Applying these observations from greatly reduced systems to the topic at hand leads to the prediction that putative strain injury thresholds must vary with flow, respiratory rate, and most importantly with end-expiratory lung volume. In fact, the classic experiments of Webb and Tierney, which underscored the lung-protective effects of PEEP, are entirely consistent with this prediction (14). Mechanical ventilation produced much less injury in anesthetized rats, when FRC was raised with PEEP, even though the lungs were inflated to similar end-inspiratory volumes. Lung-protective effects of PEEP (and consequently of increased FRC) are commonly attributed to prevention of so-called "atelect-trauma" resulting from injurious interfacial stresses (15, 16). However, given the effects of strain-amplitude on cell and tissue mechanics, it is quite possible that reductions in V_T *per se*, irrespective of the average volume about which lungs are oscillated, account for lung protection (14). Hager and colleagues' *post hoc* analysis of ARMA data, which suggested benefit of low-tidal-volume ventilation across all Pplat quartiles, may be interpreted in that context (17).

In summary, then, it seems inescapable that the risk of lung damage from mechanical ventilation, even when applied to healthy lungs, is multifactorial and cannot be linked to a single variable. As far as the strain-injury threshold is concerned it is conditional on PEEP and probably on flow and rate settings as well. This is not to distract from the conceptual advantage of quantifying lung deformation in terms of strain as opposed to V_T normalized by predicted body weight (PBW), because strain accounts for disease-related unit loss (18). Protti and colleagues appropriately caution against the uncritical application of a porcine model–derived strain threshold to patient care, but their message is unmistakable: a normal lung can tolerate fairly large tidal volumes for fairly long periods of time!

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ROLF D. HUBMAYR, M.D.
Pulmonary and Critical Care Medicine
Mayo Clinic
Rochester, Minnesota

References

1. Greenfield LJ, Ebert PA, Benson DW. Atelectasis and Surface Tension Properties of Lung Extracts Following Positive Pressure Ventilation and Overinflation. *Surg Forum* 1963;14:239–240.
2. The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 2000;342:1301–1308.
3. Protti A, Cressoni M, Santini A, Langer T, Mietto C, Febres D, Chierichetti M, Coppola S, Conte G, Gatti S, *et al.* Lung stress and strain during mechanical ventilation: Any safe threshold? *Am J Respir Crit Care Med* 2011;183:1354–1367.
4. Ranieri VM, Zhang H, Mascia L, Aubin M, Lin CY, Mullen JB, Grasso S, Binnie M, Volgyesi GA, Eng P, *et al.* Pressure-time curve predicts minimally injurious ventilatory strategy in an isolated rat lung model. *Anesthesiology* 2000;93:1320–1328.
5. Terragni PP, Rosboch GL, Lisi A, Viale AG, Ranieri VM. How respiratory system mechanics may help in minimising ventilator-induced lung injury in ARDS patients. *Eur Respir J Suppl* 2003;42: 15s–21s.
6. Fredberg JJ, Stamenovic D. On the imperfect elasticity of lung tissue. *J Appl Physiol* 1989;67:2408–2419.
7. Hildebrandt J. Pressure-volume data of cat lung interpreted by a plastoelastic, linear viscoelastic model. *J Appl Physiol* 1970;28:365–372.
8. Sugihara T, Hildebrandt J, Martin CJ. Viscoelastic properties of alveolar wall. *J Appl Physiol* 1972;33:93–98.
9. Fabry B, Maksym GN, Butler JP, Glogauer M, Navajas D, Taback NA, Millet EJ, Fredberg JJ. Time scale and other invariants of integrative mechanical behavior in living cells. *Phys Rev E Stat Nonlin Soft Matter Phys* 2003;68:041914.
10. Krishnan R, Park CY, Lin YC, Mead J, Jaspers RT, Trepat X, Lenormand G, Tambe D, Smolensky AV, Knoll AH, *et al.* Reinforcement versus fluidization in cytoskeletal mechanoresponsiveness. *PLoS ONE* 2009;4:e5486.
11. Negrini D, Passi A, Moriondo A. The role of proteoglycans in pulmonary edema development. *Intensive Care Med* 2008;34:610–618.
12. Cavalcante FS, Ito S, Brewer K, Sakai H, Alencar AM, Almeida MP, Andrade JS Jr, Majumdar A, Ingenito EP, Suki B. Mechanical interactions between collagen and proteoglycans: implications for the stability of lung tissue. *J Appl Physiol* 2005;98:672–679.
13. Albaiceta GM, Gutierrez-Fernandez A, Garcia-Prieto E, Puente XS, Parra D, Astudillo A, Campestre C, Cabrera S, Gonzalez-Lopez A, Fueyo A, *et al.* Absence or inhibition of matrix metalloproteinase-8 decreases ventilator-induced lung injury. *Am J Respir Cell Mol Biol* 2010:43:555–563.
14. Webb HH, Tierney DF. Experimental pulmonary edema due to intermittent positive pressure ventilation with high inflation pressures: protection by positive end-expiratory pressure. *Am Rev Respir Dis* 1974;110:556–565.
15. Muscedere JG, Mullen JB, Gan K, Slutsky AS. Tidal ventilation at low airway pressures can augment lung injury. *Am J Respir Crit Care Med* 1994;149:1327–1334.
16. Oeckler RA, Lee WY, Park MG, Kofler O, Rasmussen DL, Lee HB, Belete H, Walters BJ, Stroetz RW, Hubmayr RD. Determinants of plasma membrane wounding by deforming stress. *Am J Physiol Lung Cell Mol Physiol* 2010:299:L826–L833.
17. Hager DN, Krishnan JA, Hayden DL, Brower RG. Tidal volume reduction in patients with acute lung injury when plateau pressures are not high. *Am J Respir Crit Care Med* 2005;172:1241–1245.
18. Chiumello D, Carlesso E, Cadringher P, Caironi P, Valenza F, Polli F, Tallarini F, Cozzi P, Cressoni M, Colombo A, *et al.* Lung stress and strain during mechanical ventilation for acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2008;178:346–355.

