

Online supplement 7.

Concept of back-diffusion

Models of nitric oxide (NO) production and transport taking axial molecular diffusion into account allowed to enlighten on its crucial role in the transition area between alveolar zone and purely conductive airways [1,2]. Indeed, a relatively low alveolar concentration, due to the huge affinity of haemoglobin for NO, and a higher bronchial concentration, due to the contribution of airway epithelium production, result in a NO diffusion flux from airways towards alveolar zone. This flux is opposed, in direction, to the expired flow, and was, thus, called “back-diffusion”. Since it removes NO molecule from the expiration flow, this phenomenon contributes to decrease the expired NO concentration. It is inversely proportional to the length of axial diffusion pathway and proportional to the airway-alveoli concentration difference, to the lumen area through which the flux is passing, and to the molecular diffusion coefficient, the latter depending on the gas mixture in which NO is transported.

Models anticipated a 30% decrease of FENO [3,4] due to an increase of the back-diffusion phenomenon, when the molecular diffusion coefficient goes from 0.23 (NO in air) to 0.6 cm².s⁻¹ (NO in heliox, 80% helium, 20% oxygen), all other things remaining equal. These model predictions were confirmed by experiments on healthy subjects and perfectly controlled asthma patients [3,5].

Implications of back-diffusion

In the following, simulations of NO transport were performed with a model incorporating convection and axial diffusion in geometrical boundary conditions derived from Weibel's morphometrical data [6]. It was described in detail in Van Muylem *et al.* [2,7].

Implication on NO production sites

Figure 1 shows simulated F_ENO as a function of the furthest generation with an epithelial NO production. By example, for x=10, total airway NO production goes from trachea to generation 10, the more distal generations having no production. In all simulations, the total airway production was equal to 0.68 nL s⁻¹. The closed circles are simulations with a diffusion coefficient equal to 0.22 cm² s⁻¹ (NO in air) and the open circles are simulation without molecular diffusion (D=0). It may be seen that, when diffusion is not considered

(open circles), $F_E\text{NO}$ is relatively insensitive to how production is spread over the bronchial tree. This situation is equivalent to the “classical” two-compartment model, except that the latter does not consider airways geometry. On the contrary, with axial diffusion (closed circles), $F_E\text{NO}$ is highly sensitive to NO production site. When NO production does not go further than generations 11-12, the effect of diffusion is negligible because of the relatively small lumen cross-sections and of the fact that the “infinite sink” constituted by the alveolar compartment is too far. When NO production goes more distally, the effect of back-diffusion becomes more important and hugely impacts $F_E\text{NO}$.

So, a significant effect of back-diffusion implies a very distal NO production. Kerckx *et al.* [7] showed that, to reproduce a 30% decrease of $F_E\text{NO}$ when lungs are filled with heliox [3,5], about 75-80% of the NO production has to be concentrated on the terminal bronchioles.

Implication on alveolar NO

If back-diffusion is a “negative” source (removing NO molecules) for expired NO, it is a positive source for the alveolar compartment, independent from the alveolar production itself. Closed symbols and solid line on Figure 2 shows simulated alveolar NO (computed as the mean value in the last generation of the model) as a function of $F_E\text{NO}$ for baseline (circle), two-fold (square) and three-fold (triangle) increase of bronchial NO production. This relationship or its equivalent was found experimentally for healthy subjects [4] and perfectly controlled (based on standard pulmonary function and asthma control questionnaire [8]) asthma patients [3].

