Airway contribution to alveolar nitric oxide in healthy subjects and stable asthma patients

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Submitted 27 September 2007; accepted in final form 16 January 2008

Kerckx Y, Michils A, Van Muylem A. Airway contribution to alveolar nitric oxide in healthy subjects and stable asthma patients. J Appl Physiol 104: 918–924, 2008. First published January 24, 2008; doi:10.1152/japplphysiol.01032.2007.—Alveolar nitric oxide (NO) concentration (FANO), increasingly considered in asthma, is currently interpreted as a reflection of NO production in the alveoli. Recent modeling studies showed that axial molecular diffusion brings NO molecules from the airways back into the alveolar compartment during exhalation (backdiffusion) and contributes to FANO. Our objectives in this study were 1) to simulate the impact of backdiffusion on FANO and to estimate the alveolar concentration actually due to in situ production (FANO,prod); and 2) to determine actual alveolar production in stable asthma patients with a broad range of NO bronchial productions. A model incorporating convection and diffusion transport and NO sources was used to simulate FANO and exhaled NO concentration at 50 ml/s expired flow (FENO) for a range of alveolar and bronchial NO productions. FANO and FENO were measured in 10 healthy subjects (8 men; age 38 ± 14 yr) and in 21 asthma patients with stable asthma [16 men; age 33 ± 13 yr; forced expiratory volume during 1 s (FEV1) = 98.0 ± 11.9%predicted]. The Asthma Control Questionnaire (Juniper EF, Buist AS, Cox FM, Ferrie PJ, King DR. Chest 115: 1265–1270, 1999) assessed asthma control. Simulations predict that, because of backdiffusion, FANO and FENO are linearly related. Experimental results confirm this relationship. FANO,prod may be derived by FANO,prod = (FANO – 0.08·FENO)/0.92 (Eq. 1). Based on Eq. 1, FANO,prod is similar in asthma patients and in healthy subjects. In conclusion, the backdiffusion mechanism is an important determinant of NO alveolar concentration. In stable and unobstructed asthma patients, even with increased bronchial NO production, alveolar production is normal when appropriately corrected for backdiffusion.

Alveolar nitric oxide, as measured from an exhaled nitric oxide (eNO) tracing, is increasingly being used as a noninvasive measure of distal inflammation (2, 3, 7, 8, 16, 24), particularly in asthma. Alveolar nitric oxide is usually extracted from NO traces on the basis of two-compartment models that implicitly consider alveolar concentration and bronchial production as independent parameters (11, 17, 22). However, modeling studies of NO transport in the lungs (21, 23) have indicated that molecular diffusion has a crucial impact on the NO tracings from which alveolar concentration and bronchial production are derived. Indeed, due to the concentration gradient between the airways and the alveolar compartment, a part of the bronchial NO produced in the airways “backdiffuses” toward the alveolar lung zone during expiration, reducing NO in expired air. The existence of backdiffusion has been experimentally demonstrated in normal subjects (19, 20) by the inspiration of a mixture of oxygen-helium (heliox) before expiration. The diffusion coefficient of NO in heliox being greater than that of NO in air, the backdiffusion phenomenon is amplified, and, as a result, exhaled NO is smaller when exhaled from a heliox-filled lung than from an air-filled lung.

One consequence of the presence of NO backdiffusion is that models assuming two independent compartments lead to an underestimation of actual bronchial NO production (21, 23). Another consequence of NO backdiffusion is that it constitutes an additional NO source for the alveolar compartment and, hence, contributes to an increase of the alveolar NO concentration. Hence, two-compartment models that neglect backdiffusion also overestimate alveolar NO production by attributing measured alveolar NO concentration entirely to in situ production (4, 23).

