

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, FEV₁, 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.

Results: No FENO change ($|\Delta\text{FENO}| \leq 10\%$) was found in 16 patients (mean [SD]: 2.5% [5.2%]; ie, FENO= group); a ΔFENO value of greater than 10% was found in 23 patients (31.7% [20.3%]; ie, the FENO+ group); and a ΔFENO 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 2015;■■■■:■■■-■■■.)

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

Current guidelines¹ indicate that levels of nitric oxide (NO) measured in the exhaled air of asthmatic patients mark T_H2-type airway inflammation² 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 stability¹³⁻¹⁵ 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

FEF₂₅₋₇₅: 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
 SF₆: Sulfur hexafluoride

sulfur hexafluoride (SF₆), 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 (FEV₁/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 μ g 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 FEV₁ and forced expiratory flow between 25% and 75% of forced vital capacity (FEF₂₅₋₇₅) 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% SF₆ in oxygen. During expiration to residual volume, He and SF₆ concentrations were recorded as a function of expired volume by using a quadrupole mass spectrometer (LR6000; Logan Research LTD, Rochester, United Kingdom), and the slopes of phase III for each gas (S_{He} and S_{SF_6}) 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

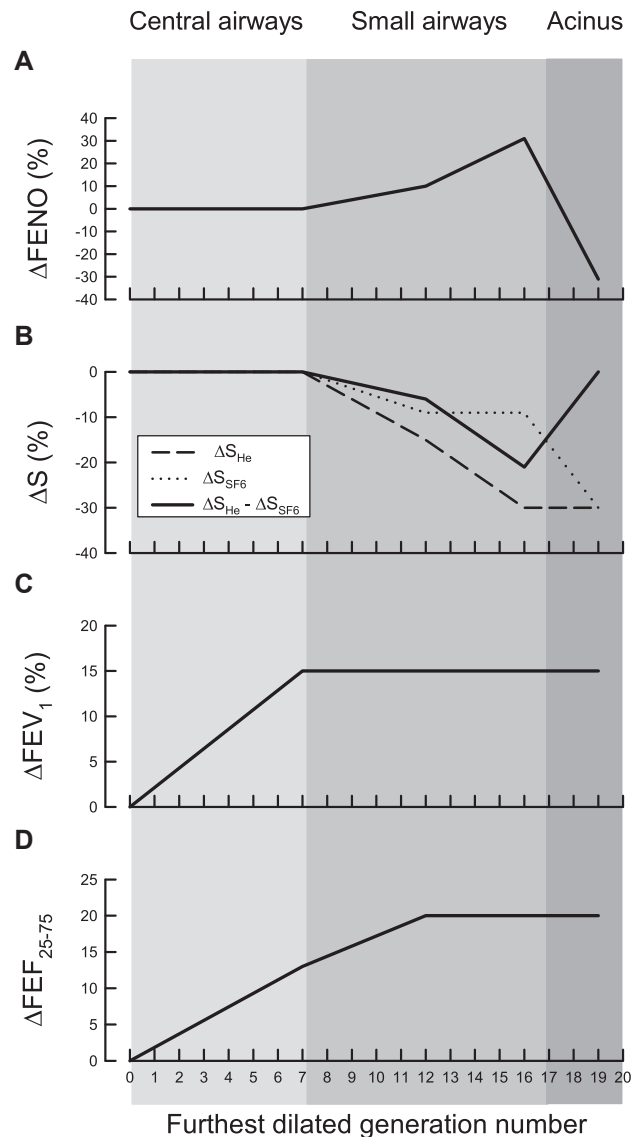


FIG 1. Theoretic model of FENO values, ventilation distribution, and pulmonary function index responses to the relief of a pre-existing airway obstruction up to a given generation (in abscissa). **A**, FENO value. **B**, ΔS_{He} , dashed line; ΔS_{SF_6} , dotted line; and $\Delta S_{He} - \Delta S_{SF_6}$, continuous line. **C**, ΔFEV_1 . **D**, ΔFEF_{25-75} .