This led the authors of the two above-mentioned papers to propose “correction” formulas allowing deriving $C_A\text{NO}$ specifically due to alveolar production ($C_A\text{NO}_{\text{prod}}$), i.e. free from airway contamination by back-diffusion.

$$C_A\text{NO}_{\text{prod}} = C_A\text{NO} - J_{\text{aw}}\text{NO}/0.74 \quad (1) \text{ from ref 4}$$

$$C_A\text{NO}_{\text{prod}} = (C_A\text{NO} - 0.08.F_E\text{NO})/0.92 \quad (2) \text{ from ref 3}$$

In Eq.1, $C_A\text{NO}$ (in ppb) and $J_{\text{aw}}\text{NO}$ (in nL s^{-1}) are the slope and the intercept of a linear regression on the \dot{V}_{NO} vs \dot{V} plot [9]. In Eq.2, $C_A\text{NO}$ was derived using a linear model on \dot{V}_{NO} vs \dot{V} plot⁹ or $F_E\text{NO}$ vs $1/\dot{V}$ [10]. Assuming, $F_E\text{NO}$ (ppb) = $22 \times J_{\text{aw}}\text{NO}$ (nL s^{-1})⁴, the outcomes of Eq.1 and Eq.2 are very similar.

Limitation of back-diffusion corrections

Both corrections formulas were experimentally derived from measurements in adult subjects with normal pulmonary function and with the support of simulations performed in the “unobstructed” Weibel’s morphometrical model [6]. However any factors that may affect back-diffusion will affect the relationship between $F_{E}NO$ (or $J_{aw}NO$) and $C_{A}NO$. The most common factor in a clinical setting is the obstruction, and, particularly in the asthma context, peripheral obstruction. Figure 2 shows, additionally to the unobstructed case (closed symbols, solid line), the simulated $F_{E}NO$ - $C_{A}NO$ relationships when an obstruction (50% reduction of the lumen) is considered in generation 17 (open symbols, dashed line) and simultaneously in generations 17-18 (gray symbols, dotted line). It may be seen that the slope of the $F_{E}NO$ - $C_{A}NO$ relationship is lowered as obstruction progresses. Consequently, if a formula assuming an absence of obstruction is applied when peripheral obstruction is actually present, over-correction may arise [11], leading to negative $C_{A}NO$ values in extreme cases.

References

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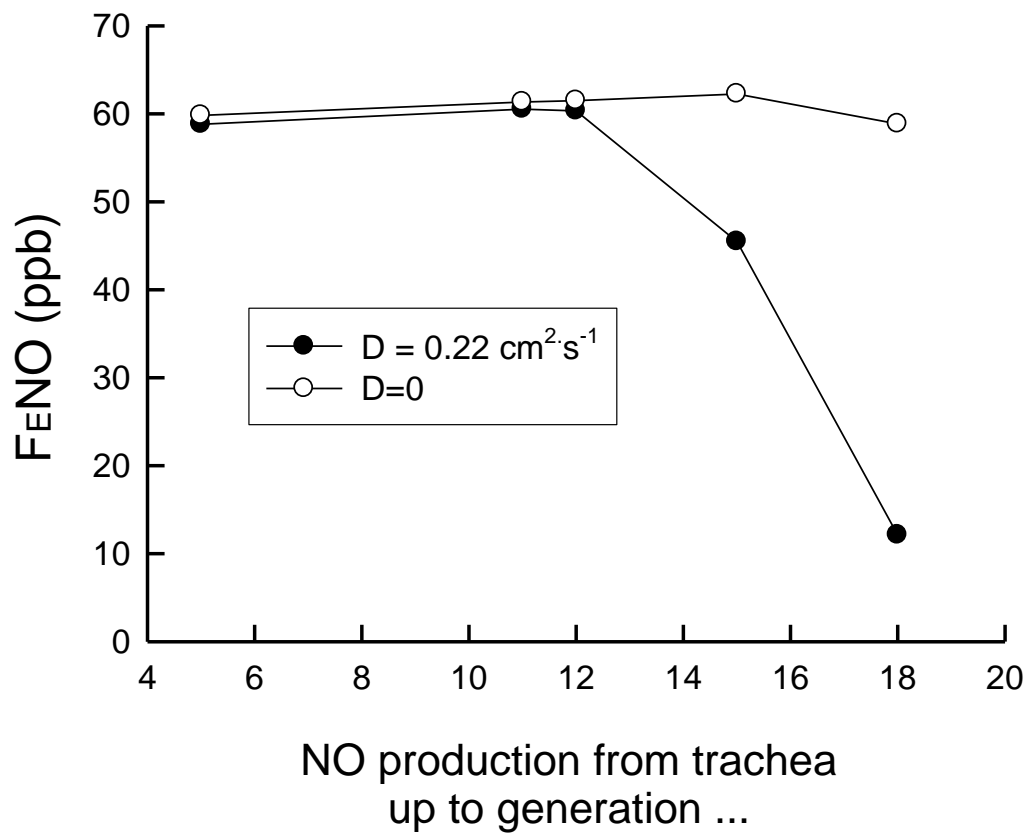