Recently, Condorelli et al. (4) demonstrated a technique to account for the impact of airway NO production on alveolar concentration and applied this technique in healthy subjects. In the present work, we aimed to establish a relationship between alveolar NO concentration and exhaled NO concentration to estimate which part of alveolar NO concentration is actually due to in situ alveolar NO production in asthma patients perfectly controlled (including no airway obstruction). We also aimed to experimentally demonstrate the link between backdiffusion and alveolar concentration in this population. This was done by first performing simulations with a model incorporating axial diffusion and considering a range of bronchial and alveolar productions relevant to clinical situations. Then the theoretical relationships obtained from the simulations were tested on experimental data obtained from healthy subjects and patients with stable asthma.

MATERIALS AND METHODS

Experimental Study

Methods. A chemiluminescence analyzer (LR 2000, Logan Research, Rochester, UK) was used to measure NO online. The instrument was calibrated daily with two calibration mixtures (103 ppb NO in nitrogen and 20 ppb in helium). Alveolar NO concentration (FANO) and the “maximal airway flux” (JNO) were computed using the method described by Pietropaoli et al. (17), i.e., as, respectively, the y-intercept and the slope of the line fitted on the exhaled NO values as a function of the inverse of the expired flow; and by the method proposed by Tsoukias et al. (22), i.e., as the slope and y-intercept of the present work, we aimed to establish a relationship between alveolar NO concentration and exhaled NO concentration to estimate which part of alveolar NO concentration is actually due to in situ alveolar NO production in asthma patients perfectly controlled (including no airway obstruction). We also aimed to experimentally demonstrate the link between backdiffusion and alveolar concentration in this population. This was done by first performing simulations with a model incorporating axial diffusion and considering a range of bronchial and alveolar productions relevant to clinical situations. Then the theoretical relationships obtained from the simulations were tested on experimental data obtained from healthy subjects and patients with stable asthma.
the line fitted on NO output (exhaled NO values times the expired flow) as a function of the expired flow. The exhaled NO tracings were obtained for flow rates of 50, 175, and 300 ml/s. Exhaled NO at 50 ml/s will be further referred to as the exhaled NO (FENO) according to American Thoracic Society/European Respiratory Society standard (1). Exhaled NO was also measured at 50 ml/s after 2 min equilibration with heliox (FENO)\textsubscript{heliox}. The decrease of exhaled NO due to heliox (\(\Delta F_{\text{NO;heliox}}\)) was expressed in absolute value (FENO - FENO\textsubscript{heliox}) and as a percentage of the value in air [(FENO - FENO\textsubscript{heliox})/FENO]. A positive \(\Delta F_{\text{NO;heliox}}\) value corresponds to a decrease of exhaled NO with heliox compared with air.

**Studied populations.** We studied 10 healthy nonsmoker subjects (8 men; age 38 ± 14 yr; range 19–56 yr) and 21 nonsmoker patients with asthma diagnosed according to standard criteria (9). Asthma control had been assessed using a French translation of the Asthma Control Questionnaire (ACQ) from Juniper et al. (13). An ACQ score below 0.75 identifies a patient whose asthma is well controlled (12). Table 1 summarizes patient characteristics. Three patients out of 21 declined to perform heliox measurements.

The local ethics committee approved the protocol, and each subject signed an informed consent.

**Model Simulation Study**

We used Eq. 1 incorporating convective and diffusive NO transport (6) and NO source terms (23) in geometrical boundaries based on the Weber’s symmetrical model (25)

\[
\frac{\partial F}{\partial t} = \frac{\partial}{\partial z} \left( \frac{D}{S} \frac{\partial F}{\partial z} \right) + \frac{1}{S} \frac{\partial}{\partial t} (QF) + \frac{V}{S} (V\text{NO} - D\text{NO}) \tag{1}
\]

where \(t\) is time, \(z\) is the distance from the alveolar end, \(F(z,t)\) is the NO concentration, and \(D\) is the molecular diffusion coefficient. \(S(z,t),\) \(s(z),\) \(V(t),\) and \(Q(z,t)\) are total cross-sectional area (airways + alveoli), airway total cross-sectional area, volume, and axial flow, respectively. \(D\) was taken to equal 0.22 cm\(^2\)/s (14). This model has been accepted as a good tool to provide a realistic description of gas concentration profiles in the lung periphery when anatomic asymmetry is neglected (6).

