ELSEVIER
DRUG DISCOVERY TODAY DISEASE MODELS

Drug Discovery Today: Disease Models
Vol. 4, No. 3 2007

*Editors-in-Chief*
**Jan Tornell** – AstraZeneca, Sweden
**Andrew McCulloch** – University of California, SanDiego, USA

Respiratory diseases

# *In silico* modeling of nitric oxide production, transport and consumption in the lungs

David A. Shelley[2,a], Hye-Won Shin[1,2,a], Steven C. George[1,2,*]

[1]Department of Biomedical Engineering, University of California, Irvine, Irvine, CA 92697, United States
[2]Department of Chemical Engineering and Materials Science, University of California, Irvine, Irvine, CA 92697, United States

**Nitric oxide (NO) mediates a vast array of physiological functions in the lungs, and our understanding of these events has improved dramatically over the past decade. Once produced enzymatically and intracellularly, NO can diffuse as a free molecule, or undergo chemical reactions to preserve and alter the biological activity. *In silico* modeling of these non-linear, multiscale and dynamic processes plays a crucial role in our understanding of, and thus our ability to manipulate, these events to treat pulmonary disease.**

**Section Editor:**
Steven C. George – Departments of Biomedical Engineering, and Chemical Engineering and Materials Science, University of California, Irvine, CA 92697-2575, USA

## Introduction

Nitric oxide (NO) is a diatomic free radical that directly mediates or significantly influences an enormous number of major biological functions in the lungs. Examples include pulmonary blood pressure, bronchial smooth muscle tone and host defense (bacteriacidal and mucocillary beat frequency). NO is produced enzymatically from one of three isoforms of nitric oxide synthase (NOS) and intracellularly by numerous resident and transient cell types (e.g. bronchial epithelium and macrophage). Once produced, NO can diffuse as a free molecule to a site of action, or can react rapidly with several substrates to preserve the biological activity. The chemical reactions influence intracellular processes, neighboring cells (paracrine functions) and whole organ functions (pulmonary blood pressure). As a result, NO metabolism and transport in the lungs are dynamic, non-linear, and occur over multiple length scales. A complete picture of NO metabolism in the lungs will emerge only by combining experimental (*in vivo* and *in vitro*) observations and *in silico* modeling. An excellent review of fundamental NO biochemistry, physiological measurement of NO and early models of NO biotransport in several organ systems appeared in 2001 [1]. The present review will focus on *in silico* modeling efforts aimed at understanding NO production, consumption and transport in the lungs at the micro-, meso- and macroscopic levels.

## Microscopic modeling

We will limit our discussion of microscopic events to those that occur intracellularly, transmembrane or within biochemical networks, and thus over length scales on the order of microns or less. The earliest reports of the biological action of NO were related to its role as the endothelium-derived relaxing factor (EDRF). Thus, much of the early modeling efforts focused on the production of NO by the vascular endothelium and diffusion to the site of action, in this case the vascular smooth muscle. The later models will be discussed in detail in Section 'Mesoscopic modeling'; however,

*Corresponding author: S.C. George (scgeorge@uci.edu)
[a] Contributed equally to this manuscript.

1740-6757/$  DOI: 10.1016/j.ddmod.2007.06.006

related to this function is the rapid reaction of NO with hemoglobin within the erythrocyte.

The observation that NO reacts extremely fast with hemoglobin (diffusion-limited) created a paradox in which potentially a large amount of NO produced from the endothelium would be scavenged by the large sink of hemoglobin present in the adjacent blood. While a fraction of NO produced by the endothelium may reach the vascular smooth muscle, it was perplexing that NO production might so inefficient. The relevance to pulmonary physiology was the potential link to idiopathic pulmonary hypertension.

Several research groups hypothesized that since hemoglobin is present within the red blood cell (RBC), the RBC may present a significant barrier for diffusion of NO from the endothelium to hemoglobin. These groups were able to demonstrate that the presence of the RBC slowed the effective rate of reaction of NO with hemoglobin by several orders of magnitude, but were not able to determine whether this experimental observation was due to an unstirred layer on the outside of the cell (extracellular), an intrinsic resistance in the membrane, or a resistance within the cytosol of the RBC (intracellular) [2–4].

Vaughn *et al.* [5] described an elegant single cell model of NO transport through the RBC membrane and subsequent reaction with hemoglobin in an effort to quantify the intrinsic RBC resistance to NO consumption. They conserved NO in the extracellular and intracellular space and utilized a simple one-dimensional reaction-diffusion model assuming a pseudosteady state and neglecting convective transport. They demonstrated that either the membrane permeability or the resistance in the cytosol was ~2100 times smaller than the permeability of a lipid bilayer or the rate of reaction of NO with oxyHb in free solution. While they could distinguish between these two parameters, they speculated that the resistance was due to a reduced membrane permeability. Nonetheless, their model was able to quantify the magnitude of RBC resistance to NO consumption and was used in subsequent mesoscopic models of intercellular NO diffusion.