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Nitrogen Balance in the Ecosystem of the Cystic Fibrosis Lung

In this issue of the *Journal*, Grasemann and coworkers (pp. 1363–1368) report that levels of asymmetric dimethyl arginine (ADMA) are increased in cystic fibrosis (CF) airways (1). ADMA inhibits cellular arginine uptake and nitric oxide (NO) synthase (NOS) activity. Levels of ADMA decrease during antibiotic therapy in association with improved lung function. This observation may prove to have therapeutic relevance. However, it is important to note that increased ADMA in CF airways may be both beneficial (through inhibition of NO production) and harmful (through inhibition of S-nitrosothiol production).

Nitric oxide in the concentrations measured in exhaled air (parts per billion) is generally irrelevant to normal lung physiology (2). However, NO can be relevant to lung pathology. It interacts with oxygen, superoxide, and other molecules to injure airway epithelium. Products of these reactions, such as nitrous acid and peroxynitrous acid, are potent cytotoxins downstream of inducible NOS activity (3).

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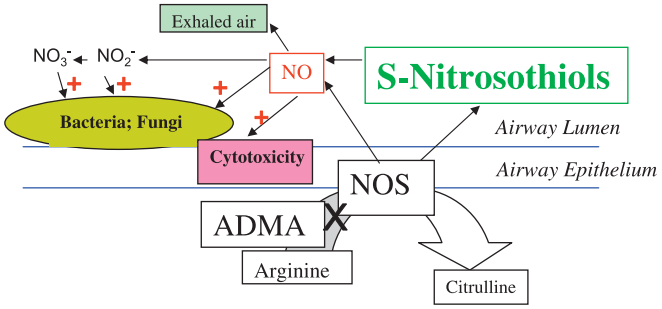

Figure 1. Cystic fibrosis airway nitrogen redox ecology. Nitric oxide (NO) synthase (NOS) isoforms in the cystic fibrosis (CF) lung produce both NO and S-nitrosothiols. NO is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-). In this issue of the *Journal*, Grasemann and coworkers show that asymmetric dimethylarginine (ADMA), which blocks arginine uptake and NOS activity, is increased in CF airways (1). High levels of ADMA could benefit the CF airways by inhibiting adverse effects of NOS (*red*), including its cytotoxicity and its promotion of the growth of denitrifying organisms. However, high levels of ADMA could also adversely affect the CF airways by inhibiting the beneficial formation of S-nitrosothiols (*green*). S-nitrosothiols are endogenous bronchodilators that inhibit microbial growth, augment ciliary beat frequency, inhibit amiloride-sensitive sodium transport, and increase expression and maturation of the delF 508 CF transmembrane regulatory protein (2, 8–13).

