Effect of airways constriction on exhaled nitric oxide

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Submitted 25 September 2007; accepted in final form 11 January 2008

EXHALED NITRIC OXIDE (eNO) has the potential to be a valuable marker of inflammation at different lung depths, making it attractive as a noninvasive monitoring tool for steroid–dependent asthma patients. eNO procedures have been standardized so as to minimize variability in eNO owing to breathing maneuver, inhaled NO level, or mouth pressure, and possible effects from alimentation or lung function testing before eNO testing have been identified (1). The potential effect of bronchodilation or bronchoconstriction on eNO, in the absence of any change in actual inflammation-related NO production, has also been investigated (3, 6, 8). For instance, de Gouw et al. (3) observed decreased eNO levels in asthma patients after acute provocation with either histamine, hypertonic saline, or AMP, and not with isotonic saline, prompting the authors to suggest that airway caliber should be taken into account when interpreting exhaled NO in lung disease.

histamine challenge; small conductive airways

could influence eNO-derived values, which would then be unduly attributed to a change in inflammation.

One mechanism that is likely to play a pivotal role in the effect of airway constriction or dilation on eNO is backdiffusion of NO from the bronchial airways toward the alveolar zone. Modeling-based experimental studies from various groups (11, 17) have demonstrated the existence of an intrapulmonary gradient between the higher airway NO concentration in the conductive airways and the lower alveolar NO concentration in the lung periphery, persisting throughout expiration. As a consequence, bronchial NO as inferred from two-compartment models (7) [e.g., using the slope of eNO vs. the reciprocal of flow (10)] is typically underestimated by 1.7- to 2.5-fold (2, 17). Indeed, when NO backdiffusion drags NO into the alveolar space, less NO is available for expiration than that predicted by a two-compartment model (which neglects any NO transfer between the so-called airways and alveolar compartment). While this effect of NO backdiffusion on the estimate of bronchial NO can be intuitively grasped (and corrected for by a multiplicative factor of 1.7–2.5), the effect of airway caliber on eNO in the presence of NO backdiffusion is less straightforward. Also, the predicted effect of airway constriction on eNO will depend on whether the NO produced in the airway wall that is available to the airway lumen is assumed to vary with airway epithelial surface area (leading to a NO flux decrease) or whether it is considered independent of airway constriction (corresponding to a constant NO flux). Hence, a proper understanding of the impact of airway caliber on eNO requires experiment-driven model simulations.

We tested the hypothesis that constriction of airways at different lung depths could have very different effects on eNO. We first investigated the effect of bronchoconstriction on eNO in a well-defined experimental situation where only conductive airways are known to be involved, namely during histamine provocation in normal nonhyperresponsive subjects (21). Since histamine not only induces an overall constriction of conductive airways, but also a parallel heterogeneity in constriction, the effect of the resulting ventilation heterogeneity on eNO can also be assessed. Experimental eNO data are compared to simulations with a trumpet model of the lung that incorporates NO backdiffusion (17) to verify which pattern of proximal vs. peripheral airway constriction is compatible with the eNO changes observed after histamine provocation. Of more general interest, the eNO simulations will serve to identify the airways that, when constricted or dilated, crucially interfere with eNO measurement in lung disease.
EXHALED NO AND AIRWAY CONstriction

MATERIALS AND METHODS

Experimental methods. The protocol was approved by the local ethics committee (B14320071296), and informed consent was obtained from all participating subjects. An identical set of measurements was performed at baseline and immediately following a cumulative dose of 2 mg histamine inhalation by 20 healthy never-smoker subjects who did not show a forced expired volume in 1 s (FEV1) decrease of >20% after 2 mg histamine. One set of measurements included, in this sequence, eNO, spirometry, multiple-breath washout, and resistance measurements. Five of the 20 subjects repeated the entire procedure on a different day, whereby isotonic saline was substituted for histamine.

