawNO. The median error by least squares fitting between modelled and actual data was significantly lower for the non linear (1.96) compared to mixed methods (3.31 and 3.62). Parameters calculated by the non-linear method using five and four flow rates were significantly different; ratio (95% CI) of CawNO was 2.02 (1.45, 2.83). NO models give different results, although CawNO is relatively model independent. Non-linear modelling has the least error, suggesting it is the best method. The number of flow rates should be standardised

Key words; chronic obstructive pulmonary disease, nitric oxide, two compartment modelling.
**Introduction**

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory condition characterised by poorly reversible airflow obstruction. The role of nitric oxide (NO) in the pathophysiology of COPD is not fully understood. NO is synthesized from L-arginine by NO synthase (NOS) enzymes [1], and can have multiple biological effects, including neurotransmission [2], vasodilation [2] and immunoregulation [3, 4]. Inducible NOS expression is increased in cells within the lumen and the airway wall of patients with COPD [5, 6, and 7]. Increased airway concentrations of NO related species may upregulate levels of oxidative stress and inflammation [8], with protein nitration and nitrosylation [9, 10] altering cell function.

The measurement of the fractional amount of lung NO that is exhaled (FeNO) at a single flow rate is a widely used biomarker of airway inflammation [11, 12 and 13]. An alternative explanation for raised FeNO levels may be alveolar capillary block, for example in emphysema. The applications of this measurement in COPD have been limited; because current cigarette smoking reduces FeNO [14] levels by either (i) inhibition of airway NOS activity [15] or (ii) an increase in levels of oxidative stress leading to the consumption of NO [8, 10]. This may explain the normal range of FeNO at a single flow rate observed in many COPD patients [16]. Nevertheless, FeNO is raised in ex-smoking COPD subjects [17, 18] and subjects with unstable disease [16] indicative of raised levels of inflammation compared to controls, and may be used to predict drug responsiveness [18].

Multiple flow rates FeNO data can be applied to a two-compartment model that allows estimation of the NO airway wall concentration (CawNO in ppb), airway wall diffusing capacity (DawNO in pl/ ppb/s) and steady state alveolar concentration (CalvNO in ppb) [19, 20, 21]. Maximal airway wall NO flux (J’awNO in pl/s) can be calculated as the product of CawNO and DawNO [19]. The
total NO flux (Jaw_NO) is a different calculation that accounts for Calv_NO as follows; Jaw_NO = Daw_NO (Caw_NO-Calv_NO). It has been reported that this model is useful for assessing inflammation in the lung periphery, as Calv_NO is raised in patients with asthma [22] and COPD [23, 24], and does not change with inhaled corticosteroid treatment [22, 24, 25]. Furthermore, it has been reported that smoking reduces Caw_NO [26]. Alterations in Daw_NO have also been described and this may be the cause of elevated Fe_NO levels in asthma regardless of the Caw_NO and Calv_NO levels [27].

Several different mathematical approaches that are based on the two-compartment model have been applied to multiple flow rates Fe_NO data: (1) linear analysis estimating a limited number of parameters; Calv_NO and Jaw_NO [20], or Daw_NO and J’aw_NO (and hence Caw_NO) [28] (2) non-linear analysis [28] allowing estimation of all parameters and (3) a combination of a linear analysis to calculate Calv_NO followed by non-linear fitting to determine the other parameters [23, 24, 29]. These models all use different mathematical approaches and so may give different results. As far as we are aware, these models have not been compared.

This paper reports multiple flow rates modelling using Fe_NO data from COPD patients and controls. We hypothesized that the different two-compartment modelling approaches may provide different results, and our primary aim was to compare the models. We also investigated the effect of the number of flow rates on the derived parameters from each method.

