

IX. Flow-Independent Parameters: Based on Transport Models

As NO is continuously formed in the airways, the concentration of NO will vary with the flow of exhaled air (Figure IX.1), a fact that has been well documented by Silkoff *et al.* [Silkoff PE *et al.* 1997] Kissoon and colleagues have measured exhaled NO levels at a series of low flow rates (4, 5, 7, 10, 15, 23, 31 and 46 mL/s) and documented changes in the concentration (101.3, 87.7, 81.1, 62.1, 74.2, 62.3, 46.4 and 36.9 ppb, respectively). [Kissoon N *et al.* 2000] It is therefore important to register the flow rate if NO is expressed as a concentration.

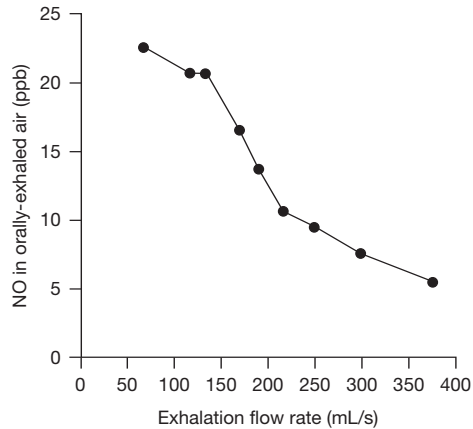


Figure IX.1. Relationship between exhalation flow rate and NO concentration in orally exhaled air from a healthy subject [Silkoff PE *et al.* 1997]

9.1. NO Transport Models

It is not feasible to construct a model that accounts for the whole complexity of the lungs. However, one can simplify by using a model that assumes that all alveoli can be united in one compartment and all bronchi in a second compartment, which forms a 'balloon on a stick' model. This partitioning of the alveolar and airway components is referred to as the two-compartment model. [Tsoukias NM and George SC 1998] This approach takes advantage of the fact that the relationship between NO output and expiratory flow is

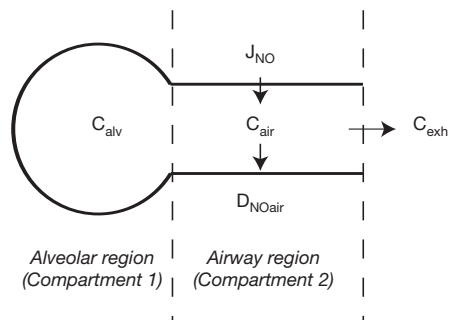


Figure IX.2. NO diffusion model. C_{alv} = steady-state alveolar concentration, J_{NO} = max influx of NO from the airways, C_{exh} = exhaled NO concentration, D_{NOair} = diffusing capacity of NO in the airways, C_{air} = concentration of NO in gas phase within the airway region [Tsoukias NM *et al.* 2001]

approximately linear above a threshold of 50 mL/s in healthy adults. [Tsoukias NM and George SC 1998; Tsoukias NM *et al.* 1998] Similar models followed the two-compartment model, as described by Pietropaoli *et al.*, [Pietropaoli AP *et al.* 1999] Silkoff *et al.* [Silkoff PE *et al.* 2000] and Högman *et al.* [Hogman M *et al.* 2000] In addition, new breathing techniques that use the two-compartment model have been developed to characterize NO exchange dynamics (Figure IX.2). [Shin HW *et al.* 2001; Tsoukias NM *et al.* 2001] These

models were reviewed in 2004 by George *et al.* [George SC *et al.* 2004] All models require exhaled NO to be measured at multiple flows over a wide range of rates. A later model published by Shin and George incorporates axial diffusion into a one-dimensional trumpet model of NO gas exchange in the lungs. [Shin HW and George SC 2002] The trumpet model predicts a significant back-diffusion of NO from the airways into the alveolar region, resulting in a significant loss of NO that would therefore not appear in the exhaled breath.

Furthermore, this back-diffusion of NO results in a significant loss of NO, which does not appear in the exhaled breath. [Shin HW *et al.* 2004a] The result is a potential underestimation of both the maximum airway flux of NO and the airway wall concentration of NO. This effect will, however, only be significant if there is substantial production of NO in the very small airways. This model has been shown to produce results that are independent of insufflating gas (e.g. air vs. heliox in which molecular diffusion of NO varies by 2.5-fold), as should be expected of flow independent parameters. [Shin HW *et al.* 2006a]

Condorelli *et al.* further developed this model to produce an algorithm which characterizes NO exchange using multiple constant flow exhalations and a model which considers the trumpet shape of the airway tree and axial diffusion (trumpet model with axial diffusion [TMAD]). [Condorelli P *et al.* 2007] Figure IX.3 depicts the mapping of airway dimensions to the 'trumpet geometry'. The TMAD was tested in 8 healthy subjects using exhalation flow rates of 100, 150, 200 and 250 mL/s. Compared to the two-compartment model, estimates from the TMAD for the maximum airway flux were statistically higher and estimates for the steady state alveolar concentration are statistically lower. The new model showed that proximal airway NO production is larger than previously predicted, and that NO in peripheral bronchioles and alveoli is near zero in healthy subjects.

