

Short and long-term effects of cigarette smoking independently influence exhaled nitric oxide concentration in asthma

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Background: The fractional concentration of nitric oxide in exhaled breath (FeNO) is elevated in asthma. FeNO measurement has been proposed as a noninvasive index of disease activity. Cigarette smoking suppresses FeNO, which limits its use in smokers.

Objective: To identify and model short-term and long-term influences of cigarette smoking on FeNO.

Methods: The smoking history, FeNO, and fractional concentration of carbon monoxide in exhaled breath (FeCO) were measured in 53 subjects with asthma and 51 control subjects. A mathematical model of the short-term and long-term effects of cigarette smoking on FeNO was derived.

Results: Subjects with asthma had higher FeNO than controls ($P < .001$). Smokers had increased FeCO ($P < .001$). The short-term effect (hours since last cigarette) was associated with increased FeNO ($P < .01$) and decreased FeCO ($P < .05$). The long-term effect (years smoked) was associated with decreasing FeNO only in the subjects with asthma ($r = -0.62$; $P = .005$). These short-term and long-term effects were independent and were combined in a model predicting FeNO, predicted $\log_{10}$ FeNO = $1.23 - 0.58e^{-0.34t} - 0.00000103 \times (\text{lifetime cigarettes})$, where t = hours since the last cigarette. This gave a convincing prediction of FeNO ($r = 0.83$; $P < .0001$).

Conclusion: Short-term and long-term effects of smoking influenced the measurement of FeNO. We defined a model that describes these effects. The use of this formula may improve the value of FeNO measurements in smokers with asthma. (J Allergy Clin Immunol 2005;116:88-93.)

Key words: Smoking, nitric oxide, asthma

Nitric oxide (NO) is present in measurable quantities in exhaled breath.¹ The fractional concentration of this endogenous exhaled NO (FeNO) may be of clinical value as a noninvasive biomarker.² For example, altered concen-

Abbreviations used

ATS: American Thoracic Society
CO: Carbon monoxide
FeCO: Fractional concentration of carbon monoxide in exhaled breath
FeNO: Fractional concentration of nitric oxide in exhaled breath
iNOS: Inducible NOS
NO: Nitric oxide
NOS: Nitric oxide synthase
UK: United Kingdom

trations are associated with some respiratory diseases such as asthma^{3,4} and chronic obstructive pulmonary disease,⁵ as well as systemic conditions such as cirrhosis,⁶ inflammatory bowel disease,⁷ and hypertension.⁸ Cigarette smoking reduces the concentration of FeNO⁹ and can obviate the clinical value of quantifying FeNO in disease among smokers.

The effects of smoking on FeNO are dynamic⁹; therefore, it may be possible to estimate these effects to understand better the clinical value of FeNO measurements among smokers. This may be important in asthma, because approximately 25% of adult patients in most developed countries are current cigarette smokers.¹⁰ The aims of the study were to quantify FeNO in patients with asthma and relate this to their recent and long-term smoking history, and to derive a mathematical model that describes these effects.

METHODS

Subjects

Fifty-three subjects with asthma (23 nonsmokers, 17 smokers, and 13 exsmokers) and 51 healthy subjects (20 nonsmokers, 20 smokers, and 11 exsmokers) were recruited from respiratory outpatient clinics and hospital staff, respectively. The diagnosis of asthma was based on the American Thoracic Society (ATS) criteria.¹¹ Patients had not received oral corticosteroids in the past 4 weeks, and none had a history of a respiratory tract infection within the preceding 4 weeks. Current treatment included inhaled short-acting β_2 -agonist ($n = 52$), inhaled corticosteroid ($n = 43$), inhaled long-acting β_2 -agonist ($n = 7$), inhaled anticholinergic ($n = 3$), leukotriene antagonist ($n = 2$), and theophylline ($n = 1$). Subjects were excluded if they were pregnant or lactating. Smokers had a greater than 10 pack-year smoking history to allow a sufficient exposure to cigarette smoke, and exsmokers had stopped smoking at least 1 year previously with a

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TABLE I. The number of subjects with asthma and control subjects and their sex, age, and indices of smoking history (years smoked, years since quitting smoking, cigarettes per day, and pack-years), percent predicted FEV₁, serum IgE concentration (IU/mL), number atopic, fractional concentrations of FeNO and FeCO in exhaled air, duration and severity of asthma, and corticosteroid therapy according to smoking history