FENO, ventilation distribution, and pulmonary function indices resulting in relief of a pre-existing airway obstruction up to a given generation.

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_{SF_6} (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_{SF_6} 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_{SF_6} changes are nonlinearly related to FENO value changes, whereas their difference mirrors FENO value changes.

FEV₁ and FEF₂₅₋₇₅ (Fig 1, C and D, respectively). We assume that FEV₁ will not be affected by airway caliber changes beyond the

central airways and FEF_{25-75} will be sensitive to changes up to small airways. Experiments using heliox instead of air support these assumptions, showing that the forced expiration curve is sensitive to central resistance and much less so to peripheral obstruction.²¹ Moreover, a magnetic resonance imaging study²² using hyperpolarized ^3He detected regional ventilation impairment after a methacholine challenge, most likely acting in the small airways area,¹² without effecting change in the forced flow-volume curve.

With the above assumptions, differences in slope changes (Fig 1, B, open circles) are theoretically expected to negatively correlate with or mirror FENO value changes, and FENO value changes are expected to be independent of FEV_1 and FEF_{25-75} changes.

Statistical methods

Baseline values. FENO values were log-transformed to achieve normal distribution and incorporate parametric statistics. A FENO value change beyond the well-known variability linked to FENO measurement methodology (ie, $\pm 10\%$ compared with baseline) was considered significant.¹ As a result, 3 groups were defined based on FENO behavior after bronchodilation: one group of patients exhibiting no significant FENO change ($|\Delta\text{FENO}| \leq 10\%$ of baseline; ie, group FENO=), a second group who exhibited a significant FENO value increase ($\Delta\text{FENO} > 10\%$ of baseline; ie, group FENO+), and a third group exhibiting a significant FENO value decrease ($\Delta\text{FENO} < -10\%$ of baseline; ie, group FENO-).

ANOVA was performed for baseline values of FENO, FEV_1 , FEF_{25-75} , S_{He} , and S_{SF6} to assess differences between the groups.

FENO and lung function changes. Mixed linear model analyses were performed to assess bronchodilation effect in each group. We considered the group identifier (FENO=, FENO+, and FENO-), the prebronchodilation/postbronchodilation status, and the interaction between these 2 elements as fixed effects. We considered the prebronchodilation value as a random effect (random intercept model).

Sensitivity model. To challenge the sensitivity model presented in Fig 1, a multiple linear regression was performed with ΔFENO as the dependent variable and $\Delta S_{\text{He}} - \Delta S_{\text{SF6}}$, ΔFEV_1 , and ΔFEF_{25-75} as independent variables.

Reproducibility of FENO behavior. Reproducibility of FENO behavior after bronchodilation was assessed in a subset of patients in the lung function laboratory department on 2 occasions by computing the intra-class correlation coefficient for ΔFENO and ΔFEV_1 (percentage of baseline) and by assessing how many patients remained in the group in which they were placed during their first evaluation (FENO=, FENO+, or FENO-).

Effect of forced maneuvers on FENO values. The effect of the forced maneuver on FENO values was assessed in a subset of patients. FENO values were measured at baseline and 15 to 20 minutes after a forced expiratory maneuver without bronchodilation.

We used R software²³ and defined statistical significance as a 2-tailed P value of less than .05.

RESULTS

We recruited a total of 68 asthmatic patients, including 10 who had received a new diagnosis and were yet untreated. After bronchodilation, 16 (23%) patients had a change in FENO values of less than 10% of baseline (FENO= group), 23 (34%) patients had an increase of greater than 10% (FENO+ group), and 29 (43%) patients had a decrease of greater than 10% (FENO- group). Fig 2 shows FENO value changes in the 3 groups.