Methods

Subjects
Fifty COPD patients (23 COPD smokers; COPD S and 27 COPD ex smokers; COPD EX) diagnosed according to current guidelines [30] with relevant symptom history (chronic cough and sputum production, dyspnoea and wheeze), a significant smoking history (> ten pack years) and spirometric measurements of forced expiratory volume in 1 second (FEV\(_1\)) < 80% and FEV\(_1\)/forced vital capacity FVC < 0.7 participated in this study. Patients were required to be able to perform technically acceptable exhaled NO measurements at all the five flow rates used in this study. A total of 77 COPD patients were recruited for this study, but 27 patients were excluded as they were not able to perform acceptable exhaled NO measurements at all five flow rates. Two healthy control groups (19 smokers (HS) and 16 non-smokers (HNS)) with no history of respiratory disease and normal spirometry were also recruited. Healthy smokers were defined based on normal lung function and a smoking history of more than ten pack years. The demography of all participants is shown in Table 1. Only subjects who had negative skin prick tests to three allergens (house dust mite, grass pollen and cat hair; ALK Abello; Denmark) were included and patients with a clinical history of asthma or atopy were excluded. Exclusion criteria were a respiratory tract infection or exacerbation of COPD in the preceding six weeks. Written and informed consent was obtained and the local ethics committee approved the study.

**Study Design**

Subjects abstained from food and caffeine for two hours, smoking for six hours, and alcohol for twelve hours prior to the measurement of FE\(_{NO}\) using a Niox chemiluminescence on-line analyser (Aerocrine, Solna, Sweden). The analyser was calibrated according to the manufacturer’s instructions every 14 days. After inhaling NO free air to total lung capacity, subjects exhaled at a constant flow rate against a resistor to collect the plateau NO concentration. Readings can only be
taken at five flow rates with the Niox machine as the manufacturer only provides resistors for the following five flow rates; 10, 30, 50, 100 and 200 ml/sec. The actual flow rates achieved were required to be within 10% of the target flow rate [31]. The exhalation times required were 20, 10, 10, 6 and 6 sec respectively at these flow rates as per manufacturers’ guidelines. These standard settings ensure that the total volume of air exhaled at each flow rate accounts for the exclusion of dead space [31]. The manufacturer’s information about the Niox analyser states that the accuracy of the FeNO measurement is ± 2.5 ppb of measured value <50 ppb and ± 5 % of measured value >50 ppb, and the linearity is <2.5 ppb integral linearity. It is possible that these parameters differ with the flow rates used, and this requires further study. Three acceptable readings were recorded according to the ATS guidelines [31] at each of the five flow rates in one sitting. Recalibration was not required after each change of resistors.

In a random subgroup of twelve COPD patients, within day variability of exhaled NO was assessed by comparing measurements taken at 10am to 1pm on the same day, and between day variability determined by comparing readings taken at 10am a week apart.

Estimation of NO model parameters

FeNO multiple flow rate data were applied to a basic two-compartment model developed to distinguish between NO generated in the airways from that in the alveoli. The model is represented by the equation A [19]; FeNO = CawNO + (CalvNO – CawNO) .exp (- DawNO/ V) with unknown variables CawNO, CalvNO and DawNO, where V is the exhalation flow rate (ml/s). J’awNO is the total maximum flux of NO from the airway wall to the lumen and is the product of DawNO and CawNO. Multiplication of equation A by V leads to equation B; V NO = V.FeNO = V.CawNO+V(CalvNO-CawNO).exp(-DawNO/V), where V NO is the elimination rate of NO from the airways. At high flow rates, the argument of the exponential function is small and may be
approximated from exp. \((Daw_{NO}/V)\) to \(1-Daw_{NO}/V\) so equation B becomes equation C; \(V_{NO}= V\). Calv_{NO} - (Calv_{NO}-Caw_{NO}).Daw_{NO}. The mathematical techniques are as described below.

1. Linear Analysis

(i). Linear method 1 *(Tsoukias)* [20]

\(V_{NO}\) is plotted against flow rate at the higher flow rates and equation C is used to deduce that Calv_{NO} is the slope of the straight line where the y-intercept is \((Caw_{NO}- Calv_{NO})\) Daw_{NO}, which represents the total airway NO flux \((Jaw_{NO})\), and only approximates to the maximum NO flux \((J’aw_{NO})\) if Calv_{NO} approaches zero.

(ii). Linear method 2 *(Silkoff 2 flow)* [28]

From the linear plot at low flow rates of \(V_{NO}\) against \(Fe_{NO}\) represented by the equation; \(V_{NO} = J’aw_{NO} – Daw_{NO}/Fe_{NO}\), Daw_{NO} is estimated from the slope and Caw_{NO} from the x-intercept.