	Healthy control subjects			Subjects with asthma		
	Smokers	Never-smokers	Exsmokers	Smokers	Never-smokers	Exsmokers
Number	20	20	11	17	23	13
Male: female	9:11	9:11	4:7	9:8	17:6	7:6
Age, y	42.5 (36.7-46.0)	32.5 (28.0-43.2)	50.0 (40.0-53.0)	40.0 (35.5-52.0)	39.0 (33.0-52.0)	52.0 (47.0-59.0)
Years smoking	25.0 (20.0-30.0)	NA	20.0 (16.0-23.0)	25.0 (21.0-31.0)	NA	25.0 (14.5-31.0)
Years exsmoking	NA	NA	4.0 (1.0-10.0)	NA	NA	8.0 (1.5-12.5)
Cigarettes per day	20.0 (20.0-25.0)	NA	NA	20.0 (17.5-27.5)	NA	NA
Pack-years	26.0 (14.5-32.6)	NA	23.0 (16.0-32.4)	27.5 (16.0-35.7)	NA	22.5 (14.5-30.0)
FEV ₁ % predicted	95.0* (86.0-105.8)	95.6* (87.3-101.1)	96.9* (86.1-112.0)	75.4 (70.3-89.1)	81.4 (72.6-86.9)	72.0 (51.7-81.8)
Total IgE IU/mL	67† (26-111)	32† (13-67)	52‡ (23-181)	254 (112-713)	112 (42-420)	535 (109-819)
Atopic, n	9	5	3	13	22	9
FeNO ppb	4.71‡ (3.85-6.82)	7.18 (5.70-10.06)	6.08 (4.32-7.94)	4.03‡ (2.96-6.47)	14.30 (10.63-27.86)	10.40 (6.47-17.47)
FeCO ppm	17.6§ (10.9-21.5)	3.2 (2.7-3.8)	4.6 (4.0-6.0)	16.3§ (10.1-22.5)	3.9 (3.2-4.3)	3.4 (3.1-4.5)
Asthma duration, y	NA	NA	NA	15 (7.5-32.5)	17 (8.0-37.5)	29 (8.7-43.0)
ATS asthma score	NA	NA	NA	4 (3-4)	3 (3-5)	5 (4-6.5)
Corticosteroids, inhaled, ug/d	NA	NA	NA	800 (100-900)	400 (0-600)	800 (400-1000)

NA, Not appropriate.

*Greater than the corresponding section in the asthma group ($P < .001$).

†Less than the corresponding section in the asthma group ($P < .001$).

‡Less than the never-smokers in the same group ($P < .01$).

§Greater than the exsmokers or never-smokers in the same group ($P < .001$).

smoking history of greater than 10 pack-years. Subjects were asked to refrain from smoking or strenuous exercise for at least 1 hour before testing and the time since their last cigarette was recorded.

The West Ethics Committee, North Glasgow University National Health Service Trust, approved the study. All subjects gave written informed consent.

Measurements

Spirometry was measured with a dry spirometer (Vitalograph Ltd, Buckingham, United Kingdom [UK]), and the best of 3 attempts was taken for analysis. FEV₁ was measured before and 15 minutes after 2.5 mg nebulized albuterol to test reversibility.

The FeNO and fractional concentration of carbon monoxide (CO) in exhaled breath (FeCO) were measured simultaneously by using a chemiluminescence analyzer (model LR2000; Logan Research Ltd, Rochester, UK), with a detection limit of 0.1 ppb NO.¹² The analyzer was calibrated with certified NO mixtures (100 ppb) in nitrogen (BOC Special Gases, Guilford, UK). The subject inspired to total lung capacity and, with no breath holding, exhaled gently into a sampling tube against a flow resistor. The subject exhaled at a constant rate to maintain a constant mouth pressure of 4 to 5 cm H₂O by observing a visual display of this pressure. All subjects maintained an exhalation flow rate of 250 mL/s. NO levels were taken from the plateau at the end of exhalation. Exhaled CO was measured by using a modified electrochemical sensor with a sensitivity of 1 to 500 ppm CO. The means of triplicate FeNO and FeCO measurements were used as the representative values.