Baseline values

Population characteristics, baseline lung function, and FENO values are summarized in Table I. Only FENO values exhibited a global group effect: a *post hoc* analysis (Tukey honest significant difference test) showed that the FENO= group had a significantly greater baseline FENO value than the FENO+ ($P = .049$) and

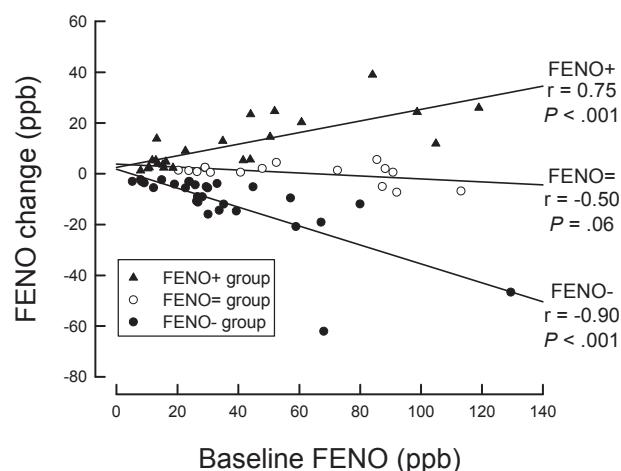


FIG 2. FENO value changes from baseline as a function of baseline values. Solid circles, open circles, and solid triangles indicate the FENO-, FENO=, and FENO+ groups, respectively. Regression lines, correlation coefficients, and their statistical significances are indicated for each group.

FENO- ($P = .03$) groups. FENO values were the same in the FENO+ and FENO- groups ($P > .99$).

FENO value and lung function changes (mean [SD])

Overall and in the FENO= group, FENO values did not significantly change (-2.1% [32.2%], $P = .54$, and 2.5% [5.2%], $P = .09$, respectively), whereas the changes in the FENO+ and FENO- groups were 31.7% (20.3%) ($P < .001$) and -31.5% (17.3%) ($P < .001$), respectively.

Moreover, as shown in Fig 2, the ordinate at the origin of FENO value changes (in parts per billion) as a function of baseline value does not differ from zero for the 3 groups ($P = .53$, $P = .055$, and $P = .27$ for the FENO-, FENO=, and FENO+ groups, respectively). Taken together, this means that FENO value absolute changes were proportional to baseline values in the FENO+ and FENO- groups.

Fig 3 shows the box plots of FEV_1 , FEF_{25-75} , and slope changes. FEV_1 significantly increased in the same manner within each group ($P = .45$), whereas FEF_{25-75} , S_{He} , and S_{SF6} showed contrasting behaviors between the groups: no change was reported in the FENO= group; FEF_{25-75} and S_{He} significantly increased and decreased, respectively, in both the FENO+ and FENO- groups; and S_{SF6} significantly decreased only in the FENO- group. The changes in the FENO+ and FENO- groups significantly differed from those in the FENO= group for FEF_{25-75} ($P = .042$ and $P = .049$, respectively) and S_{He} ($P = .006$ and $P = .04$, respectively). For S_{SF6} , changes in the FENO- group differed from changes in the FENO= ($P = .002$) and FENO+ ($P = .02$) groups.

Sensitivity model

Fig 4 shows the univariate relationship between ΔFENO and $\Delta S_{\text{He}} - \Delta S_{\text{SF6}}$ (Fig 4, A), ΔFEV_1 (Fig 4, B), and ΔFEF_{25-75} (Fig 4, C). Only $\Delta S_{\text{He}} - \Delta S_{\text{SF6}}$ is correlated with ΔFENO changes. This was also confirmed by means of a multiple regression analysis including $\Delta S_{\text{He}} - \Delta S_{\text{SF6}}$, ΔFEV_1 , and ΔFEF_{25-75} as independent variables.

