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Invited Editorial

How accurately should we estimate the anatomical source of exhaled nitric oxide?

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FOR MORE THAN A DECADE it has been recognized that nitric oxide (NO) appears in the exhaled breath and the level is altered in numerous pulmonary diseases in which inflammation plays an integral role (e.g., asthma) (1, 8). Thus the exhaled NO signal has the potential to uniquely delineate the contribution of inflammatory processes to lung disease in a noninvasive manner complementing more traditional measurements of lung function, namely, lung volumes and expiratory airflow that focus solely on structural properties of the respiratory system. This potential, combined with the relative ease with which it can be detected and the serious and ongoing epidemic of asthma and other chronic lung diseases, has imbued the measurement of exhaled NO with the promise of becoming a useful clinical tool. However, this promise has not yet been fulfilled. Skepticism regarding the measurement of exhaled NO persists due to both its variability among clinically similar subjects and the inconsistent correlations with other indexes of lung function and symptoms. This skepticism is appropriate and stems both from the relatively crude techniques currently employed to characterize exhaled NO and from our still incomplete understanding of the fundamental biological mechanisms that determine the appearance of NO in the exhaled breath.

Since the initial observation that NO appears in the exhaled breath, several research groups have made seminal contributions toward our understanding of the unique features of NO exchange in the lungs. In particular, exhaled NO has sources from both the airway and alveolar regions, which has been determined from a combined approach implementing experimental observations (5, 7, 15, 19) and "two-compartment" mathematical models (9, 14, 20, 21). The present clinical approach for exhaled NO measures the concentration during a vital capacity maneuver while holding expiratory flow and pressure constant (2). The recommended exhalation flow is low enough (50 ml/s) to cause the concentration to be predominantly of airway origin and is thus ineffective at describing the lower alveolar concentration of NO, ignoring this potentially important signal.

Although asthma is traditionally thought to be an inflammatory disease of the airways, several groups have employed the two-compartment model of NO exchange and reported an elevated alveolar concentration of NO during periods of enhanced symptoms (11, 13), or in patients who are refractory to inhaled corticosteroids and bronchodilators (3, 6, 12). The observations of increased alveolar NO are particularly relevant as these patients have proven to be difficult to manage, are hospitalized more frequently, and could well benefit from early detection of disease exacerbation and alternate therapeutic regimens.

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Since the alveolar concentration cannot be directly measured, estimating the alveolar concentration requires a model of NO exchange in the lungs that, when combined with experimental measurements, can partition the exhaled NO signal into proximal and peripheral contributions. This feature is frequently neglected when the "alveolar concentration" or "airway flux" of NO is reported, and we are led to believe that these numbers are direct experimental measurements. This concept is not new to the physiology community. Other examples include the Fick method to determine cardiac output, and the measurement of lung diffusing capacity. Buried in these measurements are mathematical models approximating the physiology. In fact, the accuracy of these estimates depends not only on the accuracy of the model but also on the experimental protocol (i.e., algorithm) and instrumentation. It is therefore pertinent in our quest to interpret exhaled NO to consider the question: how accurately can the anatomic source of NO be estimated, and at what cost? In general, as both the computational complexity and accuracy of an algorithm and model increase, the ease of clinical translation decreases.

The initial two-compartment models were extremely simple in structure, essentially describing NO gas exchange using a single expansile balloon (alveolar region) connected to a rigid tube (airways). The algorithms were equally simple involving linear fits of experimental measurements in which the slope and intercept reflected region-specific (i.e., alveolar) NO parameters (9, 14, 20, 22). While these early models were elegant in their simplicity and ability to explain the strong flow dependence of exhaled NO, they neglected potentially important physical and physiological phenomena such as axial (or longitudinal) gas phase diffusion, the trumpet shape of the airway cross-sectional area, and spatial heterogeneity in flow.

Recently, more advanced models have been developed (18, 23) and validated with new experimental measurements (16, 17) demonstrating the importance of axial diffusion of NO. In particular, the airway source of NO is large enough to create an axial gradient in NO concentration that leads to diffusion of NO from the airway tree into the alveolar region (i.e., "back-diffusion"). In other words, the alveolar region can serve as a sink for airway NO; and conversely, NO from the airway tree can contaminate the alveolar region, leading to a falsely elevated estimate of the alveolar concentration. We recently quantified this potential effect and presented a simple method to account for axial diffusion of NO on the estimation of the alveolar concentration (4); however, we only tested the model in healthy subjects. Two new studies (10, 24) are presented in the *Journal of Applied Physiology*, both of which elegantly combine experimental measurements and a mathematical model of NO exchange that advance our knowledge and understanding of NO gas exchange and thus our interpretation of the exhaled NO signal.