Serum total IgE (IU/mL) was measured by enzyme immunoassay (Pharmacia Ltd, Milton Keynes, UK). The short-term smoking history was defined as the time interval in hours between smoking the last cigarette and measuring FeNO. The long-term smoking history was defined as the cumulative smoking history in years or lifetime number of cigarettes smoked.

Statistical analysis

Data were analyzed by using a statistical software package (Minitab Ltd, Coventry, UK).

Differences between subjects with and without asthma or between smokers and nonsmokers were tested for statistical significance by using the rank-sum test for interval variables such as exhaled gas concentrations, and the χ^2 test for nominal variables such as the presence of atopy. The effects of multiple causal factors in combination, such as steroid therapy combined with smoking, or long-term and short-term effects of smoking, were investigated by using ANOVA. Optimization of the parameters in the descriptive model was performed by minimizing the sum of squares of errors in the predicted log concentration of exhaled NO. Statistical significance was accepted at the 95% level.

RESULTS

The number, sex, age, serum IgE level, atopic status, and FEV₁ in the asthma and healthy control study groups, along with the inhaled corticosteroid use, asthma duration, and ATS asthma score of the asthma subjects, are listed in Table I according to smoking history. There were no significant age differences. The asthma group had significantly reduced lung function measured by the percent of predicted FEV₁ ($P < .001$) and significantly higher IgE levels ($P < .001$) and atopy ($\chi^2 = 23.6$; $P < .001$) than the control group. The fractional exhalation of FeCO ppm and FeNO ppb are listed according to smoking and clinical history. The smokers had increased FeCO (16.41 [10.62-20.63]) compared with the never-smokers

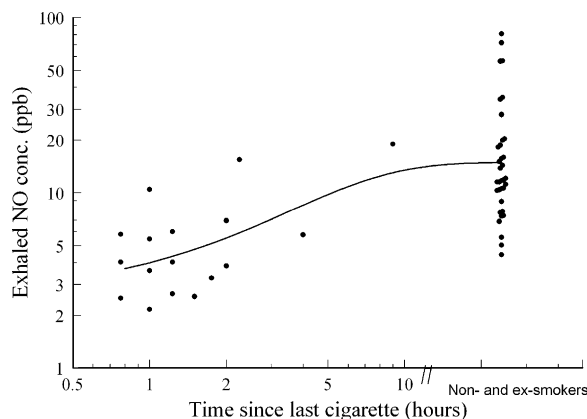


FIG 1. The fitted curve represents the mathematical model in which the log FeNO is depressed by an amount that decays exponentially with time after smoking the last cigarette.

(3.51 [3.07-4.16]) and with the exsmokers (3.99 [3.26-4.91]; $P < .001$). These values are consistent with reference ranges according to smoking history.^{13,14} The subjects with asthma had increased FeNO compared with healthy control subjects (10.53 [5.67-17.03] and 6.18 [4.36-8.96]; $P < .001$).

Association of FeCO and FeNO with smoking history

The smoking history is summarized in Table I. There was no significant difference in smoking history between the asthma and control groups. In the smokers, the short-term smoking history was, median (interquartile range), 1.0 (1.0-2.0) hours, with range 1 to 9 hours since smoking the last cigarette.

In the smokers of this study, the long-term smoking history was 25.0 (20.0-30.0) years smoking, and lifetime cigarettes smoked was 197,100 [116,800-240,900].

In all smokers (subjects with asthma plus control subjects), there was a significant association between the short-term smoking history and both log FeNO ($r = 0.443$; $P < .01$), and log FeCO ($r = -0.332$; $P < .05$). This means that the FeNO significantly increased, and the FeCO level significantly decreased, with time since smoking the last cigarette.

The association between FeNO and short-term smoking history was modeled mathematically by using all of the patients with asthma, and the best fitting curve shown in Fig 1 demonstrated that log FeNO was depressed by an amount that decays exponentially with time after the last cigarette. The model had the effect decaying with a half-life of 2.4 hours. In algebraic form the model is

$$\log_{10}(\text{FeNO}) = 1.17 - 0.76e^{-0.283t}$$

where t is the time in hours.

The long-term effect of cigarette smoking measured by number of years smoked was significantly associated with decreasing log FeNO ($r = -0.421$; $P = .009$) but was unrelated to the log FeCO level ($r = 0.017$; $P = .920$).