The last term of Eq. 1 is the NO source term: the difference between NO production (\(V\text{NO}\); in pl/s) and NO diffusion into blood (\(D\text{NO}\) being expressed in pl\(\cdot\)s\(^{-1}\)\(\cdot\)ppb\(^{-1}\) per unit volume. We considered

\[
V\text{NO}(z) = V\text{awNO}(z) + V\text{ANO}(z)
\]

and

\[
D\text{NO}(z) = D\text{awNO}(z) + D\text{ANO}(z)
\]

where \(V\text{awNO}(z)\) and \(V\text{ANO}(z)\) are airway and alveolar NO production, respectively, and \(D\text{awNO}(z)\) and \(D\text{ANO}(z)\) are airway and alveolar NO transfer factor, respectively.

**Table 1. Asthma patient characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>M/F</td>
<td>16/5</td>
</tr>
<tr>
<td>Age, yr</td>
<td>38±14*</td>
</tr>
<tr>
<td>FE\textsubscript{V1}, %predicted</td>
<td>98±0.11.9*</td>
</tr>
<tr>
<td>F\textsubscript{ENO}, ppb</td>
<td>46.5±22.1</td>
</tr>
<tr>
<td>ACQ score</td>
<td>0.1 [0-0.7]†</td>
</tr>
<tr>
<td>Current ICS dose, µg eq BDP/day</td>
<td>1,000 [0-1,000]+</td>
</tr>
<tr>
<td>LABA</td>
<td>15/21 (71%)</td>
</tr>
</tbody>
</table>

Values are means ± SD (*) or median [range] (†). M/F, men/women; FE\textsubscript{V1}, forced expiratory volume in 1 s; F\textsubscript{ENO}, exhaled NO concentration at 50 ml/s expired flow; ACQ, asthma control questionnaire of Juniper et al. (13); ICS, inhaled corticosteroids; BDP, beclomethasone dipropionate; LABA, long-acting \(\beta\text{-agonist; ppb, parts per billion.}\

In the present study, the baseline overall source terms values are 3,083 and 3,167 pl/s for \(V\text{awNO}\) and \(V\text{ANO}\), respectively, and 5.07 and 1,558 pl\(\cdot\)s\(^{-1}\)\(\cdot\)ppb\(^{-1}\) for \(D\text{awNO}\) and \(D\text{ANO}\), respectively. \(D\text{awNO}\), \(D\text{ANO}\), and \(V\text{ANO}\) values were taken from Pietropaoli et al. (17), and \(V\text{awNO}\) value was adjusted by trial and error to mimic the mean experimental FENO in our healthy subjects group. The distribution of the NO sources as a function of \(z\) and details about numerical computation of Eq. 1 solutions are described elsewhere (23). The boundary conditions are \(F = 0\) at the model entry (no NO inspired), and \(dF/dz = 0\) at the model end (15).

Simulated conditions bronchial and alveolar productions ranging from this baseline value to a fivefold increase. The limit case corresponding to a nil alveolar production was also considered.

The simulated maneuver always consisted of a 2-s inspiration at 500 ml/s from a preinspiratory volume of 3,700 ml, followed by a 20-s expiration at 50 ml/s. Before the maneuver, a steady concentration profile inside the model had been established by simulating 25 breath cycles (1-s inspiration and 1-s expiration at 500 ml/s).

The primary outcomes of the simulations were \(J\) the expired NO concentration (F\text{ENO})\textsuperscript{0} at the end of the maneuver and 2) the alveolar NO concentration (F\text{ANO})\textsuperscript{1} present in the model in the last lung generation before the maneuver. It is to be noted the simulated F\text{ANO}\textsuperscript{1} was directly available as model outcome and not computed like experimental F\text{ANO}\textsuperscript{0}. F\text{ENO}\textsuperscript{0} was also simulated using a diffusion coefficient of 0.6 instead of 0.22 cm\(^2\)/s (14). Finally, the hypothetical case \(D = 0\) (no axial diffusion) was also considered.