At least four groups have assimilated rate expressions into integrated reaction networks to gain insight into the dynamics of the production and consumption of NO and related intermediates [6–11]. Many of the individual rate constants that describe the chemical reactions involving NO have been quantified. Figure 1 depicts a typical reaction network for NO from Hu *et al.* [8]. A common feature of these models is a system of coupled ordinary differential equations describing the concentrations of chemical species as a function of time, which takes the general form,

$$\frac{d\mathbf{C}}{dt} = \mathbf{KC} \qquad (1)$$

where **C** and **K** represent the matrices of species concentrations and reaction rate constants, respectively. In each case,

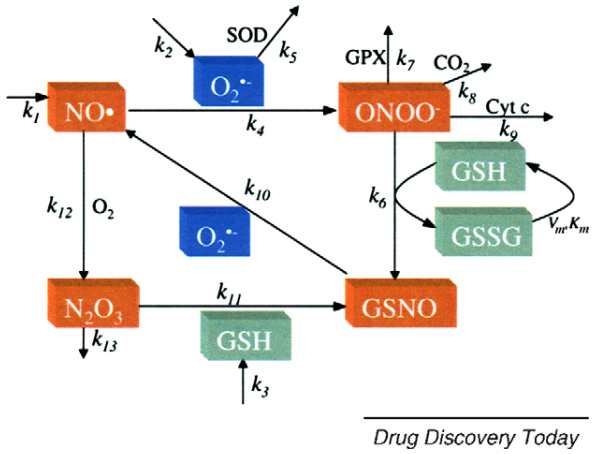


**Figure 1.** Reaction network of NO as described by Hu *et al.* [8] with numerous intermediates including $O_2^-$ (superoxide), $ONOO^-$ (peroxynitrite), GSNO (*S*-nitrosoglutathione), $N_2O_3$, GSH (glutathione) and GSSG (reduced glutathione). Thirteen reaction rate constants, $k_x$ ($1 < x < 13$), describe the dynamics of each reaction. SOD: superoxide dismutase; GPX: glutathione peroxidase; cyt *c*: cytochrome *c*. Reprinted with permission from Springer Science.

the model assumes a homogeneous cytosol, and thus does not consider spatial gradients, molecular diffusion or compartmentalization of enzymes or substrates.

Condorelli and George [7] described a kinetic model for the activation of soluble guanylyl cyclase by free NO and predicted that the concentration of NO necessary to activate sGC was much smaller than previously thought, and that the activation of sGC was much more rapid than the deactivation. Yang *et al.* [11] was also interested in the activation of sGC by NO, but focused on the production of cGMP and its subsequent effect on the calcium activated potassium channel in the vascular smooth muscle cell.

Ray and Kirschner [9] were interested in the activation of macrophages and the role of NO in this process. They predicted that NO production by the macrophage negatively regulates multiple activation pathways to achieve a balance between quiescence and activation. Hu *et al.* [8] used a similar approach to present a kinetic network model based on the conservation of seven species: NO, $O_2^-$ (superoxide), $ONOO^-$ (peroxynitrite), GSNO (*S*-nitrosoglutathione), $N_2O_3$, GSH (glutathione) and GSSG (reduced glutathione) (Fig. 1). The resulting system of seven coupled ordinary differentials equations was solved to address the role of NO, $O_2^-$ and GSH production on the level of $N_2O_3$ and hence damaging nitrosation. They predicted that GSH may act as a critical switch for $N_2O_3$ concentration – a novel mechanism that may have implications for lung function.

An earlier model by Shin and George [10] was specifically aimed at understanding NO production, consumption and transport in the airway epithelium as a mechanism leading to NO in the exhaled breath. This model was the first to examine

molecular diffusion of NO between neighboring compartments (airway epithelial cell and the mucus layer), and thus considered the physical dimensions of the compartments.

More recently, the first model to address cellular compartmentalization of enzymes and substrates relevant to NO production and consumption was presented. Chen and Popel [6] were interested in NO production and release from the endothelial isoform of NOS from endothelial cells and considered the impact of distributing the source at the membrane as opposed to being uniformly distributed throughout the cytosol. They demonstrated that compartmentalization of eNOS did not impact NO production (and hence release), but that this result depended on the assumption that all substrates are present in excess – an assumption that may break down in numerous cellular states.