Nitric oxide can also affect bacteria in the CF airway (4, 5). Indeed, the CF airway is a complex ecosystem in which nitrogen oxides, oxygen, protons, and more complex chemical species are exchanged between prokaryotic and eukaryotic cells (Figure 1; 4–7). For example, levels of NO are lower than normal in the CF airway, in part because NO is consumed by *Pseudomonas*, *Aspergillus*, and other denitrifying organisms (4). NO can serve both as an electron acceptor (dissimilatory denitrification, ultimately forming ammonia) and as a precursor for amino acid formation (assimilatory denitrification). Airway NO levels rise and NH_3 levels fall with antibiotic therapy in CF (4). Levels of oxidized NO in the form of nitrate are high in the CF airway (6); and nitrate, like NO, can feed denitrifying organisms. Together, the effects of NOS products to cause cytotoxicity and promote prokaryotic growth suggest that the high levels of ADMA should be advantageous for patients with CF, and that a decrease in ADMA levels with antibiotic therapy might be disadvantageous.

However, NOS also produces S-nitrosothiols in concentrations two log orders higher than NO (8–10). S-nitrosothiols are antimicrobial. They augment ciliary beat frequency (2). They relax human airway smooth muscle and prevent tachyphylaxis to β_2-adrenergic agonists (2, 10, 11). They inhibit amiloride-sensitive sodium transport (12). They augment expression, maturation and function of delF508 CF transmembrane conductance regulator through inhibition of the expression of Hsp70/Hsp90-organizing protein (13). S-nitrosothiol levels are decreased in the CF airway (14), consistent with high ADMA levels (1); indeed, S-nitrosothiol replacement therapy improves oxygenation in CF (9). Prevention of S-nitrosothiol formation is therefore likely to be an important disadvantage of having high levels of ADMA in the CF airway (1).

We do not know why ADMA levels are high in CF. One possible mechanism is based on what we know about the CF airway ecosystem. Anti-pseudomonal therapy decreases ADMA levels in patients with CF (1). Biochemically, ADMA levels are decreased by dimethylarginine dimethylaminohydrolases (DDAHs). DDAHs are inhibited by S-nitrosylation, or physiological protein modification by NO (15). S-nitrosylation is driven both by NOS activation and—in relatively acidic conditions in the CF airway ecosystem (5)—by nitrite protonation; at baseline, this should increase ADMA levels. Antimicrobial therapy can decrease bacterial conversion of abundant airway nitrate to nitrite. Nitrite depletion would decrease DDAH S-nitrosylation, thereby increasing ADMA breakdown during the course of therapy. However, this proposed mechanism is speculative. Much work remains to be done.

The CF airway is dark, damp, and largely anaerobic (7). It is also surprisingly well-suited to denitrifying prokaryotic species, given that NOS expression is decreased and ADMA levels are increased. To understand why—and to learn how to use nitrogen oxide redox ecology to therapeutic advantage—we need to get beyond simply modeling NO radical diffusion (2, 3). The Cystic Fibrosis Foundation has shown the benefit of promoting interactions among scientists across disciplines. The work of Grasemann and coworkers suggests that there might be much to gain by organizing a small working group to bring together nitrogen balance ecologists, biochemists, CF airway epithelial biologists, and mathematicians experienced in biochemical modeling. This group could model prokaryotic and eukaryotic outcomes of specific CF interventions to identify strategies to optimize therapeutic development in CF. The elegant insights of Grasemann and coworkers serve to alert us that both the benefits and toxicities of airway nitrogen oxides need to be better understood to improve clinical outcomes in CF.