2. Non linear analysis *(Silkoff 9 flow)* [28]

Equation A is used to determine all parameters using the Solver tool in excel. \(J’aw_{NO}\) is calculated as the product of Caw_{NO} and Daw_{NO}.

3. Mixed Linear and non linear analysis

(i) Mixed Model 1 *(Hogman)* [23]

Linear method 1 is used to determine Calv_{NO} from equation C. Data from a range of low to high flows is then applied to equation A to estimate Caw_{NO} and Daw_{NO} by non-linear regression using the solver tool in Excel. \(J’aw_{NO}\) is calculated as the product of Caw_{NO} and Daw_{NO}.

(ii) Mixed Model 2 *(Pietropaoli)* [29]
Equation C is divided by flow rate to give equation D: \( \text{FeNO} = \text{CalvNO} + (\text{CawNO} - \text{CalvNO}) \frac{\text{DawNO}}{V} \).

From the plot at high flow rates of \( \text{FeNO} \) versus \( 1/\text{flow rate} \), \( \text{CalvNO} \) is estimated from the y-intercept. \( \text{J’awNO} \) and \( \text{DawNO} \) are determined using the solver tool in Excel from the equation

\( \text{FeNO} = \frac{\text{J’awNO}}{\text{DawNO}} + (\text{CalvNO} - \frac{\text{J’awNO}}{\text{DawNO}}) \exp(-\frac{\text{DawNO}}{V}) \).

\( \text{CawNO} \) is calculated from the equation \( \text{J’awNO} = \text{CawNO} \cdot \text{DawNO} \).

**Statistical Analysis**

Natural log transformation was required to normalise all the model derived data except \( \text{DawNO} \) which remained non-parametric so geometric means and 95% confidence intervals are presented for \( \text{CalvNO}, \text{CawNO}, \text{J’awNO} \) and \( \text{FeNO} \) and medians and interquartile ranges for \( \text{DawNO} \). ANOVA followed by unpaired t tests with Welch correction as appropriate were used to compare parametric data between groups and assess the effect of inhaled corticosteroids on \( \text{FeNO} \) and model parameters. The non-parametric Kruskal Wallis test with the Dunn post test followed by Mann Whitney test was applied in the case of \( \text{DawNO} \). Repeated measures ANOVA followed by the Bonferroni multiple comparisons tests were used to assess differences between the modelling methods, and to evaluate the effect of using different numbers of flow rates for methods that required more than two flow rates to calculate parameters (i.e. non-linear analysis and mixed linear and non-linear analyses). The non-parametric Kruskal Wallis test with the Dunn post test followed by Wilcoxon matched pairs signed ranks test was applied in the case of \( \text{DawNO} \). Repeated measures ANOVA followed by the Bonferroni multiple comparisons tests were used to compare the error of modelled data to actual data using techniques that required non-linear modelling (i.e. non-linear analysis and mixed linear and non-linear analyses). The model parameters were calculated by the non linear and mixed methods using four flow rates, by
excluding data from either the lowest (10ml/s) or the highest (200ml/s) flow rates. Four flow rate
data was compared to five flow rate using paired student’s t tests or the Wilcoxon matched pairs
signed ranks test for $\text{Daw}_{\text{NO}}$. The non-parametric spearman correlation was used to analyse the
relationship between FEV$_1$ and NO. Within and between day variability of Fe$_{\text{NO}}$ and model
parameters were analysed by assessing the differences between groups using paired students t-
test, except in the case of $\text{Daw}_{\text{NO}}$ when the Wilcoxon paired test was applied P<0.05 was
considered statistically significant.