A major feature of the microscopic models of NO production, consumption and transport is the characterization of individual reaction rate constants, and how these chemical reactions behave within a network. At this point, the rate constants are measured experimentally in isolation of the intracellular network and do not adequately consider the compartmentalization of substrates and enzymes (see Outstanding issues). Advanced experimental measurements to determine the dynamic nature of substrate and enzyme compartmentalization will be necessary before improved microscopic models of NO metabolism and transport are possible.

## Mesoscopic NO modeling

While subcellular and whole organ modeling can provide unique insights into NO biology, there are important dynamics of NO biology that fall between these microscopic and macroscopic views. In particular, NO has long been thought to be produced by epithelial layers (e.g. the vascular endothelium) and diffuse either as a free molecule or as a stable intermediate (e.g. GSNO), to a distant cell type. The distance separating the source and the site may be on the order of mm.

Since accurate measurement of NO concentration *in situ* is difficult to measure over this distance, the problem is ideally suited for mathematical modeling, involving simultaneous production, diffusion and/or convection, and reaction of NO with multiple substrates in and around the vessel lumen. The question that initiated this modeling is how an NO source (endothelium) located adjacent to an NO sink (hemoglobin) can affect smooth muscle tone via activation of soluble guanylate cyclase (sGC) and is relevant to both the pulmonary and bronchial circulations.

The earliest models of this system were introduced by Vaughn *et al.* [12] and Butler *et al.* [13]. Fig. 2 depicts a typical structure of a mesoscopic model to investigate NO transport from the endothelium to the smooth muscle. Both models utilize a cylindrical geometry with the vessel lumen in the center, surrounded by the endothelium and an outer abluminal region containing the vascular smooth muscle. NO is generated by the endothelium at both the luminal and abluminal surfaces, where it can diffuse into the blood to react with hemoglobin, or into the smooth muscle to react with various possible substrates. The governing equation is a standard unsteady convective-diffusion equation with chemical reaction,

$$\frac{\partial C_{NO}}{\partial t} = D\nabla^2 C_{NO} - \nabla C_{NO} v - V_{NO} \quad (2)$$

where $C_{NO}$ is the concentration of NO in the tissue, $v$ the convective flow of NO, $D$ the molecular diffusion coefficient of NO in tissue and $V_{NO}$ is the rate of NO consumption owing to chemical reaction. Both authors account for the physiological properties of blood flow; red blood cells are located in the center of the lumen, surrounded by an RBC-free region of plasma. Using this relatively simple model, steady-state NO profiles in and around the vessel lumen can be generated by neglecting the time derivative, allowing for prediction of the effective diffusion distance of NO into the smooth muscle region. This distance bounds the region where NO concentrations are greater than $K_m$ (the Michelis–Menten rate constant defining the concentration at which the turnover of an enzyme is 50% of the maximum rate) of sGC. Accounting for the RBC-free zone adds a layer of diffusive resistance between the sites of NO production (endothelial cells) and consumption (hemoglobin in the red blood cell), which increases NO concentrations in the smooth muscle region.

Using these initial models as a starting point, several groups have added various levels of complexity to further characterize the system. Condorelli and George [14] developed a model with a structure similar to the Butler model. However, rather than simulate diffusion into an infinite space, this model adds an outer boundary to represent the airway wall and airway lumen, making the model more specific to the human pulmonary system. Additionally, a lower value for the $K_m$ of sGC was taken into account based on earlier work by Condorelli and George [7]. This model is used to demonstrate that NO produced by the airway epithelium may impact bronchial smooth muscle tone; the authors also hypothesize an optimal vessel radius and that intermittent NO signaling may increase the efficiency of sGC activation.

Tsoukias *et al.* [15] created a transient model to test the hypothesis that burst-like release of NO could affect vascular smooth muscle tone, as well as reduce NO scavenging by hemoglobin. The authors include the time-derivative of NO concentration; the governing equation becomes an unsteady convective-diffusive equation with chemical reaction in cylindrical coordinates (i.e. Eq. (2)). The authors conclude that transient burst-like NO release is a more efficient method of sGC activation and that frequency-dependent control of