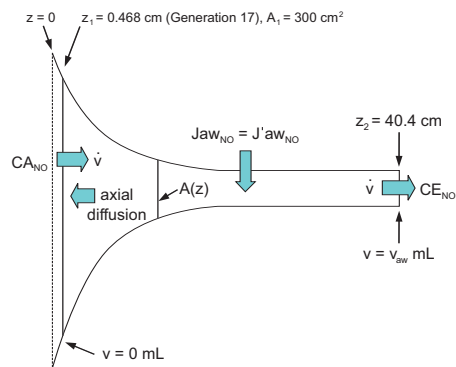


Figure IX.3. Schematic of the trumpet model. $A(z)$ = cross-sectional area at position z , CA_{NO} = NO concentration in alveolar air, CE_{NO} = NO concentration in exhaled air, $J_{aw_{NO}}$ = rate of addition of NO to the airstream (from the airway wall), $J'_{aw_{NO}}$ = maximum airway flux, \dot{v} = steady flow of NO (towards the mouth), v_{aw} = airway volume, position z_2 = mouth [Condorelli P *et al.* 2007]

All these models predict that exhaled NO levels increase as exhalation flow decreases, because of prolonged contact of the expirate with bronchial epithelium. This raises the intriguing possibility of whether variations in inspiration rate could contaminate alveolar NO with bronchial-derived NO, and thus affect exhaled NO measurements. This was investigated by Zacharasiewicz *et al.*, but they found no effect of different inhalation rates. [Zacharasiewicz A *et al.* 2004] Thus, we can be assured that current techniques for measuring exhaled NO do not need to take inhalation rates into consideration.

9.2. Potential Clinical Use

Diffusion models are now beginning to provide valuable information into a wide range of inflammatory diseases, as outlined below. Further insights are expected as this relatively new area continues to develop.

9.2.1. Asthma

Studies indicate that diffusing capacity of the airways is increased in patients with asthma. Silkoff *et al.* found the airway diffusing capacity in patients with asthma was four times that of healthy individuals. [Silkoff PE *et al.* 2000] Notably, inhaled corticosteroids had no effect on diffusing capacity. These results were confirmed by Shin and co-workers using a different technique. [Shin HW *et al.* 2004b] The underlying cause of the increase in diffusing capacity is not known, but may reflect up-regulation of iNOS in the airway epithelium, subepithelial fibrosis or up-regulation of non-adrenergic, non-cholinergic, NO-producing nerves in airways in compensation for decreased sensitivity of airway smooth muscle to the relaxed effects of endogenous NO. [Silkoff PE *et al.* 2000] Pedroletti *et al.* showed that the elevated exhaled NO seen in asthmatic children was related to an increase in both the diffusing capacity of NO in the airways and NO concentration in the airway wall. [Pedroletti C *et al.* 2003] Because diffusing capacity correlated with the volume of respiratory anatomic dead space in control subjects and exhaled NO correlates with diffusion capacity in asthmatic children, the findings suggested that a larger part of the bronchial tree produces NO in asthmatic children than in control children. Interestingly, airway diffusing capacity has been shown to correlate inversely with FEV₁ and FVC, suggesting that this parameter may reflect physiological changes in the airways that do not respond to inhaled corticosteroids. [Shin HW *et al.* 2004b]

Bronchial NO flux also appears to be relatively high in patients with asthma. In the study by Silkoff and co-workers, NO flux was 6512 pL/s in patients with asthma who were not receiving corticosteroids, compared with 1020 pL/s in healthy controls. [Silkoff PE *et al.* 2000] Corticosteroid treatment was associated with a reduction in NO flux (2416 pL/s) and the parameter was correlated to lung function. Gelb *et al.* found that abnormal large airway NO flux was associated with an increased risk of asthma (relative risk [RR] 2.4, $p = 0.04$). [Gelb AF *et al.* 2006] Again, Shin *et al.* were able to confirm the general findings of increased and corticosteroid-responsive bronchial flux, but found no correlation with lung function tests. [Shin HW *et al.* 2004b] Data from another group also suggest no correlation between bronchial flux and lung function. [Gelb AF *et al.* 2004] Although it is clear that corticosteroids have an impact on bronchial flux, how this reduction in flux affects symptoms and long-term outcomes requires further investigation. Malinovschi *et al.* demonstrated increased airway tissue concentration of NO and airway transfer of NO in IgE sensitized individuals. [Malinovschi A *et al.* 2006b]