**Statistical methods.** Paired and unpaired \(t\)-tests and regression analysis were used. The level of significance was taken as \(P < 0.05.\)

**RESULTS**

In the present study, linear regressions on the exhaled NO values as a function of the inverse of the expired flow included 50 ml/s. They presented a median \(r^2\) of 0.996 (range 0.918–0.998). Additionally, the 31 sets of values were pooled according to a standardization method allowing one to estimate occult nonlinearities (18). It appeared that nonlinear fitting on pooled data did not bring any additional variance reduction (\(r^2 = 0.986\) and 0.987 for second- and first-order adjustment, respectively).

No difference appeared between F\text{ANO}\textsuperscript{0} values computed by the method of Pietropaoli et al. (17) and by the method of Tsoukias et al. (22) for healthy subjects (3.4 ± 1.3 and 3.1 ± 1.5 ppb, respectively; \(P = 0.31\)) and for asthma patients (5.1 ± 2.9 and 4.8 ± 3.1 ppb, respectively; \(P = 0.74\)). In the following figures, only F\text{ANO}\textsuperscript{0} computed by the method of Pietropaoli et al. (17) will be displayed.

Similarly, no difference appeared between methods in J\text{ENO} computation for healthy subjects (669 ± 274 and 745 ± 311 pl/s, respectively; \(P = 0.15\)) and for asthma patients (2,067 ± 1,009 and 2,254 ± 1,150 pl/s, respectively; \(P = 0.69\)).

A regression analysis on all subjects between J\text{ENO} [determined by the method of Pietropaoli et al. (17)] and F\text{ENO} showed a nearly perfect fitting:

\[
F\text{ENO (ppb)} = 0.022 J\text{ENO (pl/s)}(r^2 = 0.997; P < 0.001) \tag{2}
\]

J\text{ENO} computed by the method of Tsoukias et al. (22) is linked to F\text{ENO} by a 0.020 factor (\(r^2 = 0.984\)). The \(r^2\) value in Eq. 2 means that J\text{ENO} and F\text{ENO} give the same information. There-
subjects group) and 3.1 ppb (vs. 3.4 ± 1.3 ppb in our healthy subjects group), respectively.

Figure 1 presents the simulated NO concentration profiles at the end of expiration as a function of the distance from alveolar end for the baseline bronchial production (curve 1) and for two different bronchial productions. A substantial NO concentration gradient is present at the level of the acinus entrance between the airways and the alveoli. This gradient increases with bronchial NO production resulting in increasing alveolar concentration.

Figure 2 shows the simulated impact of bronchial production and molecular diffusion on FENO (Fig. 2A) and FANO (Fig. 2B); in both panels, the baseline alveolar production was considered. In Fig. 2A, FENO was seen to increase with increasing bronchial production and to decrease with increasing molecular diffusion. In Fig. 2B, FANO is seen to increase with increasing bronchial production only when molecular diffusion is considered (D = 0.22 and D = 0.6 cm²/s).

The dependence of FENO on molecular diffusion depicted in Fig. 2A was verified experimentally: FENO with heliox-filled lung was consistently lower than FENO with air-filled lung. Figure 3 presents individual values of the percentage difference (ΔF_{E_{heliox}}) between heliox-filled and air-filled FENO. The mean value was equal to 35.6 ± 8.6% for healthy subjects (open circles) and to 30.3 ± 6.3% for well-controlled asthma patients (closed circles). There was no statistical difference between the two groups (P = 0.101). Pooling healthy subjects and asthma patients, the mean ΔF_{E_{heliox}} was equal to 31.9 ± 7.3%. The dashed lines are the upper and lower limits of ΔF_{E} simulated over the entire range of bronchial and alveolar productions here considered.