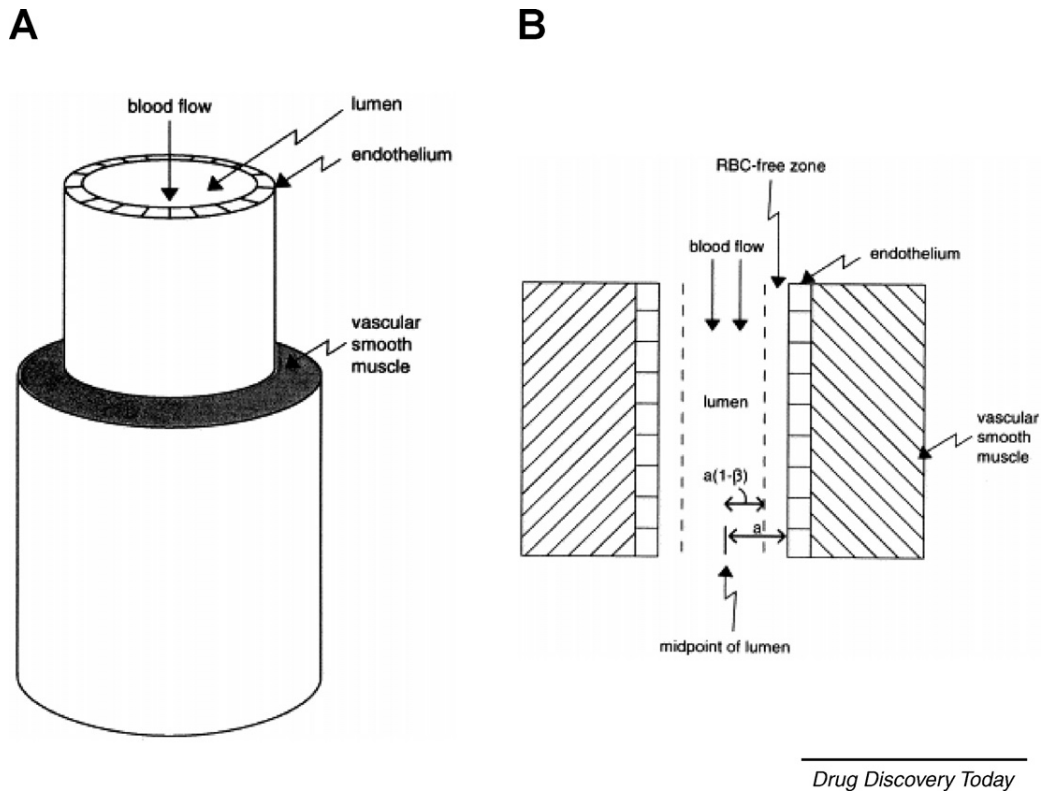


**Figure 2.** Model schematics of the vascular endothelium, lumen and surrounding smooth muscle presented in 3-dimensional (A) and 2-dimensional (B) coordinates [13]. NO is released at the luminal and abluminal surfaces of the endothelium and diffuses into the blood or smooth muscle regions. This system can be modeled using the convective-diffusion-reaction equation (Eq. (2)) in cylindrical coordinates. Reprinted with permission from Elsevier Science.

cGMP, a downstream product of sGC activation, is possible. The authors hypothesize this burst-like release could be tied to experimentally observed $Ca^{2+}$ oscillations.

Kavdia *et al.* [16] developed a model to simulate the effect of hemoglobin-based oxygen carriers (HBOC) as a blood substitute on sGC activation. The authors hypothesized that HBOC would consume NO at a much faster rate than naturally occurring hemoglobin in RBCs, causing lower amounts of NO to reach the smooth muscle resulting in hypertension. As in previous models, the authors simulate the RBC-free region adjacent to the endothelium; however, they include a reaction term to account for the HBOC circulating in the plasma. This exogenous hemoglobin is directly adjacent to the endothelium and is not contained within RBCs, removing two diffusive barriers between the sites of NO production and consumption. This results in significantly less NO diffusing away from the lumen and a severely reduced ability to activate sGC.

To understand which diffusion barrier is most significant in limiting NO consumption by hemoglobin, El-Ferra *et al.* [17] introduced a distributed multicellular model. The diffusion barriers consist of the RBC depleted zone near the endothelium surface, the diffusion layer (unstirred layer) surrounding each RBC, and the resistance of the RBC membrane. The model structure contains an RBC-free region adjacent to the endothelium, as well as simulated red blood cells within the lumen core. The number of RBCs is based on a typical hematocrit value, and the RBC distribution is randomized within the core. This modeling led the authors to the conclusion that the RBC membrane resistance is the limiting factor for NO consumption by hemoglobin and is consistent with the microscopic models of the red blood cell described earlier.

More recent advances in modeling NO in the microcirculation have incorporated coupled $NO/O_2$ transport [18,19]. This work is similar to previous models, with the addition of transport equations for oxygen. The modeling of coupled $NO/O_2$ transport is used to show that concentration changes of one species can have a dramatic impact on the transport of the other species [19]. Chen *et al.* [18] added more complexity by modeling the dynamics of coupled $NO/O_2$ transport with an arteriole-venule pair, a fashion similar to Kavidia *et al.* [20]. These models predict that decreasing the distance between the arteriole and venule causes a higher NO concentration in the venular wall than the arteriole wall. They also demonstrate that the addition of capillaries in the surrounding tissue significantly increases NO concentration in the arteriole wall.