Results

COPD Patients Compared To Controls

Single Flow Rate Fe$_{\text{NO}}$ Data

COPD S had significantly lower Fe$_{\text{NO}}$ levels at all five flow rates compared to COPD EX. The
results for Fe$_{\text{NO}0.05}$ (Fe$_{\text{NO}}$ measured at flow rate 50ml/s) are shown in Fig. 1. The mean (95%
confidence interval) for COPD S was 12.1 (9.2, 16.0), and for COPD EX was 18.41 (14.1, 24.1).
There were no other differences between the groups at any of the other flow rates. Fe$_{\text{NO}}$ levels at
all flow rates in COPD patients were not influenced by ICS use; Fe$_{\text{NO}0.05}$ was similar in ICS users
(mean 19.11, 95% CI 9.4-28.9) compared to those not taking ICS (mean 25.0, 95% CI 16.0-35.0).

Multiple Flow Rate Parameters

All the analysis methods showed that Caw$_{\text{NO}}$ was significantly reduced in COPD S compared to
COPD EX (Fig 2). All the methods except for mixed method 1 also showed a significant
reduction in COPD S compared to HNS while the mixed method 2 also showed a significant
reduction in Caw$_{\text{NO}}$ in HS compared to COPD EX.
There was evidence that cigarette smoking reduced J’aw\textsubscript{NO} (fig 3); all the methods except mixed method 1 showed that J’aw\textsubscript{NO} in COPD S and HS was reduced compared to COPD EX. Mixed method 1 showed that J’aw\textsubscript{NO} was significantly lower in HS compared to HNS.

There were no differences between groups (p>0.05 for all comparisons) in Calv\textsubscript{NO} and Daw\textsubscript{NO} using all of the modelling methods (Table 2).

Model parameters calculated by all five methods were not influenced by ICS use.

**Relationship between FEV\textsubscript{1} and NO measurements**

FEV\textsubscript{1} was not related to (i) Fe\textsubscript{NO} at all five flow rates (P >0.05 for all associations) or (ii) NO model parameters (Caw\textsubscript{NO}, Calv\textsubscript{NO}, Daw\textsubscript{NO} and Jaw\textsubscript{NO}) derived from the five modelling methods (P >0.05 for all associations) in COPD smokers or COPD ex smokers.

**Differences between Modelling Methods**

The data from COPD patients was used to compare the different modelling methods (see fig 4). Calv\textsubscript{NO} (fig. 4a) was significantly different between all the methods used i.e. no two methods gave the same result for this parameter. Daw\textsubscript{NO} data was non-parametric so mean and 95% confidence intervals could not be obtained for comparisons between methods. Non-parametric (Wilcoxon matched pair test) comparisons showed significant differences between all the methods (p<0.05 for all comparisons). J’aw\textsubscript{NO} (fig. 4b) was also different for the majority of between method comparisons, while there was more agreement between methods for the measurement of Caw\textsubscript{NO}(fig. 4c), as the majority of methods gave similar results. Similar results for comparisons between methods were obtained when using data from COPD patients and healthy subjects combined (data not shown).
Error of Non-Linear Modelling Methods

The error between modelled and actual data using the least squares fitting technique for non-linear regression was significantly lower for the non-linear method compared to the mixed methods which involved linear analysis prior to non-linear regression (Fig.5). The mixed methods produced a similar degree of error.

The Effect of Decreasing the Number of Flow Rates

Using non-linear analysis, all the parameters calculated using five flow rates were significantly different to those from the four highest or lowest flow rates (Table 3). The significant differences between groups in $Caw_{NO}$ and $J'aw_{NO}$ observed with five flow rates (see Figs 2 and 3), were no longer present when only four flow rates were used. Significant differences observed in $Caw_{NO}$ between groups, using five flow rates data becomes insignificant when only four flow rate data is applied (data not shown).

For mixed method 1, all the parameters were altered by the number of flow rates applied except for $J'aw_{NO}$ which was similar when calculated from the five and four highest rather than the four lowest flow rates. The number of flow rates used did not alter any parameters except $Calv_{NO}$ when calculated using mixed method 2.

Reproducibility of $FeNO$ and model derived parameters

The $FeNO$ readings at all five flow rates were similar when repeated on the same day or a week later (Table 4). There was no significant within or between day difference ($p>0.05$ for all comparisons) for any of the flow rates, indicating satisfactory reproducibility. As would be expected, there was also no significant within or between day variability ($p>0.05$ for all comparisons) for the model derived parameters calculated from this data by all five methods (data not shown).