The eNO measurement started with a vital capacity inhalation of NO-free air (filter type A1B2E2K1HgCONO-P3, Draeger, Luebeck, Germany) followed by exhalation via a restrictor with the subject maintaining a mouth pressure of 15 cmH2O so as to obtain eNO for 50 ml/s. Exhaled NO concentration was continuously recorded (CLD60, EcoPhysics) such that a NO plateau value (\(\text{FeNO}_{50}\)) could be readily identified. Lung function was performed using standard equipment (VmaxEncore, SensorMedics). Instrumentation and theory underlying multiple-breath washout tests of ventilation heterogeneity have been extensively reported elsewhere (5, 21). From the multiple-breath N2 tracings, N2 phase III slopes were computed in subsequent expirations, from which indexes Scond and Sacin can be derived to represent the conductive and acinar components of ventilation heterogeneity, respectively. Since Scond and Sacin derive from phase III slopes, their value increases when ventilation heterogeneity increases; normal Scond and Sacin values are below 0.042 liter−1 and 0.12 liter−1, respectively (5, 20). Sacin will increase if ventilation heterogeneity is increased in the acinar lung zone, due to an alteration of the intra-acinar asymmetry. Scond will increase when heterogeneous narrowing of conductive airways induces an alteration in the specific ventilation and/or ventilatory flow asynchrony between the lung units subtended by these airways proximal to the terminal bronchioles.

Simulations. Intrapulmonary NO transport and the resulting \(\text{FeNO}_{50}\) were simulated with a validated 23-generation trumpet model of the lung (17), using previously established values for airways NO transfer factor (5.07 pl·s−1·ppb−1) and for alveolar NO flux and transfer factor (3.167 pl/s and 1,558 pl·s−1·ppb−1) (10); airways NO flux was set to 3,083 pl/s. In this model, airway NO production was transferred to total airway epithelial surface area in each airway generation. Simulations of \(\text{FeNO}_{50}\) were performed in the absence of airway constriction and in the case of 30% constriction, which is defined as a 30% reduction in airway diameter (50% reduction in lumen cross section). Airway constriction (in all lung generations except 22 and 23) was simulated using two alternative hypotheses: 1) that NO flux decreases in proportion to constriction (i.e., a 30% reduction in epithelial surface that is in contact with the airway lumen), and 2) that NO flux is independent of constriction.

We also investigated the effects of decreased NO production in the absence of airway constriction, of a greater degree of constriction, and of ventilation heterogeneity. The latter was done by incorporating two parallel model subunits with lung volume ratio of 0.72 and corresponding inspired volume ratio of 1.69, taken from a previous study of ventilation distribution in the human lung (19). Finally, we challenged two model assumptions (homogeneity of NO production and proportionality to epithelial surface area) and assessed their effect on \(\text{FeNO}_{50}\) sensitivity to airway constriction.

Statistical analysis. Using Statistica 5.1 (StatSoft, Tulsa, OK), Wilcoxon matched pairs tests and Spearman rank correlations were performed.

RESULTS

Experimental data. The measures of spirometry, airway resistance, ventilation heterogeneity, and \(\text{FeNO}_{50}\) shown in Table 1 were normal at baseline in all 20 subjects (14 women/7 men). In particular, \(\text{FeNO}_{50}\) was within the range of values for healthy volunteers reported in the literature (9, 12, 15, 16); in the most recent of these studies, the reference range was 8–41 ppb with a geometric mean of 18 ppb (15). After histamine provocation, significant changes were observed in FEV1, forced expiratory flow (FEF) after exhalation of 75% forced vital capacity (FEF75), mean forced midexpiratory flow between 25% and 75% forced vital capacity (FEF25–75), specific airway conductance (\(s\text{Gaw}\)), Scond, and \(\text{FeNO}_{50}\) but not in Sacin or functional residual capacity (FRC). When correlating the percentage changes in any of the spirometric or ventilation distribution parameters in Table 1 to the corresponding percentage changes in \(\text{FeNO}_{50}\) (\(\Delta \text{FeNO}_{50}\)), the only significant correlations were between \(\Delta \text{FeNO}_{50}\) and \(\Delta \text{FEF75} (R = 0.48; P = 0.032; \text{Fig. 1A})\) and between \(\Delta \text{FeNO}_{50}\) and \(\Delta \text{FEF}_{25–75} (R = 0.59; P = 0.006). The absence of correlation between \(\Delta \text{FeNO}_{50}\) and ventilation heterogeneity, quantified by \(\Delta \text{Scond}\), is illustrated in Fig. 1B (P = 0.063). It can be noted from Fig. 1 that \(\text{FeNO}_{50}\) decreased in all subjects but one.