Buerk *et al.* [21] derived a model that accounts for the competition between superoxide dismutase and NO on

superoxide scavenging, coupled with NO transport from the vascular endothelium. Using Eq. (2) in cylindrical coordinates, the authors add kinetic equations for NO dependence on oxygen, superoxide, peroxynitrite, hydrogen peroxide, nitrite and nitrate. This model is more comprehensive than previous works, incorporating microscale reaction networks in a mesoscale model involving transport between multiple tissue regions. Buerk's model provides detailed insight into

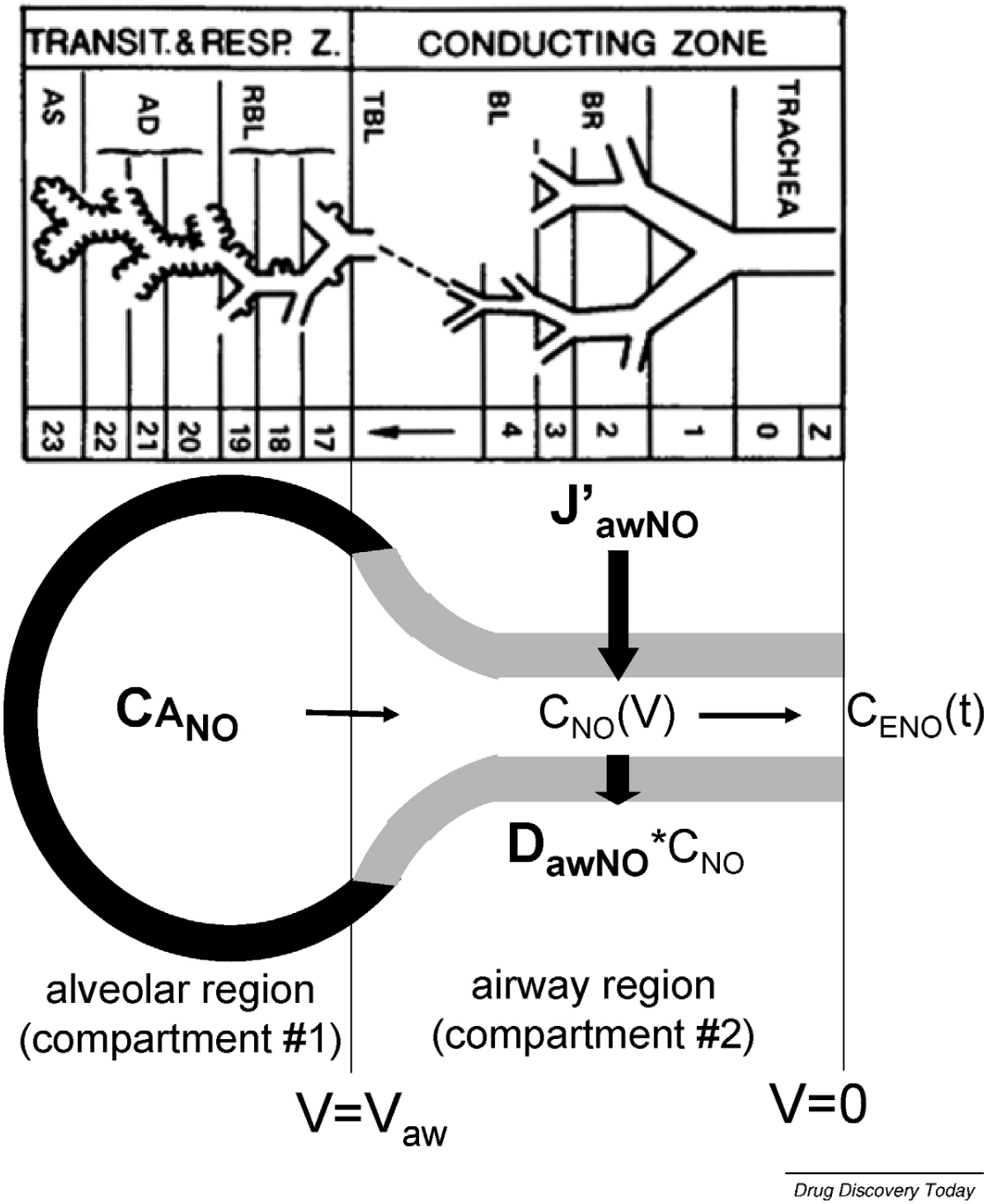


**Figure 3.** During exhalation, a steady state alveolar or peripheral concentration, $Ca_{NO}$, enters the airway compartment whereupon additional NO is transferred from the airway walls. The alveolar or peripheral concentration represents the acinar region of the lungs (Weibel generations 17–23, from [42], reprinted with permission from Springer-Verlag). The airway compartment represents the oropharynx and Weibel generations 0–16, and considers the increasing surface area per unit volume of the airway tree (i.e. trumpet shape). The contribution from the airways depends on the exhalation flow and is the sum of two terms: $J'aw_{NO} - Daw_{NO}C_{NO}$. $J'aw_{NO}$ is the maximum airway flux (pl/s). $Daw_{NO}$ is the airway diffusing capacity (or conductance). $C_{NO}$ is the airway compartment gas phase NO concentration which depends on axial volumetric position ($V$), and the airway compartment volume is $Vaw$ (BR: bronchus; BL: bronchiole; TBL: terminal bronchiole; RBL: respiratory bronchiole; AD alveolar duct; AS: alveolar sac).

the biochemical pathways affecting NO transport and provides a framework for future investigations into the relative importance of each pathway.

Mesoscopic modeling is a useful tool to gain insight into systems that cannot easily be explored directly. While significant advances have been made in NO modeling in the microcirculation, the literature lacks a mathematical analysis of how this system changes in disease states relevant to the lungs, such as asthma, where NO metabolism is significantly altered. In addition, future models must continue the trend started by Buerk *et al.* [21] that incorporates microscale information into the mesoscale model (see Outstanding issues).

## Macroscopic modeling

Macroscopic modeling includes the synthesis of production, transport and consumption of NO over length scales that represent features of the entire lung (order centimeters). This scale is dominated by models of NO gas exchange in the airway and alveolar regions of the lungs to understand the dynamics of NO that appears in the exhaled breath. NO was first detected in the exhaled breath of humans more than a decade ago [22] and remains a promising noninvasive biomarker of lung inflammation [23]. Substantial evidence suggests that both the airway and alveolar regions are significant sources of exhaled NO in contrast to other respiratory gases such as $CO_2$ that is evolved predominantly in the alveolar compartment. This unique feature of exhaled NO generates a significant flow-dependence [24,25], and thus, requires new analytical techniques (i.e. models) to characterize.