**Discussion**

Our study set out to compare data from two-compartment NO models in COPD. Our main findings were: (1) the non linear model [28] has the least error, so we propose that this should be adopted in future COPD studies, although using more than one modeling technique would be a sensible way to ensure the validity of results. (2) Calv$_{NO}$ data appears to be highly variable, challenging current opinion regarding the potential applications of Calv$_{NO}$ measurements in COPD [24, 33] (3) Data from five flow rates was more reliable than four flow rate data. These findings need to be taken into account when comparing studies using different models and also for future recommendations for guidelines on use of multiple flow NO modelling.

We assessed the degree of agreement between the different mathematical models, and found a high degree of agreement for Caw$_{NO}$, but much less for all other parameters. In particular, Calv$_{NO}$ results were extremely model dependent. There is no “gold standard” modelling method, so it is not known which mathematical model actually provides the most clinically meaningful data. For the three mathematical methods that estimate all the model parameters (i.e. those that use non-linear modelling), we observed that mixed linear and non-linear modelling methods have more error compared to pure non-linear modelling. Given the differences between the various models, the method with the best fit to the actual data (i.e. pure non-linear modelling) may be regarded as providing the most accurate results.

For mixed modelling methods, non linear fitting of data (for Daw$_{NO}$ and Caw$_{NO}$ or Daw$_{NO}$ and Jaw$_{NO}$) is constrained by the assumption already made for Calv$_{NO}$ by the initial linear part of the method. The increased error of mixed methods (compared to pure non-linear modelling) can be explained by the degree of inaccuracy associated with initially estimating Calv$_{NO}$, which is then added to the error derived by non linear fitting based on this value of Calv$_{NO}$. For linear models
only two of the three model parameters are predicted and the actual data coincides exactly with
the modelled data at the two flow rates used, hence the error could not be assessed. We have
confined our study to COPD, but similar studies should be performed in other disease states to
validate specific modelling methods.

The advantage of non linear and mixed modelling is that more parameters can be estimated
compared to linear modelling. A practical drawback is that a greater number of flows are
required, including those at the lower range which can be technically challenging for some
subjects. In the current study, only 50 patients out of 77 were able to perform readings at all five
flow rates, as they were unable to perform reliable readings at the lower flow rates (10 and / or
30ml/s). These readings are needed for modelling methods to accurately estimate $C_{aw}$ and
$D_{aw}$.

The number of flow rates that should be used for multiple flow rate modelling has not been
standardised. There is some published evidence from a small sample set that non linear modelling
is dependent on the number of flow rates used [23]. For the methods that require data from
greater than two flow rates to perform modelling, we assessed the effect of using data from
different numbers of flow rates. The non linear method and mixed model 1 showed flow rate
dependency, as results from four and five flow rates were significantly different, and these
methods were most sensitive using five flow rates i.e. significant $C_{aw}$ findings using five flow
rates became non-significant with four flow rates. In contrast, mixed model 2 was relatively flow
rate independent. This has practical implications for the use of the linear method and mixed
model 1, as the number of flow rates used should be standardised, with our data showing that five
flow rates are needed.
The flow rates used in the current study were limited by the resistors commercially available. Previous studies have used flow rates greater than 200ml/s to improve the reliability of the calculated parameters [23, 26]. The current study has proved that NO modelling results are dependent on the number of flow rates used, and it is likely that the use of additional flow rates above 200ml/s will also influence the modelled parameters.

Our FeNO0.05 data showing lower levels in COPD S compared to COPD EX is in agreement with previous published data [16, 17]. Two-compartment NO modelling showed that this difference was due to a decreased concentration of NO in the airway wall (CawNO) regardless of which model was applied. There was also evidence that smoking reduced CawNO and J’awNO in healthy smokers, although the exact pattern of differences between the four groups for both CawNO and J’awNO varied with the models used. In contrast, smoking did not affect CalvNO and DawNO.

