Different patterns of exhaled nitric oxide response to β_2-agonists in asthmatic patients according to the site of bronchodilation

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Background: In asthmatic patients undergoing airway challenge, fraction of exhaled nitric oxide (FENO) levels decrease after bronchoconstriction. In contrast, model simulations have predicted both decreased and increased FENO levels after bronchodilation, depending on the site of airway obstruction relief.

Objective: We sought to investigate whether β_2-agonists might induce divergent effects on FENO values in asthmatic patients as a result of airway obstruction relief occurring at different lung depths.

Methods: FENO, FEV_1, and the slope of phase III of the single-breath washout test (S) of He (S_{He}) and sulfur hexafluoride (S_{SF6}) were measured in 68 asthmatic patients before and after salbutamol inhalation. S_{He} and S_{SF6} decreases reflected preacinar and intra-acinar obstruction relief, respectively. Changes (Δ) were expressed as a percentage from the baseline.

Results: No FENO change ($|\Delta FENO| \leq 10\%$) was found in 16 patients (mean [SD]: 2.5% [5.2%]; ie, FENO= group); a $\Delta FENO$ value of greater than 10% was found in 23 patients (31.7% [20.3%]; ie, the FENO+ group); and a $\Delta FENO$ value of less than −10% was found in 29 patients (−31.5% [17.3%]; ie, the FENO− group). All groups had similar ΔFEV_1 values. In the FENO= group neither S_{He} nor S_{SF6} changed, in the FENO+ group only S_{He} decreased significantly (−21.8% [SD 28.5%], $P = .03$), and in the FENO− group both S_{He} (−29.8% [24.0%], $P < .001$) and S_{SF6} (−27.2% [23.3%], $P < .001$) decreased.

Discussion: Three FENO behaviors were observed in response to β_2-agonists: a decrease likely caused by relief of an intra-acinar airway obstruction that we propose reflects amplification of nitric oxide back-diffusion, an increase likely associated with a predominant dilation up to the preacinar airways, and FENO stability when obstruction relief involved predominantly the central airways. In combination, these results suggest a new role for FENO in identifying the site of airway obstruction in asthmatic patients. (J Allergy Clin Immunol 2016;137:806-12.)

Key words: *Asthma, fraction of exhaled nitric oxide, bronchodilation, ventilation distribution*

Current guidelines[1] indicate that levels of nitric oxide (NO) measured in the exhaled air of asthmatic patients mark T_H2-type airway inflammation[2] and hold potential application in asthma management.[3] However, noninflammatory changes related to airway caliber also appear to affect fraction of exhaled nitric oxide (FENO) levels. Several studies with airway challenges have demonstrated that the reduction of airway caliber reduces FENO levels in the absence of any inflammatory changes.[4-8] This is likely due to the decrease of available epithelial surface, which impairs NO diffusion from the airway epithelium into the airway lumen.[9] Involvement of the peripheral airways amplifies this reduction because the biggest NO production is concentrated in the small conductive airways,[8] which represent the vast majority of the total epithelial surface. This was confirmed in a model simulation,[9] which indicated the most marked effect of airway constriction on FENO reduction in generations 10 to 15.

Beyond generation 15, molecular diffusion generates an NO flux, with back-diffusion[10,11] from bronchioles toward the alveoli that is proportionate to acinar airways caliber and removes NO molecules from the expiratory flux. Thus airway constriction beyond generation 15 (ie, deeper than the zone affected by indirect agents)[7,12] would decrease back-diffusion and paradoxically increase FENO values. Taking all of the above into account, it is quite surprising that studies of bronchodilation do not indicate a substantial effect of β_2-agonist inhalation on FENO levels through either FENO stability[13-15] or a statistically significant but modest FENO value increase.[16] In fact, considering the aforementioned experiments and models regarding airway constriction and assuming a homogeneous baseline constriction and inflammation status in a given region, Fig 1, *A*, which was adapted from Verbanck et al,[9] shows 3 different patterns of FENO response to β_2-agonists expected to occur as bronchodilation penetrates deeper and deeper into the bronchial tree: no change, increase, or decrease with obstruction relief located centrally, up to the onset of the acinus, or inside the acinus, respectively.

We hypothesized that the apparent absence of bronchodilation's effects on FENO values could in fact mask divergent FENO behaviors. The key objective of the present study was to investigate whether β_2-mimetics can induce divergent effects on FENO values in asthmatic patients as a result of airway obstruction relief occurring at different lung depths. For the purpose of this study, we used the inert gas washout technique with He and

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Abbreviations used
FEF_{25-75}: Forced expiratory flow between 25% and 75% of forced vital capacity
FENO: Fraction of exhaled nitric oxide
FRC: Functional residual capacity
NO: Nitric oxide
S: Phase II of the single-breath washout test
SF_6: Sulfur hexafluoride

sulfur hexafluoride (SF_6), which was shown to be sensitive to specific sites of airway obstruction.[16-19]

METHODS

Subjects

Asthmatic patients exhibiting airway obstruction defined according to Global Initiative for Asthma guidelines (FEV_1/forced vital capacity ratio < 0.75)[17] were recruited from the outpatient asthma clinic (CUB-Erasme University Hospital, Brussels, Belgium). Asthma was defined according to standard criteria.[17] Asthma control level at the time of inclusion was evaluated through the Asthma Control Questionnaire.[18] Subjects were requested to halt short- and long-acting β_2-agonists for 6 and 24 hours, respectively. Participants were instructed to avoid eating and drinking 3 hours before the visit, and their diets were reviewed to ensure the absence of food with potential to interfere with FENO measurement.[1]

The study was approved by the local ethics committee of CUB Erasme University Hospital in Brussels, Belgium, and all patients signed informed consent forms.