NO exchange in the human lung was first described by Hyde *et al.* using a simple one-compartment model to understand the consumptions kinetics of inspired NO [26]; however, this model could not explain the exhaled flow dependence of endogenously produced NO. Tsoukias and George reported the first two-compartment model (Fig. 3) that captured the essential experimental observations of exhaled NO, by lumping the branching airway tree into a single path cylinder with linearly increasing surface area per unit airway volume and the alveoli into a flexible balloon [25]. The governing equation is a modified version of the convective-diffusion equation with chemical reaction shown in Eq. (2). Several similar models followed shortly thereafter [27–29]. In each case, the alveolar region is described with an alveolar concentration ($Ca_{NO}$) and the airway region is described using two parameters, the airway diffusing capacity ($Daw_{NO}$) and either the airway wall concentration ($Caw_{NO}$) or the maximum airway wall flux ($J'aw_{NO}$; equal to the product $Daw_{NO}Caw_{NO}$). A recent review describes this model in greater detail and the analytical methods (i.e. breathing maneuvers combined with the mathematical model) used to estimate the airway and alveolar parameters [30].

With the exception of the model by Tsoukias and George, the early models neglected the trumpet shape of the airways and assumed a cylindrical shape, and all of the early models neglected axial (longitudinal) diffusion of NO in the gas phase. More recently, experimental and theoretical evidence demonstrated that these physical and anatomical features of NO exchange were not negligible and should be considered in more comprehensive models of NO exchange [31–34]. In the past 2 years, Shin *et al.* [35] and Condorelli *et al.* [36] have presented simplified techniques that included a two-compartment model that considered both axial diffusion and the trumpet shape in combination with a series of breathholds (convective term neglected in Eq. (2)) or a series of constant flow steady state (time derivative neglected in Eq. (2)) breathing maneuvers.

Although the most recent NO exchange model has incorporated axial diffusion and the trumpet shape of the airway tree, a more advanced model to characterize NO exchange in diseased airways such as asthma is necessary. Perhaps the next most important step is to consider ventilation and NO production heterogeneity (see Outstanding issues). There is significant experimental evidence that proximal and distal airways in asthma empty and fill in a heterogeneous pattern [37,38] and that NO production may also vary significantly within different regions of the airways [39–41].

## Model translation to humans

The translation of the NO production, consumption and transport models to humans is well underway at the macroscopic level. The use of exhaled NO concentration is rapidly entering the clinical arena, and the analytical instrument produced by Aerocrine, Ltd (Sweden) has received FDA approval. Furthermore, numerous studies have applied the two-compartment model to disease or altered physiological states such as asthma, sarcoidosis, COPD, cystic fibrosis, lung transplant and smoking. The next two critical steps are to (1) align specific features of the model to features of a disease (e.g. heterogeneous ventilation patterns in asthma) and (2) integrate microscopic and mesoscopic experimental data and models into the macroscopic model to gain a systems-level understanding of NO metabolism in the lungs (see Outstanding issues).