Our findings of a reduction in NO production in the airway wall of smokers, rather than the alveolar region, is in agreement with previous data [23, 26]. It has been reported that CalvNO is increased in COPD patients [23, 24], and is related to disease severity but is not influenced by current smoking [24]. Our COPD patients did not have severe disease, so we were less able to evaluate whether CalvNO was related to disease severity. However, our data shows that CalvNO is an extremely model dependent parameter and so CalvNO results obtained from one model may be less robust than CawNO results, which are less model dependent. Furthermore, the modelling method used by Brindicci et al [24] apparently uses the linear 1 method to estimate J’awNO, whereas this method actually estimates (CawNO- CalvNO) DawNO which is JawNO. This subtle but important change to the accepted method probably alters subsequent non-linear calculations.

Given the evidence from the current study that smoking reduces airway wall NO production, it is interesting to speculate on the mechanisms involved. The inhibition of NOS activity in the airway
wall epithelium [15] is a possibility. Airway arginases and the metabolism of asymmetric dimethyl arginine, superoxides [8] and nitrosoglutathione [9, 10] may also be affected by smoking by upregulation of NO metabolites. Alternative nitration pathways are also proposed whereby nitrites are oxidised by myeloperoxidase or xanthine oxidase [10]. In smokers with COPD, the level of airway wall NO may be further complicated by the effects of airway pH and bacterial airway colonisation on nitrogen oxides [32]; reduced airway pH aids in the conversion of nitrite to NO.

An important methodological point to raise is that the actual FeNO reading which is accepted from the exhalation trace is dependent on whether the peak or plateau is used. This is particularly important at higher flow rates, as these are essential for accurate estimation of CalvNO. However, the procedure used in clinical practice is variable, depending on the NO analyser set up and operator preference, and in the future it would be preferable to have some standardisation of this procedure. Additionally, ATS guidelines recommend that the mean of three NO plateau measurements that are within 10% are taken for a FeNO reading [31]. However, this may not be practically feasible for some patients if the absolute readings are low, as seen at the higher flow rates.

The advantages of NO modelling over single flow rate data is that the location of altered FeNO production can be identified. We, in agreement with others, have shown that CalvNO [24, 33] and DawNO [24, 26] are unaffected by smoking, and so may have potential for assessing inflammation in COPD. In particular, CalvNO has been proposed as a measurement that could be indicative of small airway inflammation [24, 33]. However, our data concerning the variation in CalvNO data between models underscores the need for method harmonisation so that results from different studies can be compared. There is also the possibility that NO modelling may be a more sensitive way of detecting early signs of a COPD exacerbation [33]. Furthermore, as inflammation is
heterogeneously distributed in the COPD lung, NO modelling could be used for more accurate location of inflammation [33], so enabling more specific targeting of treatments. These potential applications of NO modelling techniques in the future will rely on well validated and robust methodology, which the current paper set out to address. Axial diffusion is important in accurately evaluating NO transport in the airways, and a three compartmental NO model has been used to include this variable [34]. Our paper focused on comparisons of two compartment models, but it would certainly be of interest for future work to similarly evaluate the three compartment model in COPD.

This study can be used to make suggestions for standardizing NO multiple flow rate modelling: (1) In the absence of a “gold standard” NO modelling method, we recommend that future studies use more than one method to ensure the validity of the results. This would appear to be particularly important for CalvNO. If estimation of all modelling parameters is needed (CawNO, J’awNO, CalvNO and DawNO), then our results indicate that the non-linear method has the least error, with the greatest fit to the actual data. (2) It appears that data from more flow rates is likely to be more accurate, and certainly for the non-linear and mixed model 1 it seems that five flow rates are needed. At present, NO modelling is more useful for research studies rather than clinical practice. We hope that our recommendations for the harmonisation of NO modelling methods will increase the value of this technique in airway inflammation research, enabling data from different investigators to be directly compared.
References


Table 1

Subject Demographics

<table>
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<tr>
<th>Subject</th>
<th>n</th>
<th>ICS use</th>
<th>Gender M/F</th>
<th>Age (years)</th>
<th>Smoking history (pack years)</th>
<th>FEV1 (%) Predicted</th>
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<tr>
<td>COPD smokers (COPD S)</td>
<td>23</td>
<td>6</td>
<td>15/8</td>
<td>60 (6)</td>
<td>42 (10)</td>
<td>64 (11)</td>
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<tr>
<td>COPD ex smokers (COPD EX)</td>
<td>27</td>
<td>18</td>
<td>17/10</td>
<td>65 (7)</td>
<td>49 (30)</td>
<td>58 (12)</td>
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<tr>
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<td>58 (7)</td>
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<td>9/10</td>
<td>60 (11)</td>
<td>N/A</td>
<td>113 (11)</td>
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</tbody>
</table>