Figure 4 shows simulated (Fig. 4A) and experimentally deduced (Fig. 4B) FANO as a function of FENO. In Fig. 4A, FANO is seen to increase with both bronchial production and alveolar production and to be linearly linked to FENO for a given alveolar production. The alveolar concentration entirely due to in situ production (FANO_{prod}) corresponds to a nil bronchial production. Indeed, in this case, NO produced in the alveoli would be affected neither by backdiffusion nor by the airways transit (blood recapture during airways transit is negligible), and FENO would be equal to FANO_{prod} (identity line on Fig. 4A). The open diamond on Fig. 4A corresponds to FANO_{prod} for baseline alveolar production (1.8 ppb). It is to be noted that, for a given alveolar production, FANO_{prod} is the lowest possible expired concentration. Hence, the gray area on the left side of the identity line is a zone of physically meaningless pairs of FANO and FENO values.

For any given alveolar production, the simple relationship between FANO and FENO on the right side of the identity line can be captured by:
The 0.92 factor expresses that, for a nil bronchial production, FANO and FENO are equal to FANO,prod \[FANO,prod = (0.92FANO + 0.08FENO)/0.92\].

Conversely, Eq. 3 also enables us to derive FANO,prod, i.e., the alveolar concentration actually due to alveolar production, from experimentally accessible indexes, which are FENO and FANO, by a very simple equation:

\[FANO,prod = (FANO - 0.08FENO)/0.92\]  \hspace{1cm} (4)

Regression line obtained from the entire population on Fig. 4B (with its 95% confidence interval) is presented alongside the simulations that consider nil (dashed line) and baseline alveolar production (dotted line). Figure 5 shows individual FANO and FANO,prod for healthy subjects, asthma patients with FENO \(\leq 50\) ppb \(n = 12\), and asthma patients with FENO \(> 50\) ppb \(n = 9\). Table 2 shows, in the three groups, the average values \((\pm SD)\) of FENO, FANO, and FANO,prod. Cases a and b correspond to FANO computed according to the method of Pietropaoli et al. (17), and FANO,prod computed by Eq. 4, respectively. Cases c and d correspond to FANO computed according to the method of Tsoukias et al. (22) and FANO,prod computed from the correction formula of Con-dorelli et al. (4), respectively. The latter correction formula uses JNO computed by the method of Tsoukias et al. (22). The two approaches give quantitatively and statistically similar results, i.e., FANO,prod identical in the three groups after correction.

Figure 6A shows simulated FANO as a function of the absolute value of \(\Delta F_{Eheliox}\) (in ppb) for a range of bronchial production and alveolar production. For a given alveolar production, FANO is linearly linked to \(\Delta F_{Eheliox}\) when bronchial production varies. An increase of alveolar production results in an upward translation of the linear relationship between FANO and \(\Delta F_{Eheliox}\). In Fig. 6B, this relationship between FANO and \(\Delta F_{Eheliox}\) is assessed experimentally, using data from healthy subjects (open squares) and well-controlled asthma patients (closed squares). As in Fig. 4B, the regression line (with its 95% confidence interval) obtained from the entire study population (solid line) is plotted alongside the simulations that consider a nil alveolar production (dashed line) and a baseline alveolar production (dotted line).

**DISCUSSION**

The present work confirms the important role of nitric oxide backdiffusion and highlights, by experimental evidence, its impact on NO alveolar concentration in healthy subjects and in stable asthma patients. Moreover, experiments and simulations not only predict that NO bronchial production is an important determinant of alveolar concentration, but also lead to the conclusion that stable asthma patients have an alveolar production similar to healthy subjects.

We computed FANO (and JNO) by including exhaled NO measured at 50 ml/s. Pietropaoli et al. (17) demonstrated a linear relationship between the inverse of the flow and exhaled NO for flows greater than 70 ml/s. However, huge nonlinearity only arises for very low flows \(< 20\) ml/s. In the present study,
an $r^2$ value greater than 0.98, in individual adjustments as well as in adjustment on pooled data (18), strongly suggests an acceptable linearity.