## Conclusions

Over the past 20 years our knowledge of the physiological and biological functions that NO mediates in the lungs has steadily increased. It is clear now that NO has major roles in vascular tone (bronchial and pulmonary circulations), bronchomotor tone and the immune response. As such, manipulating NO biology pharmacologically has exciting opportunities to modulate the pathology of such prevalent diseases as asthma and COPD. A critical step in our understanding has been *in silico* modeling of NO production, transport and consumption at significantly different length

scales. Further integration of micro-, meso- and macroscopic models with experimental observations promises a more complete understanding of NO biology in the lungs.

### Outstanding issues

- Experimental data on intracellular compartmentalization of enzymes and substrates which manipulate NO biology.
- Microscopic models which integrate enzymatic and non-enzymatic chemical reactions of nitrogen oxide intermediates.
- Experimental data on NO production rates from specific cell types *in vitro* and *in vivo*.
- Integration of microscopic experimental data and models into mesoscopic and macroscopic models of NO production, transport and consumption.
- Macroscopic models of NO metabolism which are more closely aligned with the pathophysiology of lung diseases.

## Acknowledgement

This work was supported by grants from the National Institutes of Health (HL070645, HL67954).

## References

1 Buerk, D.G. (2001) Can we model nitric oxide biotransport? A survey of mathematical models for a simple diatomic molecule with surprisingly complex biological activities *Annu. Rev. Biomed. Eng.* 3, 109–143
2 Liu, X. *et al.* (1998) Diffusion-limited reaction of free nitric oxide with erythrocytes. *J. Biol. Chem.* 273, 18709–18713
3 Liu, X. *et al.* (2002) Nitric oxide uptake by erythrocytes is primarily limited by extracellular diffusion not membrane resistance. *J. Biol. Chem.* 277, 26194–26199
4 Vaughn, M.W. *et al.* (2000) Erythrocytes possess an intrinsic barrier to nitric oxide consumption. *J. Biol. Chem.* 275, 2342–2348
5 Vaughn, M.W. *et al.* (2001) Erythrocyte consumption of nitric oxide: competition experiment and model analysis [Erratum in: *Nitric Oxide* 2001 August;5(4):425]. *Nitric Oxide* 5, 18–31
6 Chen, K. and Popel, A.S. (2006) Theoretical analysis of biochemical pathways of nitric oxide release from vascular endothelial cells. *Free Radic. Biol. Med.* 41, 668–680
7 Condorelli, P. and George, S.C. (2001) In vivo control of soluble guanylate cyclase activation by nitric oxide: a kinetic analysis. *Biophys. J.* 80, 2110–2119
8 Hu, T.M. *et al.* (2006) Kinetic modeling of nitric-oxide-associated reaction network. *Pharm. Res.* 23, 1702–1711
9 Ray, J.C. and Kirschner, D.E. (2006) Requirement for multiple activation signals by anti-inflammatory feedback in macrophages. *J. Theor. Biol.* 241, 276–294
10 Shin, H-W. and George, S.C. (2001) Microscopic modeling of NO and S-nitrosoglutathione kinetics and transport in human airways. *J. Appl. Physiol.* 90, 777–788
11 Yang, J. *et al.* (2005) Mathematical modeling of the nitric oxide/cGMP pathway in the vascular smooth muscle cell. *Am. J. Physiol. Heart Circ. Physiol.* 289, H886–H897
12 Vaughn, M.W. *et al.* (1998) Effective diffusion distance of nitric oxide in the microcirculation. *Am. J. Physiol.* 274 (5 Pt 2), H1705–H1714
13 Butler, A.R. *et al.* (1998) Diffusion of nitric oxide and scavenging by blood in the vasculature. *Biochim. Biophys. Acta* 1425, 168–176
14 Condorelli, P. and George, S.C. (2002) Free nitric oxide diffusion in the bronchial microcirculation. *Am. J. Physiol. Heart Circ. Physiol.* 283, H2660–H2670
15 Tsoukias, N.M. *et al.* (2004) A theoretical model of nitric oxide transport in arterioles: frequency- vs. amplitude-dependent control of cGMP formation. *Am. J. Physiol. Heart Circ. Physiol.* 286, H1043–H1056
16 Kavdia, M. *et al.* (2002) Model of nitric oxide diffusion in an arteriole: impact of hemoglobin-based blood substitutes. *Am. J. Physiol. Heart Circ. Physiol.* 282, H2245–H2253