Data expressed as mean (sd).
Table 2

(i) Calv\textsubscript{NO} and (ii) Daw\textsubscript{NO} in COPD and healthy subjects calculated by different methods

(i) Calv\textsubscript{NO}

<table>
<thead>
<tr>
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<th>Linear 1</th>
<th>Non linear</th>
<th>Mixed 1</th>
<th>Mixed 2</th>
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<td>2.3 (1.7, 3.1)</td>
<td>3.0 (2.4, 3.8)</td>
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<td>2.8 (1.9, 4.1)</td>
<td>3.7 (2.7, 5.1)</td>
<td>2.8 (1.9, 4.1)</td>
<td>2.9 (2.1, 4.1)</td>
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<td>HS</td>
<td>3.0 (2.2, 4.1)</td>
<td>3.6 (2.8, 4.6)</td>
<td>3.0 (2.2, 4.1)</td>
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<td>2.4 (1.6, 3.5)</td>
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(ii) Daw\textsubscript{NO}

<table>
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<th>Non linear</th>
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<th>Mixed 2</th>
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<td>7.4 (5.8- 11.8)</td>
<td>8.9 (6.8-14.8)</td>
<td>10.6 (7.6-18.8)</td>
<td>10.5 (5.6-21.1)</td>
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<td>7.7 (1.7-13.5)</td>
<td>7.4 (2.6-13.9)</td>
<td>9.9 (9.1-11.1)</td>
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<tr>
<td>HS</td>
<td>6.6 (5.7-8.8)</td>
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</tr>
<tr>
<td>HNS</td>
<td>6.7 (5.1-8.6)</td>
<td>7.4 (4.5-10.3)</td>
<td>9.4 (8.3-11.2)</td>
<td>7.8 (6.7-8.3)</td>
</tr>
</tbody>
</table>

COPD S: COPD smokers; COPD EX: COPD ex smokers; HS: Healthy smokers HNS: Healthy non smokers. Data are presented as geometric means (95% confidence intervals) for Calv\textsubscript{NO} and medians (interquartile ranges) for Daw\textsubscript{NO}.
Table 3

Effect of number of flow rates on the derived NO model parameters: - Comparison of model parameters calculated using the five or four highest or lowest flow rates by the non linear and mixed methods.

<table>
<thead>
<tr>
<th></th>
<th>5 v 4 highest flows</th>
<th>5 v 4 lowest flows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Non linear</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caw$_{NO}$</td>
<td>2.02 (1.45, 2.83) **</td>
<td>0.70 (0.61, 0.81) ***</td>
</tr>
<tr>
<td>Cal$_{NO}$</td>
<td>1.99 (1.43, 2.75) **</td>
<td>0.86 (0.79, 0.92) ***</td>
</tr>
<tr>
<td>Daw$_{NO}$</td>
<td>19.55 ***</td>
<td>-1.24 ***</td>
</tr>
<tr>
<td>J’aw$_{NO}$</td>
<td>0.72 (0.58, 0.90) **</td>
<td>1.10 (1.06, 1.14) ***</td>
</tr>
<tr>
<td><strong>Mixed 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caw$_{NO}$</td>
<td>1.68 (1.34, 2.11) **</td>
<td>0.56 (0.38, 0.83) *</td>
</tr>
<tr>
<td>Cal$_{NO}$</td>
<td>#</td>
<td>0.76 (0.61, 0.94) *</td>
</tr>
<tr>
<td>Daw$_{NO}$</td>
<td>-4.24 ***</td>
<td>-1.01 *</td>
</tr>
<tr>
<td>J’aw$_{NO}$</td>
<td>0.91 (0.76, 1.08) NS</td>
<td>1.12 (1.03, 1.23) *</td>
</tr>
<tr>
<td><strong>Mixed 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caw$_{NO}$</td>
<td>0.92 (0.72, 1.18) NS</td>
<td>1.12 (0.89, 1.41) NS</td>
</tr>
<tr>
<td>Cal$_{NO}$</td>
<td>#</td>
<td>0.68 (0.53, 0.88) *</td>
</tr>
<tr>
<td>Daw$_{NO}$</td>
<td>0.650 NS</td>
<td>0.38 NS</td>
</tr>
<tr>
<td>J’aw$_{NO}$</td>
<td>1.27 (0.96, 1.68) NS</td>
<td>0.97 (0.86, 1.09) NS</td>
</tr>
</tbody>
</table>