Also shown in the open symbols of Fig. 1, A and B, is the variability in \(\Delta \text{FeNO}_{50}\) response to the experimental procedure when saline is inhaled instead of histamine, and the absence of any such response for \(\Delta \text{FEF75}\) or \(\Delta \text{Scond}\). One subject showed no \(\text{FeNO}_{50}\) response to saline (−2% post-saline vs. −36% post-histamine; big squares), three subjects showed a \(\text{FeNO}_{50}\) response to saline that was intermediate to that of histamine (−13%, −15%, −20% post-saline, respectively, vs. −59%, −25%, −35% post-histamine; triangles, diamonds, small squares), and one subject showed a similar response to saline and to histamine (−35% post-saline vs. −36% post-histamine; small circles). On average, in these five subjects, the testing procedure with histamine and saline, respectively, elicited a 38% and a 17% decrease in \(\text{FeNO}_{50}\) implying that approxi-

Table 1. Baseline and post-histamine spirometry, ventilation distribution, and exhaled NO

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Histamine</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Lung function</td>
<td></td>
<td></td>
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<tr>
<td>FEV1, %predicted</td>
<td>104±13</td>
<td>95±15</td>
<td>&lt;0.001</td>
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<tr>
<td>FEF25, %predicted</td>
<td>106±27</td>
<td>85±28</td>
<td>&lt;0.001</td>
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<tr>
<td>FEF25–75, %predicted</td>
<td>98±22</td>
<td>80±24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sGaw, cmH2O l−1 s−1</td>
<td>0.086±0.014</td>
<td>0.079±0.014</td>
<td>0.002</td>
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<tr>
<td>Ventilation distribution</td>
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<tr>
<td>FRC, ml</td>
<td>3001±919</td>
<td>2940±979</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Sacin, l−1</td>
<td>0.065±0.018</td>
<td>0.066±0.018</td>
<td>&gt;0.1</td>
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<tr>
<td>Scond, l−1</td>
<td>0.030±0.008</td>
<td>0.053±0.024</td>
<td>&lt;0.001</td>
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<tr>
<td>Exhaled NO</td>
<td></td>
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<tr>
<td>(\text{FeNO}_{50}), ppb</td>
<td>15.8±8.0</td>
<td>12.2±7.5</td>
<td>&lt;0.001</td>
</tr>
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</table>

Values are means ± SD. FEV1, forced expired volume in 1 s; FEF75, forced expiratory flow after expiration of 75% forced vital capacity; FEF25–75, mean forced midexpiratory flow between 25% and 75% forced vital capacity; sGaw: specific airway conductance; FRC: functional residual capacity measured by multiple-breath washout; Sacin and Scond, multiple-breath washout-derived index of acinar and conductive ventilation heterogeneity, respectively (see text); \(\text{FeNO}_{50}\), exhaled nitric oxide at 50 ml/s; ppb, parts per billion. P values indicate significant differences in a Wilcoxon matched pair test between baseline and post-histamine values.
mately one-half of the histamine-induced FeNO,50 decrease was due to our particular test procedure, which did not induce any significant changes in FEV₁ (−0.1%), FEF75 (+2.7%), or Scnd (−5.5%) (P > 0.1 for all).