In a recent work addressing treated asthma, Gelb et al. (8) showed that alveolar NO may still be reduced by systemic treatment after inhaled treatment. The authors raised the issue of the alveolar spaces contamination by NO coming from airways. Indeed, they found a weak but significant correlation between NO bronchial flux and alveolar concentration. However, in addition to a slightly different FANO determination method, the asthma control status of their patients was not defined as in the present study, and most of them, although stable, presented a certain degree of airways obstruction. The stronger relationship seen in the present work (Fig. 4B) is likely due to the fact that well-controlled asthma was specifically addressed, implying an absence of obstruction (mean FEV1: 98% predicted). This allowed isolating the effect of increased bronchial NO production, avoiding the confounding effect of airway caliber reduction. The latter has been shown, for not yet elucidated reasons, to decrease exhaled NO during bronchoprovocation challenge (5, 10). Our asthma group is free of airway obstruction and presents a broad range of values in air (Fig. 3). This reduction, which is in quantitative agreement with values observed by Shin et al. (20) in healthy subjects, is similar in patients with stable asthma and in healthy subjects.

Table 2. Average values of $F_{ENO}$, $F_{ANO}$, and $F_{ANO, prod}$ in three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>$F_{ENO}$</th>
<th>$F_{ANO}$ (a)</th>
<th>$F_{ANO, prod}$ (b)</th>
<th>$F_{ANO}$ (c)</th>
<th>$F_{ANO, prod}$ (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td>16.7±5.1</td>
<td>3.5±1.3</td>
<td>2.3±1.4</td>
<td>3.1±1.5</td>
<td>2.2±1.5</td>
</tr>
<tr>
<td>Asthma patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_{ENO} \leq 50$ ppb</td>
<td>30.9±10.9</td>
<td>&lt;0.001*</td>
<td>4.0±2.4</td>
<td>0.86*</td>
<td>1.7±1.9</td>
</tr>
<tr>
<td>$F_{ENO} &gt; 50$ ppb</td>
<td>67.3±14.2</td>
<td>0.013*</td>
<td>6.8±2.7</td>
<td>0.013*</td>
<td>1.8±2.5</td>
</tr>
</tbody>
</table>

Data are means ± SD presented as: a) alveolar NO concentration ($F_{ANO}$) determined by method of Pietropaoli et al. (17); b) alveolar NO concentration due to in situ production ($F_{ANO, prod}$) computed from a by Eq. 4; c) $F_{ANO}$ determined by method of Tsoukias et al. (22); and d) $F_{ANO, prod}$ computed from c by formula of Condorelli et al. (4) [maximal airway flux ($J_{NO}$) computed from method of Tsoukias et al. (22)]. *P value refers to comparison with healthy subjects group.

Fig. 6. A: simulations of $F_{ANO}$ as a function of $\Delta F_{\text{heliox}}$, for increasing bronchial (joined symbols from left to right) and alveolar (from bottom to top) productions. B: individual values of measured $F_{ANO}$ as a function of measured $\Delta F_{\text{heliox}}$ for the healthy subjects (open squares) and the asthma patients (closed squares). The solid line is the regression line on the whole population. The gray zone is the 95% confidence interval of the regression line. Dashed and dotted lines are simulations with nil and baseline alveolar production, respectively.
duced by the simulations, suggesting that factors other than NO production affect $\Delta F_{\text{heliox}}$.

Backdiffusion also constitutes a positive source for the alveolar compartment, being expected to increase the steady-state alveolar concentration independently from in situ production. Simulations of Fig. 2B show that, for a constant alveolar production, an increase of bronchial production hugely affects alveolar concentration when axial diffusion is considered ($D = 0.22$ or $D = 0.6$ cm$^2$/s). When $D = 0$, a fivefold increase of bronchial production does not affect $F_{\text{ANO}}$. A greater diffusivity ($D = 0.6$ cm$^2$/s), as it decreases $F_{\text{ENO}}$, increases $F_{\text{ANO}}$ by increasing the backdiffusion flux.

