

Exhaled nitric oxide in wheezy infants: A marker of inflammation determined by airways acidification and S-nitrosothiol degradation?

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In this issue of the *Journal*, Debley et al¹ report that in infants and toddlers with frequent early wheeze, single-breath exhaled nitric oxide (NO) concentrations correlate with the magnitude of bronchodilator responsiveness and decrease in FEV_{0.5} over the subsequent 6 months. Furthermore, exhaled NO concentration was superior to either lung function variable in predicting later exacerbations of wheezing requiring systemic corticosteroid treatment. These findings are in accord with the results of a recent clinical study wherein increased exhaled NO values in preschool children with moderate-to-severe intermittent wheezing were associated with an increased risk of respiratory tract illness in the subsequent year and with aeroallergen sensitization.² Likewise, exhaled NO levels are higher in nonwheezing atopic infants,³ high in 3- to 4-year-olds with recurrent wheeze and a positive predictive index for asthma,⁴ increase during exacerbations of infants,⁵ and decrease in response to treatment with fluticasone⁶ and montelukast.^{7,8} Thus expired NO has promise as a useful marker of asthma activity in preschool children, a population in whom it is difficult to noninvasively assess airways inflammation.

What is the source of exhaled NO in the lung, and can we confidently link its biochemistry to airways inflammation? Clinical studies in children with asthma suggest that exhaled NO is primarily a biomarker for atopy. Exhaled NO levels correlate positively with the magnitude of blood eosinophilia, serum IgE levels, numbers of positive skin prick test responses to aeroallergens, airway hyperresponsiveness, and disease severity.^{9,10} There is a wide gap between these clinical correlations and NO biochemical pathways in the human lung. The traditional view holds that NO is primarily formed in the respiratory epithelium through T_H2 cytokine-induced stimulation of inducible NO synthase. However, most cytokines that upregulate inducible NO synthase

are T_H1 cytokines. Exhaled NO values correlate most robustly with eosinophil counts in the lung, but eosinophils are not the principal cells in the airway that express inducible NO synthase. Furthermore despite significant airways inflammation, exhaled NO levels are not increased in many adults with severe asthma compared with levels seen in those with mild-to-moderate asthma.¹¹ As perplexing, exhaled NO levels in emergency department-treated patients with acute exacerbations do not correlate with standard measures of asthma severity.¹²

Detailed analysis of NO biochemical pathways in the lung should help resolve these inconsistencies and guide the interpretation of exhaled NO levels in patients. Arginine is the primary nitrogen donor that forms NO and its reaction products in the lung. In adults with severe asthma, but not healthy subjects, arginine bioavailability highly correlates with the degree of airflow obstruction but not inflammation.¹³ NO is highly reactive and, in the presence of molecular oxygen at normal pH, forms nitrite/nitrous acid (acid dissociation constant = 3.6). Nitrite is an important reservoir of nitrogen species in the lung and can be measured in micromolar concentrations in exhaled breath condensate and bronchoalveolar lavage fluid samples. Indeed, when volunteers with asthma inhale buffer solutions, expired NO levels acutely decrease as more nitrite is formed when airway pH increases.¹⁴ Another vital NO reaction in the lungs involves loss of an electron to form the nitrosonium ion (NO⁺), a strong oxidizing agent that can then react with cysteine residues of proteins to form S-nitrosothiols. One of these is S-nitrosoglutathione (GSNO), a protective anti-inflammatory and bronchodilator compound endogenous to the airway surfaces that is depleted in children with severe asthma.¹⁵ The catabolism of GSNO in the lung is regulated in part by two reductase enzymes. The first, a GSNO reductase, is increased in the airways of subjects with mild asthma.¹⁶ The second enzyme is a carbonyl reductase.¹⁷ Whereas NO is an important degradation product of GSNO, the rate of GSNO catabolism is likely a major determinant of exhaled NO levels in humans. Indeed therapeutic inhalation of GSNO acutely increases exhaled NO levels in children with cystic fibrosis.¹⁸ Thus challenge testing with arginine, buffer, GSNO, and other determinants of NO metabolism might identify candidate subpopulations who might respond to S-nitrosothiol replacement therapy.

Thus before exhaled NO concentrations in wheezing infants can be directly linked to specific inflammatory pathways, the biochemistry of NO in the lung must be considered. Exhaled NO levels can be high with acute acidification and enhanced catabolism of GSNO, but low when important nitrogen reservoirs, such as GSNO, are depleted or there is impaired degradation of endogenous GSNO. The association of these biochemical events with inflammatory mechanisms is highly preliminary and deserving of further study so as to improve the application of exhaled NO levels to the care of children with asthma.

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