Model simulations. The baseline FeNO,50 simulated with the trumpet model (in the absence of any constriction) amounted to 17 ppb, which compares well to our experimental value [15.8 ± 8.0(SD) ppb; Table 1]. Figure 2 shows the NO concentration profiles inside the model at the end of expiration, from which it can be inferred that FeNO,50 (corresponding to NO concentration in generation 0) is decreased when a 30% constriction is imposed on generation 13, and increased when a 30% constriction is imposed on generation 19. Both of these simulations of airway constriction in Fig. 2 were obtained by considering that NO flux decreases in proportion to the airway constriction, and they show that the location of maximum NO concentration gradient inside the model is virtually unaffected by airway constriction.

Figure 3 illustrates the effect of cumulatively constricting airways in successive lung generations on simulated FeNO,50, by either considering NO flux to reduce in proportion to a 30% airway constriction, or not. When NO flux is decreased by 30% (case a), there is a negligible effect on FeNO,50 of airways constriction up to generation 9, a FeNO,50 decrease induced by constriction of airways between generations 10 and 15, a FeNO,50 increase due to airways constriction between generations 16 and 20, and almost no effect on FeNO,50 beyond generation 20. When NO flux remains independent of constriction (case b), there is a negligible effect on FeNO,50 of airways constriction up to generation 14, a marked FeNO,50 increase due to airways constriction between generations 15 and 20, and virtually no effect on FeNO,50 beyond generation 20. Thus, when considering airway constriction in the absence of a concomitant reduction in NO flux, its effect on FeNO,50 is consistently positive, as opposed to our experimental observation of a decreased FeNO,50 after histamine provocation (Table 1; Fig. 1). Finally, case c in Fig. 3 illustrates the effect of a simple 30% reduction in NO production, in the absence of any airway constriction, showing a negligible effect on FeNO,50 when NO production is reduced in airways proximal to generation 10 or peripheral to generation 20.

When simulating a 50% constriction (with an associated 50% reduction in NO flux), a maximum cumulative FeNO,50 decrease is also observed in generations 14 and 15 (as in case a of Fig. 3), yet its absolute effect on FeNO,50 is almost doubled (−16% for 50% constriction vs. −8% for 30% constriction). Beyond generation 19, the maximum cumulative effect on FeNO,50 amounts to +172% for a 50% constriction (vs. +65% for a 30% constriction). The model was also used to simulate the effect on FeNO,50 from induced conductive...
ventilation heterogeneity (which can potentially arise between any 2 lung units subtending from the 1st to the 15th branching generation). With respect to a homogeneously ventilated lung model, ventilation heterogeneity generated between lung units subtending from branch points in subsequent airway generations led to a FENO,50 change ranging from H/1 to H/100% (generation 1) to H/100% (generation 15).

Finally, Fig. 4 shows the effect of modifying two model assumptions (related to the distribution of NO production over the lungs) on the magnitude of changes observed for case a. First, the NO production available to the airway lumen was assumed to vary with airway lumen volume rather than with airway epithelial surface area, while keeping in situ NO production homogeneously distributed over the lungs (closed squares); in this case, overall airways NO flux was set to 2,245 pl/s. Second, the distribution of in situ NO production was modified from a homogeneous one to one that attributes 20% of total NO production to generations 0–2, while the remaining 80% was spread homogeneously over the 21 more peripheral airway generations; in this case, both the distribution of the 20% NO production over generations 0–2 and the distribution of the 80% NO production over generations 3–23 were done according to epithelial surface area (closed triangles); overall airways NO flux was set to 1,850 pl/s. Both alternative simulations showed a marked effect of airway constriction on FENO,50 up to generation 2, an effect that was not observed with the reference simulation (dotted line). Beyond generation 2, there was a gradual increase in constrictive effect that got amplified beyond generation 10, similar to what was observed in the reference simulation. Beyond generation 15, both alternative simulations predicted FENO,50 increases that were attenuated with respect to the reference simulation.