17 El-Farra, N.H. *et al.* (2003) Analysis of nitric oxide consumption by erythrocytes in blood vessels using a distributed multicellular model. *Ann. Biomed. Eng.* 31, 294–309
18 Chen, X. *et al.* (2007) A model of NO/O(2) transport in capillary-perfused tissue containing an arteriole and venule pair. *Ann. Biomed. Eng.* 35, 517–529
19 Lamkin-Kennard, K.A. *et al.* (2004) Interactions between NO and O2 in the microcirculation: a mathematical analysis. *Microvasc. Res.* 68, 38–50
20 Kavdia, M. and Popel, A.S. (2006) Venular endothelium-derived NO can affect paired arteriole: a computational model. *Am. J. Physiol. Heart Circ. Physiol.* 290, H716–H723
21 Buerk, D.G. *et al.* (2003) Modeling the influence of superoxide dismutase on superoxide and nitric oxide interactions, including reversible inhibition of oxygen consumption. *Free Radic. Biol. Med.* 34, 1488–1503
22 Gustafsson, L.E. *et al.* (1991) Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochem. Biophys. Res. Commun.* 181, 852–857
23 Deykin, A. *et al.* (2002) Exhaled nitric oxide as a diagnostic test for asthma: online versus offline techniques and effect of flow rate. *Am. J. Respir. Crit. Care Med.* 165, 1597–1601
24 Silkoff, P.E. *et al.* (1997) Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. *Am. J. Respir. Crit. Care Med.* 155, 260–267
25 Tsoukias, N.M. and George, S.C. (1998) A two-compartment model of pulmonary nitric oxide exchange dynamics. *J. Appl. Physiol.* 85, 653–666
26 Hyde, R.W. *et al.* (1997) Determination of production of nitric oxide by lower airways of humans-theory. *J. Appl. Physiol.* 82, 1290–1296
27 Hogman, M. *et al.* (2000) Exhaled nitric oxide partitioned into alveolar, lower airways and nasal contributions. *Respir. Med.* 94, 985–991
28 Pietropaoli, A.P. *et al.* (1999) Simultaneous measurement of nitric oxide production by conducting and alveolar airways of humans. *J. Appl. Physiol.* 87, 1532–1542
29 Silkoff, P.E. *et al.* (2000) Airway nitric oxide diffusion in asthma: Role in pulmonary function and bronchial responsiveness. *Am. J. Respir Crit. Care Med.* 161 (4 Pt 1), 1218–1228
30 George, S.C. *et al.* (2004) Modeling pulmonary nitric oxide exchange. *J. Appl. Physiol.* 96, 831–839
31 Shin, H.W. *et al.* (2006) Examining axial diffusion of nitric oxide in the lungs using heliox and breath hold. *J. Appl. Physiol.* 100, 623–630
32 Shin, H.W. *et al.* (2004) Probing the impact of axial diffusion on nitric oxide exchange dynamics with heliox. *J. Appl. Physiol.* 97, 874–882
33 Shin, H.W. and George, S.C. (2002) Impact of axial diffusion on nitric oxide exchange in the lungs. *J. Appl. Physiol.* 93, 2070–2080
34 Van Muylem, A. *et al.* (2003) Modeling of impact of gas molecular diffusion on nitric oxide expired profile. *J. Appl. Physiol.* 94, 119–127
35 Shin, H.W. *et al.* (2005) A new and more accurate technique to characterize airway nitric oxide using different breath-hold times. *J. Appl. Physiol.* 98, 1869–1877
36 Condorelli, P. *et al.* (2007) A simple technique to characterize proximal and peripheral nitric oxide exchange using constant flow exhalations and an axial diffusion model. *J. Appl. Physiol.* 102, 417–425
37 Venegas, J.G. *et al.* (2005) Self-organized patchiness in asthma as a prelude to catastrophic shifts. *Nature* 434, 777–782
38 Verbanck, S. *et al.* (1999) Evidence of acinar airway involvement in asthma. *Am. J. Respir. Crit. Care Med* 159 (5 Pt 1), 1545–1550
39 Condorelli, P. *et al.* (2004) Characterizing airway and alveolar nitric oxide exchange during tidal breathing using a three-compartment model. *J. Appl. Physiol.* 96, 1832–1842
40 DuBois, A.B. *et al.* (1999) Nitric oxide production and absorption in trachea, bronchi, bronchioles, and respiratory bronchioles of humans. *J. Appl. Physiol.* 86, 159–167
41 Silkoff, P.E. *et al.* (1998) A significant proportion of exhaled nitric oxide arises in large airways in normal subjects. *Respir. Physiol.* 113, 33–38
42 Weibel, E. (1963) *Morphometry of the Human Lung,* Springler-Verlag