Considering the smokers in separate asthma and control categories, the relationship between long-term effects of

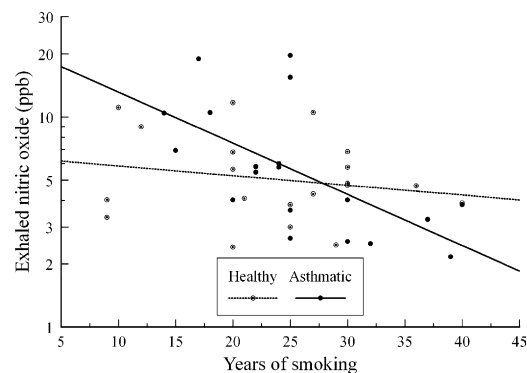


FIG 2. The association between years smoked and FeNO in asthmatic subjects and controls.

smoking on FeNO is shown in Fig 2. This demonstrated an inverse association between years smoked and FeNO in the subjects with asthma ($r = -0.62$; $P = .005$) but not in the control subjects ($r = -0.2$; $P = .4$).

Effect of age on FeNO

Log FeNO did not correlate convincingly with age ($r = -0.265$; $P = .055$).

Effect of corticosteroid therapy on FeNO

The inhaled corticosteroid dose correlated with the ATS score ($r = 0.37$; $P = .007$). In the subjects with asthma, the amount of corticosteroid used was not significantly different in smokers compared with the nonsmokers. The inhaled corticosteroid dose did not correlate with the FeNO irrespective of smoking history, and ANOVA did not support a significant contribution of the inhaled corticosteroid dose to the inhibitory effects of smoking on FeNO.

There was no effect of any other medication on FeNO levels.

Effect of atopy on FeNO

Atopy was defined as having significantly raised IgE antibody to any of the common allergens house dust mite, grass pollen, or cat dander. The atopic subjects ($n = 62$) had significantly higher FeNO than the nonatopic subjects ($n = 32$), with median (interquartile range) 8.94 (5.35-15.13) and 5.85 (4.45-9.26; $P = .021$).

The total serum IgE was log-normally distributed and correlated significantly with FeNO in the whole study group. The regression equation was $\log \text{FeNO} = 0.780 + 0.163 \log [\text{IgE}]$ ($r = 0.219$; $P = .034$). This correlation remained significant only in the subgroup of nonsmoking subjects, which included the asthma and control subjects ($n = 43$; $r = 0.392$; $P = .012$).

Association of FeNO with lifetime cigarettes smoked

Instead of number of years smoked, we used the lifetime total number of cigarettes smoked (cigarettes smoked per day $\times$ 365 $\times$ years smoked) to analyze the long-term effects of smoking, considered in isolation, on FeNO. This association, illustrated in Fig 3, is compatible with a linear relationship ($r = -0.43$; $P < .001$).

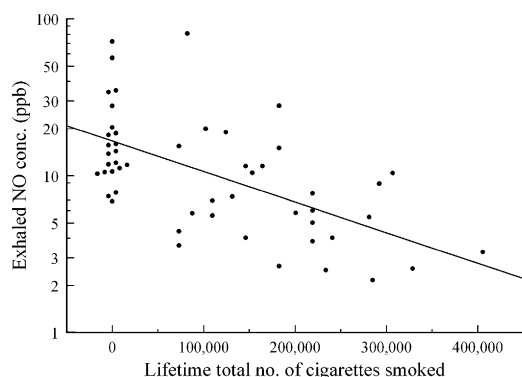


FIG 3. The long-term effects of smoking on exhaled NO concentration in patients with asthma.

Independent short-term and long-term effects of smoking on FeNO

We established that hours since smoking the last cigarette and number of years smoked were unrelated variables ($r = -0.126$; $P = .465$). Thereafter, 3 lines of exploratory analysis indicated that there were distinct short-term and long-term effects of cigarette smoking on FeNO measurement.

First, if we use log FeCO concentration as a measure of short-term exposure to smoking, and the lifetime total number of cigarettes smoked as a measure of long-term exposure, then the partial correlation coefficient of log FeNO with long-term exposure, adjusted for the effects of short-term exposure, is -0.426 ($P = .001$). The partial correlation coefficient of log FeNO with short-term exposure, adjusted for the effects of long-term exposure, is -0.326 ($P = .017$). Therefore, short-term and long-term effects appear to be independently significant.