Kerckx and colleagues (10) have independently developed a similar method to account for axial diffusion of NO into the

alveolar region. Their technique involves a simple correction that requires knowledge of the exhaled concentration at a flow of 50 ml/s ($F_{ENO,50}$) and an estimate of the alveolar concentration (acquired from the analysis of multiple constant-flow exhalations). The technique utilizes the same trumpet model, requires qualitatively the same information, and leads to similar estimations of the alveolar concentration in healthy subjects as that described by Condorelli et al. (4). However, Kerckx and colleagues (10) demonstrate for the first time that the alveolar NO concentration in mild well-controlled (unobstructed) asthmatic subjects is not elevated. Importantly, if axial diffusion of NO is neglected using the two-compartment model, the predicted alveolar concentration of the asthmatic subjects with an elevated $F_{ENO,50}$ (>50 parts per billion) in this study would be artificially elevated.

Verbanck and colleagues (24) present a second study from the same group of investigators in which they further test the model of NO exchange, which accounts for axial diffusion in a group of healthy subjects at baseline and following a histamine challenge. The histamine challenge reduces $F_{ENO,50}$, and the magnitude of the reduction correlates with a decrease in forced expiratory flow after exhalation at 25–75% forced vital capacity ($FEF_{25–75}$), but does not correlate with forced expiratory volume in 1 s (FEV_1). The authors then use the mathematical model to predict that this reduction is consistent with bronchoconstriction in airway generations 10–15, suggesting that $F_{ENO,50}$ may be a specific marker of inflammation in the small airways during bronchoconstriction. They are able to rule out even smaller airways due to the interesting prediction that bronchoconstriction in airway generations >15 reduces the cross-sectional area of the airway tree and actually increases exhaled NO by reducing the loss of airway NO to the alveolar region by backdiffusion (the net rate of axial diffusion is proportional to the cross-sectional area).

The implications of broadening our understanding of the unique gas-exchange mechanisms of NO using a combined approach of experiment and modeling are significant. NO clearly arises from multiple anatomic locations in the lungs, and current methods have only begun to explore this potential. The current American Thoracic Society/European Respiratory Society guidelines recommend measuring the exhaled concentration at a single constant flow of 50 ml/s (i.e., $F_{ENO,50}$), a remarkably simple protocol, and thus easily translatable to the clinic, but may be limiting the potential to utilize exhaled NO as an inflammatory marker. While we understand that exhaled NO is predominantly from the airways at this flow, Verbanck and colleagues (24) suggest that we might more precisely locate the airway source of NO with an accurate mathematical model. Similarly, Kerckx and colleagues (10) use the same model to enhance our understanding of how to accurately determine the small, but potentially important, alveolar concentration.

Further testing of these algorithms and models are clearly required. The potential exists to phenotype asthmatics by airway and alveolar NO levels and utilize region-specific anti-inflammatory therapy. However, at the present time, even the most advanced models of NO exchange remain relatively simple, considering the lungs as a single-path trumpet. As lung disease (e.g., asthma or chronic obstructive pulmonary disease) and inflammation progress, ventilation heterogeneity increases, eroding the accuracy of the single-path model. The utility of exhaled NO as an inflammatory marker of the lungs strongly depends on our ability to accurately pinpoint the anatomic origin. The balance between model complexity and ease of clinical translation is not yet optimal and provides exciting opportunities for the future.

