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 = $1.23 - 0.58e^{-0.34t} - 0.00000103 \times$ (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.

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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 concentrations 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 FeNO[9] and can obviate the clinical value of quantifying FeNO in disease among smokers.

The effects of smoking on FeNO are dynamic[9]; 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.

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

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 β2-agonist (n = 52), inhaled corticosteroid (n = 43), inhaled long-acting β2-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 FEV_1, 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. FEV_1 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 H_2O 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 FEV_1 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 FEV_1 ($P < .001$) 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