**DISCUSSION**

We have identified the location of airways that, either by airway constriction or by a local reduction in NO production, or a combination of both, induce distinct FENO,50 changes. In particular, we found that in normal subjects the experimental FENO,50 decrease in response to histamine provocation is due to...
airway constriction, with an associated reduction of NO flux, in the small conductive airways ranging from generations 10 to 15 (Fig. 3). While larger airways were also affected by histamine challenge (as seen from FEV$_1$ and sGaw changes), model simulations clearly indicate that neither constriction nor lower NO production in airways up to generation 9 have any repercussion on FENO$_{50}$. On the other hand, the constriction of airways peripheral to generation 15 after histamine in normal subjects is highly unlikely, since its predicted effect is to increase FENO$_{50}$, in contrast to what is observed experimentally. The model prediction that the small conducting airways are the predominant site of airway constriction inducing a FENO$_{50}$ decrease is also consistent with the experimental correlation between $\Delta$FENO$_{50}$ and changes in end-expiratory flows ($\Delta$FEF$_{75}$ or $\Delta$FEF$_{25-75}$) and with absence of any such correlation between $\Delta$FENO$_{50}$ and for instance $\Delta$FEV$_1$ or $\Delta$sGaw.

A second objective of the present experiments was to study the effect of ventilation heterogeneity on FENO$_{50}$, since ventilation heterogeneity is known to be elicited by histamine provocation in normal subjects, but is also recognized as a characteristic feature of asthma (5,18). We did not find experimental evidence of a correlation between the change of conductive ventilation heterogeneity ($\Delta$Scond) and $\Delta$FENO$_{50}$ (Fig. 1B). This is consistent with simulations in a homogeneously vs. a heterogeneously ventilated lung model, which did not reveal any meaningful FENO$_{50}$ differences.

Several authors have investigated the confounding effect of experimental procedures that include repeated forced expiration maneuvers on exhaled NO measurement, with somewhat variable outcomes (3, 4, 8, 12, 14). In 10 healthy nonatopic nonhyperresponsive subjects, Deykin et al. (4) observed an average FENO$_{385}$ decrease by 16% within 15 min after two forced expiratory maneuvers. In seven healthy control subjects, Silkoff et al. (14) found a maximal FENO$_{45}$ decrease (ranging from −4% to −25%) 1 min after three forced expiratory maneuvers, with recovery to the initial FENO$_{45}$ value taking ~1 h. In small groups of asthma patients (ranging from 5 to 11 subjects), spirometry-related eNO changes were found to either average 30% but with a huge variability in response (8) or to be negligible (3, 12). In the present study, we observed a variable but consistently negative contribution to FENO$_{50}$ in a subgroup of five normal subjects owing to the experimental procedure itself, which included forced expiration and O$_2$ inhalation (multiple-breath washout test). Whatever the exact magnitude of this confounding effect (which could not be avoided in the present protocol) there is a definite contribution from the mechanical effect of airway constriction on FENO$_{50}$ decrease, as confirmed by the correlation between changes in FENO$_{50}$ and end-expiratory flows in the entire group (Fig. 1A).

There are at least four potential contributors to a modification in FENO$_{50}$ with airway constriction, and their effect may vary with the location of constriction in the airway tree. First, there is a volumetric effect, whereby the NO is simply concentrated in a smaller (constricted airway) volume, tending to increase FENO$_{50}$. Second, at constant expiratory flow, convective velocity is increased with constriction, resulting in a shorter transit time, which tends to reduce FENO$_{50}$. Since convective gas transport is predominant in the first airway generations, the transit time effect will be most marked in the proximal lung. Third, NO backdiffusion (i.e., the NO diffusive flow from proximal to peripheral generations; Fig. 2), which normally decreases NO available for exhalation, gets hampered by a reduced diffusive cross section. Consequently, constriction of peripheral airways situated along the NO gradient will tend to increase FENO$_{50}$. Fourth, the effect of a reduction in NO flux due to an actual in situ decrease in NO production (e.g., by a neural pathway-mediated forced expiratory maneuver effect) or because NO available to the airway lumen decreases with constriction, both tend to decrease FENO$_{50}$. This effect is linked to the degree of epithelial surface area in the various airway generations (Fig. 2).