REFERENCES

1. **Alving K, Weitzberg E, Lundberg JM.** Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J* 6: 1368–1370, 1993.
2. **American Thoracic Society and European Respiratory Society.** ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 171: 912–930, 2005.
3. **Berry M, Hargadon B, Morgan A, Shelley M, Richter J, Shaw D, Green RH, Brightling C, Wardlaw AJ, Pavord ID.** Alveolar nitric oxide in adults with asthma: evidence of distal lung inflammation in refractory asthma. *Eur Respir J* 25: 986–991, 2005.
4. **Condorelli P, Shin HW, Aledia AS, Silkoff PE, George SC.** 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, 2007.
5. **DuBois AB, Kelley PM, Douglas JS, Mohsenin V.** Nitric oxide production and absorption in trachea, bronchi, bronchioles, and respiratory bronchioles of humans. *J Appl Physiol* 86: 159–167, 1999.
6. **Gelb AF, Taylor CF, Nussbaum E, Gutierrez C, Schein A, Shinar CM, Schein MJ, Epstein JD, Zamel N.** Alveolar and airway sites of nitric oxide inflammation in treated asthmatics. *Am J Respir Crit Care Med* 170: 737–741, 2004.
7. **Guo FH, De Raeve HR, Rice TW, Stuehr DJ, Thunnissen FB, Erzurum SC.** Continuous nitric oxide synthesis by inducible nitric oxide synthase in normal human airway epithelium in vivo. *Proc Natl Acad Sci USA* 92: 7809–7813, 1995.
8. **Gustafsson LE, Leone AM, Persson MG, Wiklund NP, Moncada S.** Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochem Biophys Res Commun* 181: 852–857, 1991.
9. **Hogman M, Drca N, Ehrstedt C, Merilainen P.** Exhaled nitric oxide partitioned into alveolar, lower airways and nasal contributions. *Respir Med* 94: 985–991, 2000.
10. **Kerckx Y, Michils A, Van Muylem A.** Airway contribution to alveolar nitric oxide in healthy subjects and stable asthma patients. *J Appl Physiol* (January 24, 2008). doi:10.1152/japplphysiol.01032.2007.
11. **Lehtimaki L, Kankaanranta H, Saarelainen S, Turjanmaa V, Moilanen E.** Peripheral inflammation in patients with asthmatic symptoms but normal lung function. *J Asthma* 42: 605–609, 2005.
12. **Mahut B, Delclaux C, Tillie-Leblond I, Gosset P, Delacourt C, Zerah-Lancner F, Harf A, de Blic J.** Both inflammation and remodeling influence nitric oxide output in children with refractory asthma. *J Allergy Clin Immunol* 113: 252–256, 2004.
13. **Paraskakis E, Brindicci C, Fleming L, Krol R, Kharitonov SA, Wilson NM, Barnes PJ, Bush A.** Measurement of bronchial and alveolar nitric oxide production in normal children and children with asthma. *Am J Respir Crit Care Med* 174: 260–267, 2006.
14. **Pietropaoli AP, Perillo IB, Torres A, Perkins PT, Frasier LM, Utell MJ, Frampton MW, Hyde RW.** Simultaneous measurement of nitric oxide production by conducting and alveolar airways of humans. *J Appl Physiol* 87: 1532–1542, 1999.
15. **Robbins RA, Barnes PJ, Springall DR, Warren JB, Kwon OJ, Buttery LD, Wilson AJ, Geller DA, Polak JM.** Expression of inducible nitric oxide in human lung epithelial cells. *Biochem Biophys Res Commun* 203: 209–218, 1994.
16. **Shin HW, Condorelli P, George SC.** Examining axial diffusion of nitric oxide in the lungs using heliox and breath hold. *J Appl Physiol* 100: 623–630, 2006.
17. **Shin HW, Condorelli P, Rose-Gottron CM, Cooper DM, George SC.** Probing the impact of axial diffusion on nitric oxide exchange dynamics with heliox. *J Appl Physiol* 97: 874–882, 2004.
18. **Shin HW, George SC.** Impact of axial diffusion on nitric oxide exchange in the lungs. *J Appl Physiol* 93: 2070–2080, 2002.
19. **Silkoff PE, McClean PA, Caramori M, Slutsky AS, Zamel N.** A significant proportion of exhaled nitric oxide arises in large airways in normal subjects. *Respir Physiol* 113: 33–38, 1998.
20. **Silkoff PE, Sylvester JT, Zamel N, Permutt S.** Airway nitric oxide diffusion in asthma. Role in pulmonary function and bronchial responsiveness. *Am J Respir Crit Care Med* 161: 1218–1228, 2000.

21. **Tsoukias NM, George SC.** A two-compartment model of pulmonary nitric oxide exchange dynamics. *J Appl Physiol* 85: 653–666, 1998.
22. **Tsoukias NM, Tannous Z, Wilson AF, George SC.** Single-exhalation profiles of NO and CO_2 in humans: effect of dynamically changing flow rate. *J Appl Physiol* 85: 642–652, 1998.
23. **Van Muylem A, Noel C, Paiva M.** Modeling of impact of gas molecular diffusion on nitric oxide expired profile. *J Appl Physiol* 94: 119–127, 2003.
24. **Verbanck S, Kerckx Y, Schuermans D, Vincken W, Paiva M, Van Muylem A.** Effect of airways constriction on exhaled nitric oxide. *J Appl Physiol* (January 24, 2008). doi:10.1152/japplphysiol.01019.2007.