TABLE I. Patients' characteristics, baseline lung function, and FENO values

	Total (n = 68)	FENO= group (n = 16)	FENO+ group (n = 23)	FENO- group (n = 29)	P value
Age (y)	51 (16)	45 (15)	53 (17)	51 (16)	.30
Sex ratio (F/M)	35/33	10/6	10/13	15/14	.50
ICS dose	650 (0-2000)	500 (0-2000)	800 (0-2000)	500 (0-2000)	.50
ACQ score	1.7 (0-5.2)	2.3 (0.3-4.8)	1.7 (0.3-5.2)	1.8 (0-5.2)	.45
FENO (ppb)	31.8 (14.6-69.0)	50 (27.9-89.6)	27.9 (11.9-65.0)	27.4 (13.2-57.2)	.03
FEV ₁ (% predicted)	66 (16)	68 (15)	66 (14)	66 (18)	.79
FEV ₁ /FVC ratio (%)	61 (11)	65 (6)	61 (6)	60 (14)	.19
FEF ₂₅₋₇₅ (% predicted)	38 (18)	40 (22)	39 (16)	37 (19)	.91
S _{He} (%L ⁻¹)	5.3 (3.9)	4.2 (2.7)	4.7 (2.7)	6.4 (5.0)	.14
S _{SF6} (%L ⁻¹)	6.2 (4.1)	5.2 (2.9)	5.4 (3.5)	7.5 (4.8)	.09

Data are presented as mean (SD), except for ICS dose (median [range]), ACQ score (median [range]), and FENO value (geometric mean [geometric interval]); geometric mean is antilog[mean]; geometric interval is defined as follows: Antilog[mean - SD] - antilog[mean + SD]. ICS dose is expressed in μg beclomethasone equivalent.day⁻¹. The *P* value is the statistical significance of group effect in an ANOVA analysis, except for sex ratio (χ^2 test), ICS dose, and ACQ score (Kruskal-Wallis test).

ACQ, Asthma Control Questionnaire; F, female; FVC, forced vital capacity; ICS, inhaled corticosteroid; M, male.

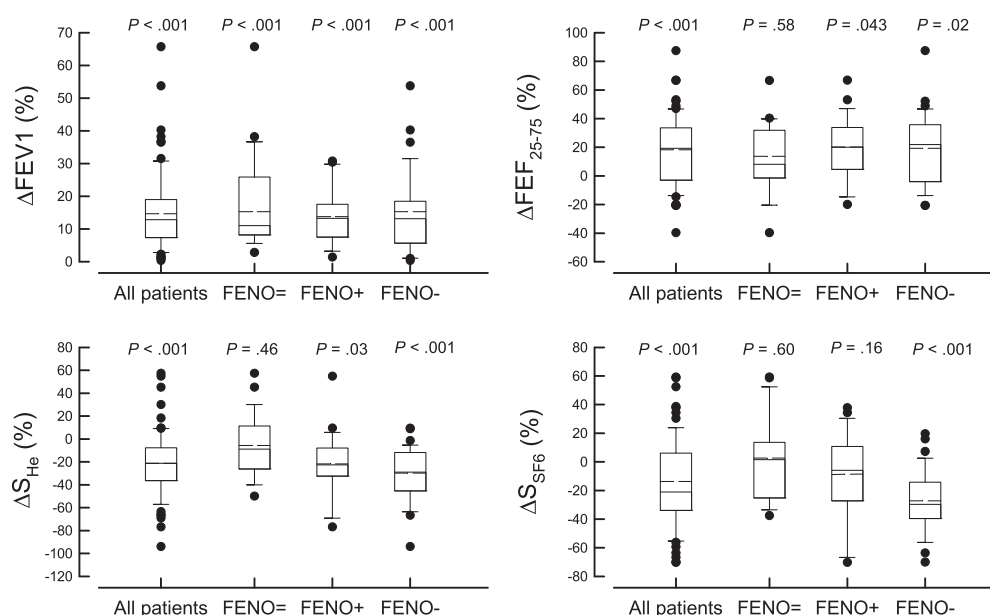


FIG 3. For each panel, changes in percentages of baseline values are represented for all patients and for the FENO=, FENO+, and FENO- groups. Boxes correspond to the interquartile interval. The solid and dashed horizontal lines correspond to the median and mean, respectively. Error bars indicate the 10th to 90th percentile intervals. Solid circles are outliers. Significance of changes from baseline is also indicated.