Let us now examine from Fig. 3 the net effect on FENO$_{50}$ from the above factors on constriction of airways at different lung depths. Between generations 0 and 9, transit time and volumetric effects are predominant, and since these effects precisely cancel out, this results in virtually no effect on FENO$_{50}$. Between generations 10 and 15, with considerable epithelial surface, a FENO$_{50}$ decrease can arise due to a reduction in NO flux, either because of an actual in situ decrease in NO production (case c) or because of a decrease in epithelial surface area (case a). From generation 15 onward, the NO backdiffusion effect starts to take over, such that constriction actually leads to an FENO$_{50}$ increase; this effect is most marked in generation 19, where the NO gradient for backdiffusion is maximal. Finally, airway constriction in generations 21–23, with negligible epithelial surface area and situated at the tail of the NO diffusion front, have virtually no impact on FENO$_{50}$.

Several model assumptions crucially interfere with the outcome in terms of FENO$_{50}$, some of which are as yet impossible to verify experimentally. While the assumption concerning the reduction of NO flux with airway constriction is a controversial hypothesis (3, 8), its influence appears to merely pertain to airways in generations 10–15 (Fig. 3). In the case of histamine challenge studied here, the negative $\Delta$FENO$_{50}$ could only be explained if NO flux were assumed to decrease with airway constriction (case a). The exact magnitude of the FENO$_{50}$ decrease in case a potentially depends on the model assumptions regarding the distribution of NO production over the lungs. Silkoff et al. (13) have measured in four normal men that ~50% of the eNO concentration stems from airway generations 0–2. There are at least two ways to modify NO distribution within the model to better mimic this experimental observation: by imposing 20% of total NO production on the first three generations (Fig. 4, solid triangles) and by imposing NO production to be proportional to airway lumen volume (Fig. 4, solid squares). Both interventions affect the relative FENO$_{50}$ response to constriction in various segments of the bronchial tree, but the overall pattern of FENO$_{50}$ change is essentially the same in that the greatest FENO$_{50}$ decrease is found between generations 10 and 15, consistent with the experimental correlation between FENO$_{50}$ and FEF$_{75}$ decrease after histamine. In both the alternative simulations of Fig. 4, an additional effect appears at the level of generations 0–2, yet it is unrealistic to assume that the main mechanical effect of histamine-induced reduction in FENO$_{50}$ would be confined to the trachea or just beyond it.

Besides the identification of airways that affect eNO, the present study also revealed that the index FENO$_{50}$ has a potential to sample airways that are not easily accessible by other noninvasive techniques. Previous experimental studies had indicated that the small conductive airways were at least partially responsible for the observed changes in spirometry and venti-
lation heterogeneity following histamine provocation (21). However, spirometry is known to poorly represent airway caliber of the smallest conductive airways, and inert gas tests suffer an intrinsic inability to differentiate between large and small conductive airways heterogeneity. Indeed, all conductive airways (i.e., airways proximal to the inert gas diffusion front), which spreads out from generation 15 into the lung periphery (Fig. 2) now enables us to actually identify constriction of the smaller conductive airways (in generations 8–15) via their mechanical effect on the parameter $F_{\text{ENO},50}$.

In summary, the interpretation of eNO experiments relies on several assumptions that are difficult to verify directly, and this is where a model-based approach can help. For instance, the model simulations compatible with our experimental observation of a decreased $F_{\text{ENO},50}$ following histamine challenge indicated that in the case of normal subjects, this is potentially due to airways constriction in generations $<15$ with the most likely contribution from airways constriction in generations 10–15. We also showed that a $F_{\text{ENO},50}$ decrease can only be obtained if NO flux is assumed to decrease with airway constriction. Irrespective of whether the latter assumption holds in all lung diseases, airway constriction between generation 15 and 20 is shown to markedly increase $F_{\text{ENO},50}$. Finally, the effect of ventilation heterogeneity on $F_{\text{ENO},50}$ is shown to be negligible.

GRANTS

This study was funded by the Fund for Scientific Research-Flanders and the NO Microgravity Application Project of the European Space Agency.

REFERENCES