Shin *et al.* investigated flow-independent NO exchange parameters in steroid-naïve patients with mild intermittent asthma and exercise-induced bronchoconstriction compared with healthy controls. [Shin HW *et al.* 2006b] Maximum airway wall flux and airway diffusing capacity were elevated in subjects with exercise-induced bronchoconstriction compared with controls, while alveolar concentrations of NO were not significantly different between the two groups (Figure IX.4). Exercise challenge reduced the airway wall flux in patients with bronchoconstriction. Changes in lung function, as assessed by spirometry, did not correlate with the airway NO parameters. The findings suggest that elevated exhaled NO at baseline and reduced exhaled NO in acute exercise-induced bronchoconstriction occur by distinct mechanisms and are independent of lung function.

Alveolar NO does not appear to be consistently increased in asthma.

Lehtimäki *et al.* found that alveolar NO levels were similar to healthy individuals and patients with asthma, but were significantly increased in patients with asthma symptoms. [Lehtimäki L *et al.* 2005; Lehtimäki L *et al.* 2000] The same group report that alveolar NO levels are increased in asthma patients with nocturnal symptoms, but not in those who do not have nocturnal asthma. [Lehtimäki L *et al.* 2002] Others have also found that alveolar NO is only increased in asthma patients with recent symptoms. [Mahut B *et al.* 2004a] However, Gelb and colleagues demonstrated that an abnormal increase in alveolar NO concentration increased the risk of asthma (RR 3.0, $p = 0.04$). [Gelb AF *et al.* 2006] Berry *et al.* showed that alveolar NO was increased in patients with refractory asthma (7.1 ppb) in comparison with mild-to-moderate disease (3.4 ppb) and healthy controls (3.4 ppb). [Berry MA *et al.* 2005] Alveolar NO has been shown to correlate to airway remodelling as assessed by TGF- β levels in bronchoalveolar lavage fluid and inflammatory markers. [Mahut B *et al.* 2004b] However, de Blic *et al.* showed no correlation between alveolar NO and bronchial-wall thickness in asthmatic children, although there was a correlation between airway wall NO flux and thickness. [de Blic J *et al.* 2005] Alveolar NO levels appear to respond to oral corticosteroid treatment in patients with asthma refractory to inhaled corticosteroids. [Mahut B *et al.* 2004b] In a study by Parshakis *et al.* alveolar NO was significantly greater in asthmatic children treated with steroids (2.22 ppb) compared with atopic children (1.21 $p = 0.0002$)

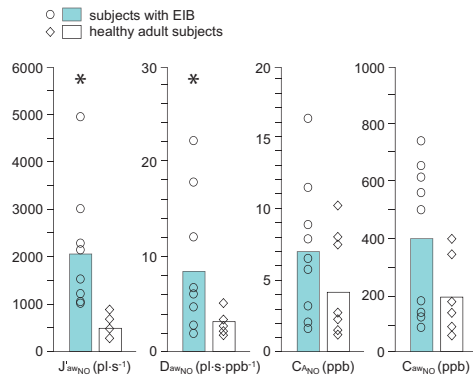


Figure IX.4. Mean values of J'_{awNO} (maximum total volumetric flux of NO from the airways), D_{awNO} (diffusing capacity of NO in the entire airway tree), $C_{A_{wNO}}$ (mixed or average fractional concentration of NO in the gas phase of the alveolar region), C_{awNO} (airway wall concentration of NO), in subjects with exercise-induced bronchoconstriction (solid green bars with open circles) and healthy adults (open bars with open squares)

* Statistically different at baseline from healthy controls [Shin HW *et al.* 2006b]

and healthy controls (1.64 ppb, $p = 0.002$) (Figure IX.5). [Paraskakis E *et al.* 2006] Children with poorly controlled asthma had higher alveolar NO than patients with well-controlled disease ($p = 0.03$). Van Veen showed that patients with oral steroid-dependent asthma had higher alveolar NO levels (2.7 ppb) compared with other patients with severe (0.6 ppb) and mild-to-moderate asthma (0.3 ppb). [van Veen IH *et al.* 2006] In this study, higher levels of alveolar NO were associated with measures of peripheral airway dysfunction.