Reproducibility of FENO behavior

Eighteen patients attended the lung function laboratory department on 2 occasions with a median time interval of 389 days. Their baseline features did not differ from those of the remaining 50 patients in terms of sex ratio (*P* > .99), age (*P* = .41), Asthma Control Questionnaire score (*P* = .65), FENO value (*P* = .18), FEV₁ (*P* = .36), FEF₂₅₋₇₅ (*P* = .13), S_{He} (*P* = .23), and S_{SF6} (*P* = .23). On first evaluation, 10 patients were in the FENO= group, 6 were in the FENO+ group, and 2 were in the FENO- group. The intraclass correlation coefficient was equal to 0.75 for ΔFENO and 0.73 for ΔFEV₁ (percentage of baseline), which might be considered as a reflection of strong reproducibility.²⁴ Fifteen (83%) patients had similar FENO behaviors or were in the same groups during the second evaluation.

Effect of forced maneuvers on FENO values

FENO values were measured in 12 patients before and 15 to 20 minutes after a forced expiratory maneuver without bronchodilation. ΔFENO was -0.2% (7.2%) of baseline value, which is not significantly different from zero (*P* = .77). An *F* test showed that the variance of FENO value changes in this subgroup did not differ from the variance in the FENO= group (*P* = .18).

DISCUSSION

This study documents 3 different patterns of FENO response to β_2 -agonist action in patients with uncontrolled asthma, and the responses most likely depend on the site of pre-existing airway obstruction. A large proportion of subjects exhibit a FENO value decrease that seems related to the relief of airway obstruction

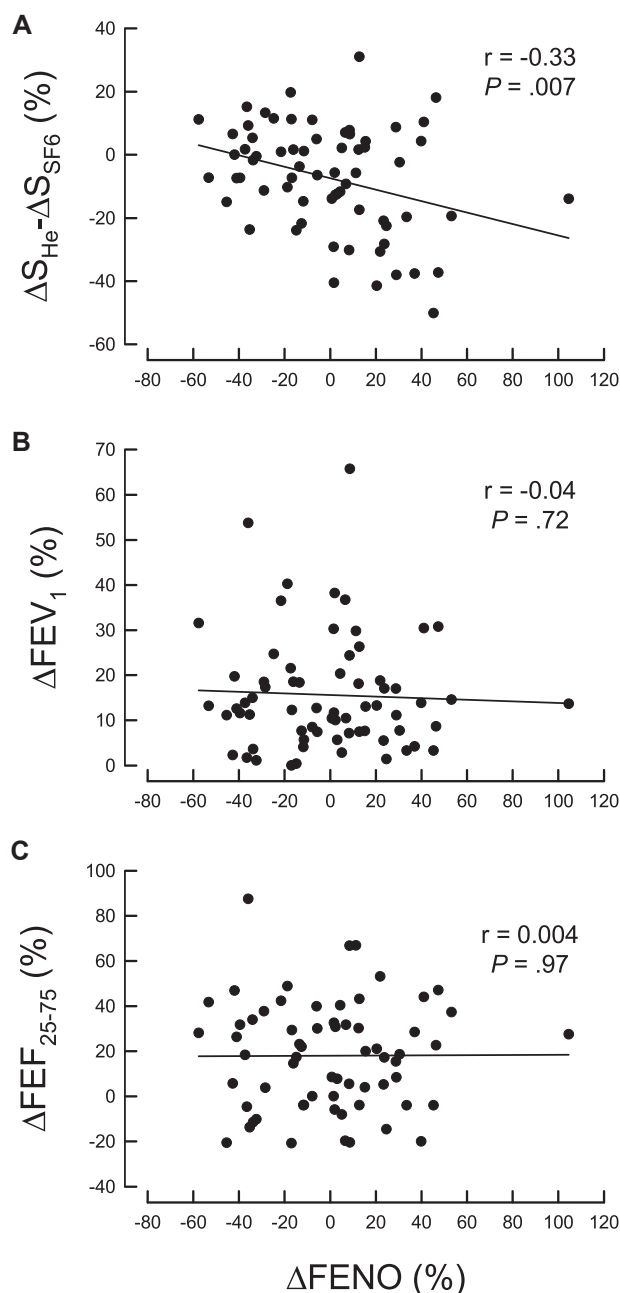


FIG 4. Observed relationships between $\Delta FENO$ (in abscissa) and $\Delta S_{He} - \Delta S_{SF_6}$ (A), ΔFEV_1 (B), and ΔFEF_{25-75} (C). Correlation coefficients and statistical significance are indicated.