The link between $F_{\text{ENO}}$ and $F_{\text{ANO}}$, via backdiffusion, is addressed in Fig. 4. Despite the simultaneous involvements of convection and diffusion transports in a relatively complex geometrical structure, simulations of Fig. 4A predict a linear relationship between $F_{\text{ANO}}$ and $F_{\text{ENO}}$. Indeed, in steady-state conditions, fast arising during expiration (23), the backdiffusion flux, i.e., the airway source of alveolar NO, is governed by the Fick’s law of the first order, i.e., directly proportional to the gradient between airways and alveolar concentrations. This leads to a first-order relationship between alveolar and airway concentration. The last term of Eq. 3 indicates that 8% of $F_{\text{ENO}}$ contributes to alveolar concentration. Instead of numerically solving a time-dependent transport equation (Eq. 1), Condorelli et al. (4) found an equivalent formula (based on bronchial production instead of $F_{\text{ENO}}$) by analytically solving a steady-state equation incorporating axial diffusion in Weibel morphometrical data. This formula, combined with Eq. 2, predicts 6% contribution of $F_{\text{ENO}}$ to alveolar concentration. This smallest impact of airway production in the equation of Condorelli et al. (4) likely comes from a relatively smaller NO production at the onset of the acinus in their governing equation, slightly reducing the impact of backdiffusion. It is to be noted that the nearly perfect link between $J_{\text{NO}}$ and $F_{\text{ENO}}$ was already pointed out by Paraskakis et al. (16). The predictions of our simulations are confirmed by experimental values of alveolar and exhaled concentrations (Fig. 4B). The regression line has a slope of 0.075, nearly identical to the simulations. Importantly, the regression line is nearly superimposed to the simulation line considering baseline alveolar production (dotted line), which gives simulated alveolar concentration very close to the average experimental value in normal subjects. Moreover, the 95% confidence interval is above the simulation line considering nil production (dashed line). This suggests that an in situ production actually occurs, even if some individual $F_{\text{ANO}}$ of asthma patients as well as of healthy subjects may be compatible with a very low NO production in the alveoli, as concluded by Condorelli et al. (4) for healthy subjects. Conversely, some patients, especially with high $F_{\text{ENO}}$, present an elevated alveolar concentration, even after correction, as illustrated on Fig. 5. Nonetheless, $F_{\text{ANO}}$ values are significantly different in asthma patients with $F_{\text{ENO}} > 50$ ppb and in healthy controls, whereas $F_{\text{ANO,prod}}$ are, in average, equivalent. Computations made according to the findings of Condorelli et al. (4) leads to the same conclusions (Table 2).

Finally, the impact of backdiffusion on $F_{\text{ANO}}$ may be assessed by the link between $F_{\text{ANO}}$ and the absolute difference between exhaled NO in air and in heliox ($\Delta F_{\text{heliox}}$ in ppb), the latter being the only marker of backdiffusion amplitude that is experimentally accessible. Results are presented on Fig. 6.

Simulations predict a linear relationship between $F_{\text{ANO}}$ and $\Delta F_{\text{heliox}}$ for a given alveolar production (Fig. 6A). Measured values of $F_{\text{ANO}}$ and $\Delta F_{\text{heliox}}$ (Fig. 6B) confirm these predictions. Their good correlation ($r = 0.68$; $P < 0.001$) attests that backdiffusion is an important determinant of the alveolar concentration. Moreover, in accordance with Fig. 4B, the regression line is close to the simulation with the baseline alveolar production (dotted line).

In conclusion, the present study brings experimental evidence that backdiffusion is a link between alveolar concentration and bronchial production in healthy subjects and in stable asthma patients. A very simple relationship between alveolar concentration and exhaled NO measured at 50 ml/s expired flow allows estimation of the part of alveolar concentration actually due to in situ production (Eq. 4). Importantly, comparisons between simulations and experiments allow concluding that patients with stable and unobstructed asthma have the same alveolar NO production as healthy subjects.

GRANTS

This study was funded by a Microgravity Application Programme project from European Space Agency: Airway NO in Microgravity. AstraZeneca provided a grant for the exhaled biomarker laboratory.

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