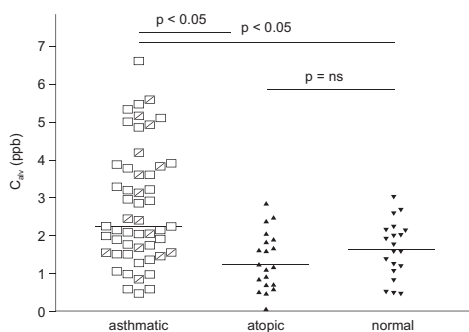


Figure IX.5. Alveolar NO concentration (C_{alv}) in asthmatic, atopic, and normal children [Paraskakis E *et al.* 2006]

Delclaux *et al.* have investigated the association between flow independent NO parameters and hyperresponsiveness in patients with asthma. [Delclaux C *et al.* 2005] They found that airway hyperresponsiveness was mainly associated with an increase in NO of distal origin. This could explain some of the inconsistent results found in correlations of hyperresponsiveness with exhaled NO, as the contribution of distal NO to total exhaled NO is dependent on flow.

9.2.2. Cystic Fibrosis

Patients with CF have an increased diffusing capacity of NO in the airways, decreased mean tissue concentration of NO in the airways, and decreased steady-state alveolar concentration compared to healthy age-matched children. [Shin HW *et al.* 2002]

9.2.3. Allergic Alveolitis

Bronchial NO flux is increased in asthma in comparison with alveolitis and healthy controls. Alveolar NO concentration is higher in alveolitis than in asthma and healthy controls. [Lehtimaki L *et al.* 2001]

9.2.4. Scleroderma

Girgis *et al.* confirmed that bronchial flux of NO was increased in scleroderma (SSc), regardless of whether interstitial lung disease (ILD) and pulmonary hypertension were present. [Girgis RE *et al.* 2002] Alveolar NO was also increased in SSc, regardless of whether ILD or pulmonary hypertension was present.

9.2.5. Liver Cirrhosis

Patients with liver cirrhosis have been shown to have an increased alveolar NO concentration (8.3 ± 0.9 ppb) compared with healthy subjects (4.7 ± 0.3 ppb). [Delclaux C *et al.* 2002; Rolla G *et al.* 2004] In patients who have cirrhosis associated with Sjogren syndrome, bronchial flux is also increased. [Rolla G *et al.* 2004]

9.2.6. Sjogren Syndrome

Sjogren syndrome is an autoimmune disorder that is often associated with other autoimmune conditions. Bronchial flux, but not alveolar NO, is increased in this condition. [Rolla G *et al.* 2004]

9.2.7. COPD

Using a model based on the classic Fick’s first law of diffusion to partition NO in the lungs, the alveolar levels of NO in COPD patients were found to be increased (4 ± 2 ppb; $p < 0.001$) compared with control patients. [Hogman M *et al.* 2002b] This finding has been confirmed by Brindicci *et al.* who demonstrated that alveolar NO increased with increasing severity of COPD. [Brindicci C *et al.* 2005] Thus, such measures may be useful in determining progression of COPD.

9.2.8. Pulmonary Hypertension

Pulmonary hypertension is associated with low levels of exhaled NO. Girgis and co-workers have used a diffusion model to determine the source of this reduction. [Girgis RE *et al.* 2005] Their results show that airway wall concentration is low in patients with pulmonary hypertension compared with healthy controls (33 vs. 104 ppb). Treatment with bosentan increased airway wall NO (and thus exhaled NO, Figure IX.6), suggesting that the reduced NO levels are related to endothelin.

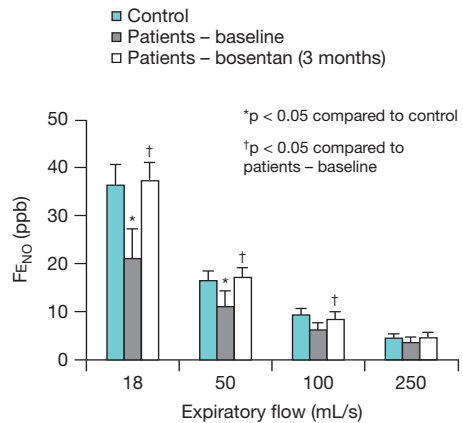


Figure IX.6. Exhaled NO levels at different flow rates in patients with pulmonary hypertension and controls [Girgis RE *et al.* 2005]

9.2.9. PCD

It is established that nasal NO levels are reduced in PCD, but exhaled NO levels are only slightly reduced. Mahut *et al.* have shown that maximum airway wall flux and alveolar NO are both reduced in PCD compared with healthy individuals. [Mahut B *et al.* 2006] Airway wall flux was associated with airflow limitation in this study.

9.2.10. Tobacco Use

A study by Malinowski *et al.* demonstrated that current smokers had lower exhaled NO, airway tissue NO concentration, and total airway NO flux compared with those who had never smoked. [Malinowski A *et al.* 2006a] Ex-smokers also had lower exhaled NO and NO flux than never-smokers. There was a negative association between current smoking and alveolar NO concentration. It appeared that the reduction in exhaled NO levels seen in ex- and current smokers could be attributed to a lower total airway NO flux in ex-smokers and reduced airway and alveolar NO concentrations in current smokers.

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