Second, a similar conclusion was derived when the short-term exposure to smoking was measured not by log FeCO as above but by the reciprocal of time since last cigarette. The reciprocal was used so that nonsmokers could be included in the calculation. It was found by partial correlation analysis that log FeNO correlated significantly with short-term exposure ($r = -0.446$; $P = .001$) when corrected for long-term effects, and log FeNO also correlated significantly with long-term exposure ($r = -0.36$; $P = .008$) when corrected for short-term effects.

Third, multiple linear regression analysis to express log FeNO as a function of lifetime total number of cigarettes smoked, along with time since last cigarette, indicated that smoking had both short-term and long-term effects, with each statistically significant after correcting for the other ($P = .002$ and $.01$, respectively).

Statistical model that combines the short-term and long-term effects

A statistical model that combines the short-term and long-term effects may be written algebraically as follows.

Equation 1:

$$\text{Predicted } \log_{10} \text{ FeNO} = 1.23 - 0.579e^{-0.344t} - 0.00000103 \times (\text{cigs})$$

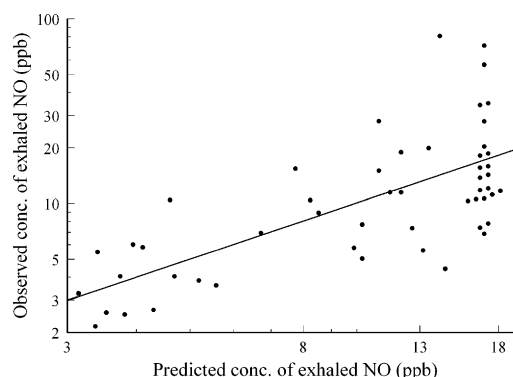


FIG 4. The relationship between predicted and measured exhaled FeNO in subjects with asthma.

where t = time since last cigarette in hours, cigs = lifetime total number of cigarettes smoked, and e is the constant 2.72, which is the base of the natural logarithm.

The fitted model implies that the short-term effect decays with a half-life of about 2.4 hours, and the long-term effects suggest that smoking about 300,000 cigarettes reduces FeNO by a factor of 2. The constants in this predictive model may be revised with the increased power of larger studies. Meanwhile, the predictive value ($r = 0.83$; $P < .0001$) is illustrated in Fig 4.

DISCUSSION

In this cross-sectional study, we have demonstrated coincidental short and long-term effects of cigarette smoking on the measurement of NO concentration in exhaled breath. These effects were independent and could be modeled mathematically.

We found that the FeNO was reduced in smokers with and without asthma compared with their nonsmoking counterparts, which is in agreement with other studies.^{4,8,9,15,16} We extended these observations by confirming a decrease in FeNO with increasing years smoked and lifetime total number of cigarettes smoked, and by showing an additional dynamic effect in which there was a short-term depression of the FeNO concentration associated with recent smoking. This depression appeared to decay exponentially with a half-life of 2.4 hours since the last cigarette.

These potentially conflicting short-term and long-term effects will have a confounding influence on the assessment of FeNO among smokers. To resolve this, we confirmed that these effects were independent and could therefore be modeled mathematically to generate an algorithm with which to estimate the FeNO concentration, taking into account the effects of cigarette smoking. It is suggested that the value of the measured FeNO concentrations would probably be improved by expressing the concentrations relative to the values predicted from the patient's smoking history. One way to do this is to estimate from Equation 1 the predicted value of log FeNO and

subtract this from the observed value of log FeNO. The residual variation in FeNO concentrations, after correcting for smoking history, presumably reflects, among other factors, the inflammatory state of the airways. There is a precedent for this: residual FeNO normalized after steroid therapy was found to reflect airway hyperreactivity.¹⁷

The increased power of a larger prospective study may also clarify the contribution of other factors to FeNO measurement. For example, inhaled corticosteroid therapy has been reported to reduce FeNO^{18,19}; however, in the current study, this had no additive influence with smoking on reducing FeNO. The reasons for this are unclear. One part explanation may be the different study structures; the inhibitory effect of corticosteroids on FeNO reported in the literature is mainly based on longitudinal studies. Our study was cross-sectional, and therefore, any inhibitory changes in FeNO within individuals might still be seen over time associated with corticosteroid therapy. Furthermore, corticosteroid therapy does not prevent the strong expression of airway inducible NOS (iNOS) in corticosteroid-dependent asthma,²⁰ nor the FeNO in bronchiectasis,²¹ and the airway-diffusing capacity for NO is elevated in asthma independent of corticosteroid therapy.²² Thus, the relationship between corticosteroids and NO metabolism is not completely understood.