up to the lung periphery (ie, from the central up to the intra-acinar airways), as assessed by changes occurring in ventilation distribution indices. In contrast, a FENO value increase is observed when bronchodilation appears to involve more proximal airways (ie, from the central airways up to terminal bronchioles), and an absence of change in FENO values seems to be associated with dilation of the central airways only.

Some previous studies have indicated the absence of a deep effect of β_2 -agonist inhalation on FENO values, with either a FENO value stability¹³⁻¹⁵ or a modest FENO value increase¹⁶ associated with an FEV₁ increase. Our study partially confirms these results

by indicating no significant bronchodilation effect on FENO values in the entire group of patients. However, this global result actually masks 3 different patterns of response to bronchodilation. One group of patients exhibited FENO value stability in spite of a FEV₁ increase. A second group displayed a FENO value increase expected to occur when FEV₁ increases. Finally, in a significant proportion of patients, a paradoxical and concomitant FENO value decrease was observed with an FEV₁ increase.

The results of our ventilation distribution tests indicate that the 3 groups are likely to differ by the site of obstruction relief induced by β_2 -mimetics. They did so in a manner predicted by a model simulation regarding airway constriction.⁹

Studying ventilation distribution with inert gases might provide some insight into the location of airway alterations. When a nonresident inert gas is inhaled and exhaled, after exhalation of dead space volume, the expired concentration reaches a plateau the slope of which reflects the ventilation heterogeneities. Magnetic resonance imaging with noble gases showed that small lung regions were involved in these ventilation heterogeneities.²² These experimental results were anticipated by theoretic considerations²⁰ and gas transport modeling,²⁵ along with an anatomic knowledge of bronchial tree dimensions.^{26,27} These indicated that the slope of the alveolar plateau is particularly sensitive to changes in regional clearance because of uneven airway caliber changes. These occur where diffusion transport balances convection transport, sometimes called a “diffusion front.” Given that He and SF₆ differ only in terms of their diffusive properties, their respective diffusion fronts have different locations, the terminal bronchioles for He and the respiratory bronchioles and alveolar ducts for SF₆,²⁰ as documented in well-defined pathologic conditions, such as chronic rejection after lung transplantation or chronic obstructive pulmonary disease.²⁸⁻³¹ These slope sensitivities are illustrated in Fig 1, B. In the present study the relief of ventilation heterogeneities caused by regional clearance enhancement, as induced by the bronchodilatory action of β_2 -mimetics, resulted in different patterns of slope decreases. The absence of a significant decrease of both the He and SF₆ slopes in the group of patients exhibiting no FENO value change after bronchodilation suggests a central obstruction relief that the FRC+I| single-breath washout test did not detect, with He and SF₆ slopes essentially sensitive to peripheral changes.^{32,33} The decrease of the He slope with no change in the SF₆ slope in the group exhibiting a FENO value increase suggests an obstruction relief up to the terminal bronchioles. The similar decrease of both slopes in the group of patients exhibiting FENO value reduction supports the notion of an obstruction relief occurring in the region extending to the very peripheral airways, thus involving both the terminal bronchioles and acinar airways. This can amplify the NO back-diffusion process,^{34,35} resulting in a FENO value decrease associated with an increase in airway caliber, as mentioned earlier. Together, our results appear to identify 3 groups of asthmatic patients related to 3 different extents of airway obstruction process: one group with a more central airway obstruction, another with airway obstruction up to the onset of the acinus, and a third group with an obstruction process also involving acinar airways. In fact, although a β_2 -agonist usually penetrates deep into the bronchial tree in all subjects, its action might be considered significant only where airway obstruction takes place.²¹ The above interpretation relies on the model in Fig 1 that allowed for the prediction of a linear relationship of $\Delta FENO$ with $\Delta S_{He} - \Delta S_{SF_6}$ across the

entire group and no relation with ΔFEV_1 and ΔFEF_{25-75} . It must be stressed that these behaviors were experimentally observed.