* P<0.05; ** = P<0.001; *** = P<0.0001; NS= not significant

Data for Caw$_{NO}$, Cal$_{NO}$ and J’aw$_{NO}$ presented as ratio of the 2 groups (95% confidence interval of the ratio) Data for Daw$_{NO}$ presented as median difference.

#; For the mixed methods, Cal$_{NO}$ is calculated using the highest flow rates; 200 and 100ml/s, and hence is the same whether obtained using the 5 or the 4 highest flow rates.
Table 4
Within and between day reproducibility of FeNO in COPD (N=12).

<table>
<thead>
<tr>
<th>FeNO (ml/s)</th>
<th>Week 1 (10am)</th>
<th>Week 1 (1pm)</th>
<th>Week 2 (10am)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>47.6 (23.5 – 71.7)</td>
<td>49.8 (25.7-73.9)</td>
<td>50.6 (16.7-84.5)</td>
</tr>
<tr>
<td>30</td>
<td>21.5 (13.3-29.7)</td>
<td>21.1 (13.6-28.7)</td>
<td>23.6 (10.4-36.7)</td>
</tr>
<tr>
<td>50</td>
<td>17.3 (10.8-23.8)</td>
<td>16.1 (10.0-22.2)</td>
<td>18.5 (8.6-28.3)</td>
</tr>
<tr>
<td>100</td>
<td>9.7 (6.3-13.0)</td>
<td>9.8 (6.6-13.0)</td>
<td>10.8 (5.5-16.1)</td>
</tr>
<tr>
<td>200</td>
<td>6.5 (4.3-8.7)</td>
<td>6.5 (4.6-8.5)</td>
<td>7.0 (4.1-9.9)</td>
</tr>
</tbody>
</table>

Data are presented as geometric means (95% confidence intervals)
**Figure Legends**

Figure 1

![Box plot](image)

P = 0.03

$\text{FeNO}_{0.05}$ in COPD and healthy subjects.
CS: COPD smokers; CEX: COPD ex smokers; HS: Healthy smokers HNS: Healthy non smokers.
The box extends from the 25th to the 75th percentile. The middle line represents the median and error bars extend from the lowest to the highest values.

Figure 2

NO airway wall concentration ($C_{awNO}$) in COPD and healthy subjects calculated by four different methods: (i) linear (ii) non linear (iii) mixed 1 and (iv) mixed 2.

CS: COPD smokers; CEX: COPD ex smokers; HS: Healthy smokers; HNS: Healthy non smokers. Horizontal bars (−) represent geometric means.
Maximal NO flux ($J'_{aw_NO}$) in COPD and healthy subjects calculated by 4 different methods: (i) linear 2 (ii) non linear (iii) mixed 1 and (iv) mixed 2.

CS: COPD smokers; CEX: COPD ex smokers; HS: Healthy smokers; HNS: Healthy non smokers. Horizontal bars (−) represent geometric means.

Figure 4
Differences in a) Calv$_{NO}$, b) Jaw$_{NO}$ and c) Caw$_{NO}$ between the NO modelling methods in COPD.

L1: Linear 1, L2: Linear 2, M1: Mixed 1, M2: Mixed 2 and NL: Non linear.

The middle horizontal bar (-) represents the ratio of the two groups and the upper and lower bars represents the 95% confidence interval of the ratio.

Figure 5
Error between modelled data and actual data derived for NO model parameters: Comparison of the mixed and full non linear methods.

M1: mixed method 1; M2: mixed method 2; NL: non linear method. Horizontal bars (-) represent geometric means.