Figure 1. Simulated $F_{E}NO$ as a function of the furthest generation with an epithelial NO production, the latter beginning in the trachea. In all simulations, the total airway production was equal to 0.68 nL s^{-1} . Open circles represent simulations when axial diffusion was not considered ($D = 0$), and closed circles represent simulations when diffusion of NO in air ($D = 0.22 \text{ cm}^2 \text{ s}^{-1}$) is considered.

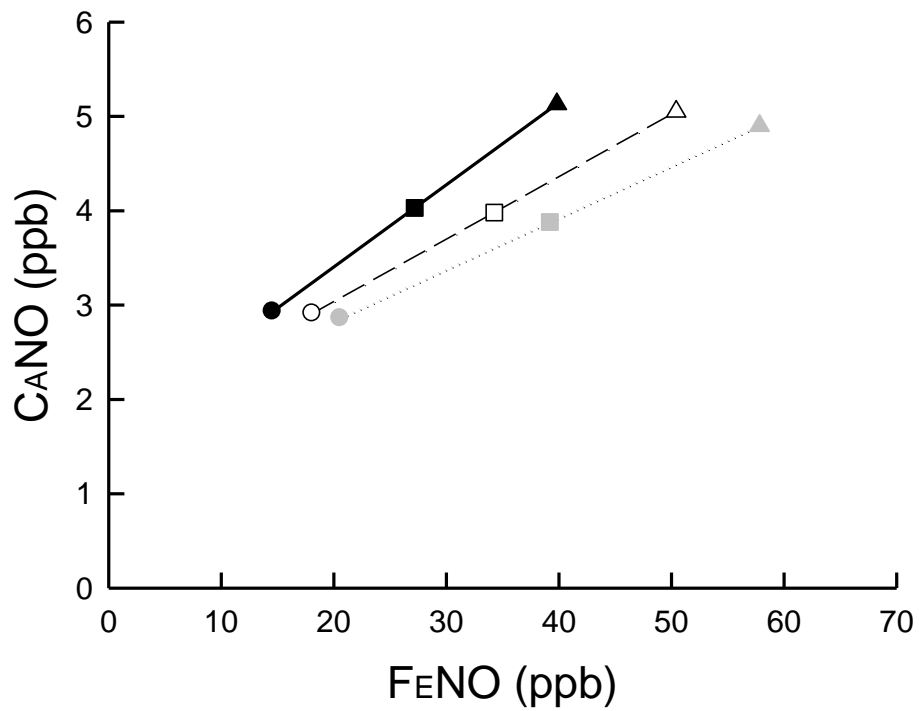


Figure 2. Simulated relationships between C_ANO and F_ENO in the unobstructed Weibel's model (solid line and closed symbols by decreasing by 50% the airway lumen area in generation 17 (dashed line, and open symbols), and by simultaneously decreasing by 50% the airway lumen area in generations 17-18 (dotted line, gray symbols). Circles, squares, and triangles correspond to $J_{aw}NO = 0.68, 1.36, \text{ and } 2.04 \text{ nL s}^{-1}$, respectively.