An alternative nonexclusive mechanism must be mentioned: Fig 1, A, assumes a homogeneous baseline constriction and inflammation status in a given region, which is an oversimplification. Intraregional constriction heterogeneities were evidenced²² and were probably associated with heterogeneous inflammatory status. This can also lead to divergent FENO behaviors depending on whether dilation acts on open or constricted airways. However, according to Suresh et al,³⁶ the sensitivity of FENO values to ventilation heterogeneities is unlikely to account for the changes observed in the present study.

It should be noted that most patients tested again approximately 1 year later under similar airway caliber conditions, and asthma control levels showed the same pattern of FENO response after acute bronchodilation. This suggests a rather permanent process, potentially identified as a “phenotype” linked to differences in depths of obstruction sites in patients with uncontrolled asthma. An alternative hypothesis, a difference in β_2 -receptor distribution along the airways, cannot be excluded but is less likely because such a difference was not reported in a study with ferrets that investigated receptors distribution on airway smooth muscle.³⁷

It is also unlikely that the FENO evolutions observed after acute bronchodilation were significantly influenced by the 10% FENO value reduction sometimes,^{16,38,39} but not always,¹⁵ reported when forced maneuvers are repeated during spirometry. In fact, although a slight shift cannot be ruled out, it should be noted that the FENO value reduction reported in the only study that included asthmatic patients using current methodology to measure FENO values was shown to occur no longer than 15 minutes after spirometry,¹⁶ which was also approximately the point at which our study measured postbronchodilator FENO values. Furthermore, to tease out the effects of bronchodilation from those of spirometry, we performed forced maneuvers without bronchodilation in a subset of patients and observed no significant FENO value changes after 15 to 20 minutes.

A limitation of this study might be the potentially arbitrary threshold ($\pm 10\%$ change) chosen to define the 3 groups because it relies on normal subject variability.¹ However, in the subset of asthmatic patients tested without bronchodilation (and with no significant FENO value changes), a similar variance in FENO value change was observed. Another potential limitation might be the study's open design. However, considering that we evaluated the immediate effect of β_2 -agonist inhalation on objective indices, a placebo effect appeared less likely to interfere with the results.

Finally, the potential effects of the present data on clinical practice must be emphasized. FENO values are usually regarded as a marker of airway inflammation, and their clinical role in asthma management is based on monitoring inflammation. However, as mentioned above, FENO values are known to be significantly affected by FEV_1 reduction,⁴⁻⁶ which might hamper their ability to reflect airway inflammation, as recently demonstrated in an experiment based on allergen challenge.⁷ In the present study an acute increase in FEV_1 is shown to affect FENO values in divergent ways according to the dilation site, emphasizing the complex interaction between FENO and FEV_1 . Therefore FENO should no longer be considered solely a marker of airway inflammation but rather as a marker that integrates both airway inflammation and lung function changes. In addition, our study also suggests that the use of short-acting β_2 -agonists should be avoided before

FENO value measurement because they might induce a FENO value increase or decrease of greater than 30%, a magnitude of change previously shown to demonstrate better or poorer asthma control.⁴⁰ Finally, in the present study we suggest a procedure to assess the airway obstruction site: measuring FENO values before and after bronchodilation.

In conclusion, 3 different patterns of FENO value change were observed in response to bronchodilation, allowing identification of 3 groups of asthmatic patients according to the depth of the obstruction processes within their airways. As a result, a new role for FENO measurement in identifying the site of airway obstruction in asthmatic patients is proposed.

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Clinical implications: FENO response patterns to β_2 -agonists could be used to identify airway obstruction sites in patients with uncontrolled asthma.

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