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Nadzeya V. Marozkina, M.D., Ph.D.
Benjamin Gaston, M.D.
Division of Pediatric Respiratory Medicine
University of Virginia School of Medicine
Charlottesville, Virginia

References

1. Grasemann H, Al-Saleh S, Scott JA, Shehnaz D, Mehl A, Amin R, Rafii M, Pencharz P, Belik J, Ratjen F. Asymmetric dimethylarginine contributes to airway nitric oxide deficiency in patients with cystic fibrosis. *Am J Respir Crit Care Med* 2011;183:1363–1368.
2. Gaston B, Singel D, Doctor A, Stamler JS. S-Nitrosothiol signaling in respiratory biology. *Am J Respir Crit Care Med* 2006;173:1186–1193.
3. Ricciardolo F, Sterk P, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. *Physiol Rev* 2004;84:731–765.
4. Gaston B, Ratjen F, Vaughan JW, Malhotra NR, Canady RG, Snyder AH, Hunt JF, Gaertig S, Goldberg JB. Nitrogen redox balance in the cystic fibrosis airway: effects of anti-pseudomonal therapy. *Am J Respir Crit Care Med* 2002;165:387–390.
5. Yoon SS, Coakley R, Lau GW, Lymar SV, Gaston B, Karabulut AC, Hennigan RF, Hwang SH, Buettner G, Schurr MJ, *et al.* Anaerobic killing of mucoid *Pseudomonas aeruginosa* by acidified nitrite under conditions characteristic of the cystic fibrosis airway. *J Clin Invest* 2006; 116:436–446.
6. Grasemann H, Ioannidis I, Tomkiewicz RP, de Groot H, Rubin BK, Ratjen F. Nitric oxide metabolites in cystic fibrosis lung disease. *Arch Dis Child* 1998;78:49–53.
7. Worlitzsch D, Tarran R, Ulrich M, Schwab U, Cekici A, Meyer KC, Birrer P, Bellon G, Berger J, Weiss T, *et al.* Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J Clin Invest* 2002;109:317–325.
8. Lui L, Hausladen A, Zeng M, Que L, Heitman J, Stamler JS. A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans. *Nature* 2001;410:490–494.
9. Snyder A, McPherson ME, Hunt JF, Johnson M, Stamler JS, Gaston B. Acute effects of aerosolized S-nitrosoglutathione in cystic fibrosis. *Am J Respir Crit Care Med* 2002;165:922–926.
10. Whalen EJ, Foster MW, Matsumoto A, Ozawa K, Violin JD, Que LG, Nelson CD, Benhar M, Heys JR, Rockman HA, *et al.* Regulation of

beta-adrenergic receptor signaling by S-nitrosylation of G-protein-coupled receptor kinase 2. *Cell* 2007;129:511–522.
11. Gaston B, Drazen JM, Jansen A, Sugarbaker DA, Loscalzo J, Stamler JS. Relaxation of human bronchial smooth muscle by S-nitrosothiols in vitro. *J Pharmacol Exp Ther* 1994;268:978–984.
12. Jain L, Chen XJ, Brown LA, Eaton DC. Nitric oxide inhibits lung sodium transport through a cGMP-mediated inhibition of epithelial cation channels. *Am J Physiol* 1998;274:475–484.
13. Marozkina NV, Borowitz M, Yemen S, Liu L, Sun F, Islam R, Erdmann-Gilmore P, Townsend RR, Lichti CF, Mantri S, *et al.* S-Nitrosoglutathione targets Hsp 70/Hsp 90 organizing protein as a corrector therapy for cystic fibrosis. *Proc Natl Acad Sci USA* 2010;107:11393–11398.
14. Grasemann H, Gaston B, Fang K, Paul K, Ratjen F. Decreased levels of nitrosothiols in the lower airways of patients with cystic fibrosis and normal pulmonary function. *J Pediatr* 1999;135:770–772.
15. Leiper J, Murray-Rust J, McDonald N, Vallance P. S-nitrosylation of dimethylarginine dimethylaminohydrolase regulates enzyme activity: further interactions between nitric oxide synthase and dimethylarginine dimethylaminohydrolase. *Proc Natl Acad Sci USA* 2002;99:13527–13532.

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The Brain in Sleep-Disordered Breathing
A Vote for the Chicken?