Study design

The study, which was designed as a prospective open study, was carried out in the Chest Department of the Erasme University Hospital in Brussels, Belgium.

FENO values, spirometric parameters, and ventilation distribution using single-breath washout tests were sequentially measured before and 15 minutes after inhalation of short-acting β_2-agonists (400 μg of salbutamol administered through a metered-dose aerosol device).

Study procedures

Spirometry. Lung function was measured with a Zan 300 spirometer (Zan, Oberthulba, Germany), according to standard guidelines.[19] FEV_1 and forced expiratory flow between 25% and 75% of forced vital capacity (FEF_{25-75}) were expressed as percentages of predicted value.[19]

Ventilation distribution tests with inert gases. Ventilation distribution was assessed by using the phase III of the single-breath washout test (S), consisting of a 1-L inspiration from functional residual capacity (FRC) of a gas mixture containing 2 inert gases: 5% He and 5% SF_6 in oxygen. During expiration to residual volume, He and SF_6 concentrations were recorded as a function of expired volume by using a quadrupole mass spectrometer (LR6000; Logan-Sinclair, Rochester, United Kingdom), and the slopes of phase III for each gas (S_{He} and S_{SF6}) were computed. The test was performed in triplicate, with a variation coefficient not exceeding 10%. Detailed procedures and processing can be found in the study by Michils et al.[12]

FENO. FENO (expressed in parts per billion) values were measured before forced expiratory maneuvers by using a daily calibrated LR 2000 chemiluminescence analyzer (Logan Research LTD, Rochester, United Kingdom) at a flow rate of 50 mL/s, according to American Thoracic Society/European Respiratory Society standard.[1]

Model of parameters' sensitivity to airway obstruction relief. Fig 1 schematically presents the hypothesized responses of FENO, ventilation distribution, and pulmonary function indices resulting in relief of a pre-existing airway obstruction up to a given generation.

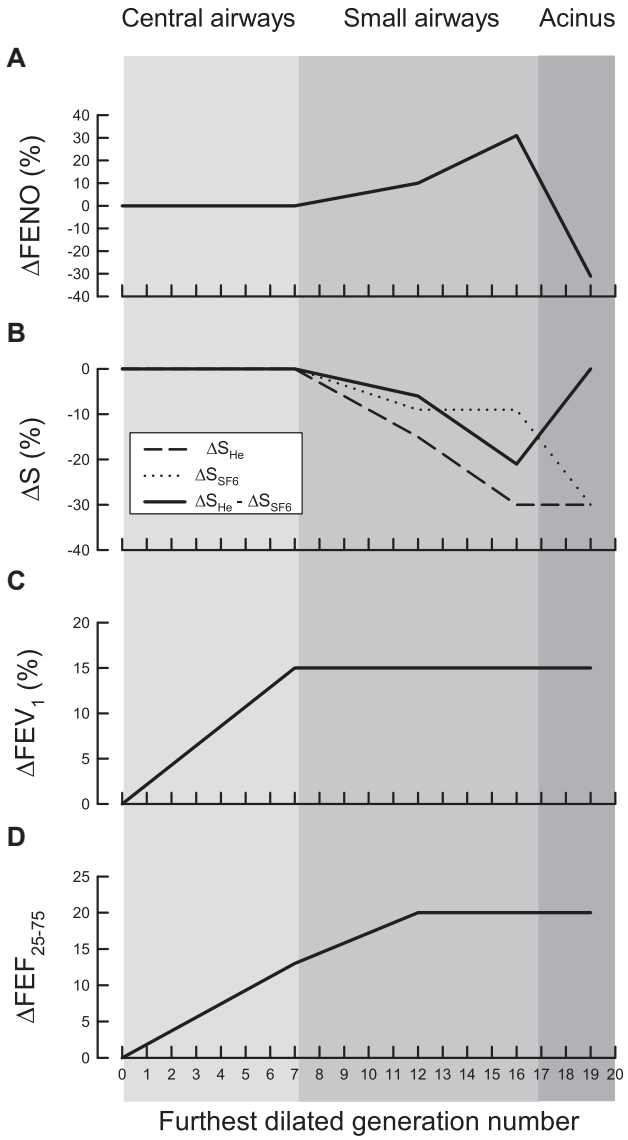

FIG 1. Theoretic model of FENO values, ventilation distribution, and pulmonary function index responses to the relief of a pre-existing airway obstruction up to a given generation (in abscissa). **A,** FENO value. **B,** ΔS_{He}, *dashed line*; ΔS_{SF6}, *dotted line*; and $\Delta S_{He} - \Delta S_{SF6}$, *continuous line*. **C,** ΔFEV_1. **D,** ΔFEF_{25-75}.

FENO (Fig 1, A). FENO response to an airway constriction relief is a mirror of the theoretic findings of Verbanck et al,[9] which simulated constriction up to a given generation. There was very little change when the relief occurred in the central airways, a FENO increase was predicted if obstruction relief progressively involved the small airways, and a FENO decrease was expected if obstruction relief penetrated the acinus.

S_{He} and S_{SF6} (Fig 1, B). Based on the diffusion front theory,[20] slope decreases are assumed to be maximal for S_{He} when dilation relieves obstruction up to generation 16 (onset of the acinus) and for S_{SF6} when dilation relieves obstruction up to generations 18 and 19 (intra-acinar airways). In the latter case no further effect is expected on S_{He}. S_{He} and S_{SF6} changes are nonlinearly related to FENO value changes, whereas their difference mirrors FENO value changes.

FEV_1 and FEF_{25-75} (Fig 1, C and D, respectively). We assume that FEV_1 will not be affected by airway caliber changes beyond the