Other factors may be involved. For example, the inhibitory effect of corticosteroids on FeNO may be partly attenuated by β -agonists,²³ and atopy rather than asthma was associated with elevated FeNO.²⁴ In the current study, atopy was associated with increased FeNO in the whole group, and total serum IgE correlated with FeNO in the nonsmokers of a combined group with and without asthma. Atopy itself was associated with asthma; therefore, clarifying these complex interactions would require a higher-powered study. This could resolve the conflicting reports on FeNO being affected by age.^{25,26} In the current study, we found that the association between age and FeNO did not quite reach statistical significance. Several other variables may also transiently reduce FeNO. These include recent exposure to environmental tobacco smoke,^{27,28} alcohol,²⁹ and caffeine,³⁰ which can often occur simultaneously. The influence of all of these factors could be identified in a larger study, in the light of which we would advise that the numerical constants in Equation 1 be revised.

Quantifying FeNO is important for 2 main reasons: first, as an adjunct to clinical monitoring,¹⁸ and second, as a probe to investigate disease pathogenesis. As a clinical adjunct, the concentration of FeNO in healthy subjects is not affected by age or sex.²⁵ However, in addition to asthma,^{3,4} it is altered in several respiratory and systemic diseases. FeNO is increased in chronic obstructive pulmonary disease,⁵ chronic bronchitis,³¹ and allergic alveolitis.³² In contrast, FeNO is decreased in cystic fibrosis³³ and in HIV pneumonitis.³⁴ FeNO is also altered in some extrapulmonary diseases. It is increased in Sjogren syndrome,³⁵ cirrhosis,⁶ and Crohn disease,⁷ and in contrast, FeNO is lower than normal in hypertension.⁸ Many of these diseases are associated with smoking. Therefore, using our equation to adjust

for the effects of smoking may help clarify the changes in FeNO that may be disease-associated.

The mechanism by which smoking causes reduced FeNO is unknown.²⁸ NO is a highly reactive free radical molecule that can be oxidized or complexed with other biomolecules depending on the microenvironment. In the air-fluid interface of the airways, NO generation may include the formation of the stable end products of NO metabolism, nitrite and nitrate.³⁶ These are decreased in saliva³⁷ and serum³⁸ of smokers and are transiently reduced (1 hour) after smoking a single cigarette.³⁹ Cigarette smoke itself contains high concentrations of NO,^{40,41} which may reduce endogenous NO synthesis by feedback inhibition,⁹ and in smokers, the expression both of endothelial NO synthase (NOS) in pulmonary arteries⁴² and of inducible NOS in lung epithelial cells⁴³ is reduced.

The NO concentration in exhaled breath is determined by epithelial iNOS⁴⁴ and NOS3⁴⁵ activities. The gene for neuronal NOS^{46,47} is an additional candidate gene for asthma. In studies of asthma susceptibility, the allele frequencies of a specific polymorphism in the nNOS gene (G894T, in a region of chromosome 12q24 shown in family studies to be linked to the diagnosis of asthma) were significantly different between subjects with asthma and controls⁴⁷ and played an important role in determining FeNO levels.⁴⁵ Also, patients with an iNOS polymorphism (multiple trinucleotide AAT repeat sequences in intron 20) had homogeneously raised FeNO compared with the heterogeneity of this measurement in the sample population.⁴⁶ We would suggest that in this and other studies—for example, coronary artery disease⁴⁸—the FeNO measurement could be adjusted for the effects of smoking by using Equation 1 to define better the study cohorts used for genetic analysis.

In conclusion, measuring the concentration of exhaled NO provides a useful index for monitoring clinical progress in asthma and has similar potential for other diseases.⁴⁹ It also provides a scientific probe into the pathogenesis of pulmonary and systemic diseases. It is important for the accuracy of these measurements that any confounding effects be accommodated. Despite FeNO being increasingly established as a clinical adjunct, the biological complexity of this molecule⁵⁰ and the limitations to our current understanding are recognized.⁵¹ To address this, we describe a mathematical model based on objective indices of smoking history that describes the effects of smoking on exhaled NO. This may permit analysis of the relative contribution of smoking and inflammation to the measured NO concentration.

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