Structural brain changes in patients with obstructive sleep apnea (OSA) were first described in the *Journal* almost 10 years ago (1). Macey and colleagues used voxel-based morphometery (VBM) to detect diffuse reductions in gray matter across the brain, including the frontal and parietal cortex, temporal lobe, anterior cingulate, hippocampus, and cerebellum. Although interpretation of these data is somewhat confounded by the inclusion of patients with known co morbidities, they did lead to the interesting speculation that primary neural deficits may cause OSA, rather than OSA producing deficits—the so-called "chicken and egg" debate that remains unresolved (1, 2). They also raised the questions: What (if any) are the functional consequences of the neural deficits in OSA, and does treatment reverse the deficits and improve cognitive function?

In this issue of the *Journal*, Canessa and colleagues (pp. 1419–1426) make advances in answering these questions (3). They found that reduced gray matter volume in the left hippocampus, left posterior parietal cortex, and right superior frontal gyrus was associated with cognitive dysfunction. Moreover, continuous positive airway pressure (CPAP) treatment resulted in increases in gray matter in the hippocampus and frontal brain regions that were correlated with improvements in executive function and short-term memory. The changes in brain morphology are consistent with some, but not all, previous imaging studies using VBM (Table 1).

The discrepancies between the studies listed in Table 1 are most likely due to differences in statistical thresholds and the development of imaging software over the years. The apparently conflicting results of Macey and coworkers (1) and O'Donoghue and colleagues (4) highlight the impact of choosing different thresholds. In the former study, the VBM analysis was uncorrected for multiple comparisons at $P < 0.001$, whereas the latter study found no significant gray matter loss at a threshold of $P < 0.05$ corrected for multiple comparisons. Thus, while opinions differ regarding statistical adjustments, what is clear is that those citing these studies should understand the limitations of the technique. VBM is an automated process, but the pre-processing and choice of thresholds can influence the results obtained. Canessa and colleagues have admirably addressed this issue by repeating the analysis of their data using the older techniques of O'Donoghue and coworkers (4). No significant differences in gray matter concentration were detected between patients with OSA and control subjects, nor pre- and post-CPAP, when the more stringent threshold of O'Donoghue and colleagues was employed. If the presence of neural lesions can be altered by changing a statistical threshold, perhaps refocusing attention on examining the functional consequences of the structural deficits may be of more value.

Patients with OSA often report mild cognitive deficits (5–7) that can cause difficulties in work efficiency and performing tasks such as driving (8, 9). The Canessa study makes the link between reductions in performance, disease severity, and brain structure prior to treatment. In the left parahippocampal gyrus, the reduction in gray matter volume was associated with errors on the Stroop executive function test, and in the left posterior-parietal cortex deficits were correlated a reduction in the Raven abstract reasoning test. These reductions in cognitive function may be due to reduced attention, or an inability to process external information (e.g., sensory input) to form a neural representation (encoding). Alternatively, patients with OSA may experience difficulty with processing information to form memories during sleep. It is still questionable whether interruption of these processes is related to sleep deprivation or intermittent hypoxia (10). The Canessa study supports a relationship between loss of gray matter volume and hypoxia, as does the compelling study of Gozal and colleagues, which demonstrated that intermittent hypoxia induces oxidative stress and cell apoptosis in the C1A hippocampal region, an area associated with spatial memory (11).

VBM analysis attained notoriety in 2000 with the publication of the Ig Nobel–winning study by Maguire and colleagues (12). This study showed that London taxi drivers who had taken "the knowledge"—a test that involves several years of preparation to memorize the streets of London—had increased hippocampal gray matter volume compared with drivers who had not taken the test. Notably, the study showed that brain plasticity could occur in focal areas of a healthy adult human brain in response to environmental stimuli. Juggling also increases gray matter proportional performance (13). Importantly, this latter study also showed that removal of the stimulus for 3 months led to a reduction in the brain gray matter.

Whether treatment of OSA can reverse the neurodegeneration reported in VBM studies is unclear. The data from Canessa and colleagues imply that this is indeed the case, with CPAP treatment resulting in an increase in gray matter volume in the hippocampus and frontal cortex. In the left hippocampus the increased volume was correlated with an increase in performance on the Stroop test. In the right enthorinal cortex the increase in volume was also correlated with improved neural function. Thus, CPAP treatment may reduce oxidative stress in the systemic circulation, since increased expression of neuronal progenitors and mature neurons in the hippocampus have been

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