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Hypercapnia and Acidosis in Sepsis

A Double-edged Sword?

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ABSTRACT

Acute respiratory distress syndrome is a devastating disease that causes substantial morbidity and mortality. Mechanical ventilation can worsen lung injury, whereas ventilatory strategies that reduce lung stretch, resulting in a "permissive" hypercapnic acidosis (HCA), improve outcome. HCA directly reduces nonsepsis-induced lung injury in preclinical models and, therefore, has therapeutic potential in these patients. These beneficial effects are mediated *via* inhibition of the host immune response, particularly cytokine signaling, phagocyte function, and the adaptive immune response. Of concern, these immunosuppressive effects of HCA may hinder the host response to microbial infection. Recent studies suggest that HCA is protective in the earlier phases of bacterial pneumonia-induced sepsis but may worsen injury in the setting of prolonged lung sepsis. In contrast, HCA is protective in preclinical models of early and prolonged systemic sepsis. Buffering of the HCA is not beneficial and may worsen pneumonia-induced injury.

ACUTE respiratory distress syndrome (ARDS) is a devastating disease that causes thousands of deaths annually, causes considerable morbidity, and imposes a major financial burden on already stretched health budgets.[1] Mechanical ventilation, while necessary to maintain gas exchange and support life, can worsen or even cause lung damage by repetitive overstretching of the remaining functional lung tissue that must bear the brunt of the entire tidal volume. The mechanisms by which mechanical ventilation may contribute to acute lung injury (ALI) are increasingly well understood. Importantly, ventilator strategies that reduce lung stretch have been demonstrated to improve survival from ALI and ARDS.[2,3] These "protective" ventilatory strategies, which aim to reduce tidal volume and airway pressures, generally involve some degree of hypercapnia ($Pa_{CO_2} > 45$ mmHg). This strategy is termed "permissive hypercapnia" and was first demonstrated to reduce injury in patients with severe asthma.[4] The clear survival benefit demonstrated by reducing lung stretch has resulted in a shift in clinical paradigms regarding hypercapnia—from avoidance to tolerance—with hypercapnia increasingly permitted to realize the benefits of low lung stretch. This hypercapnia is accompanied by an acidosis in its acute phase, termed hypercapnic acidosis (HCA), which is gradually buffered over time by tissue and renal buffering. Evidence from clinical studies attests to the safety of and lack of detrimental effects with HCA.[5] In addition, a secondary analysis of data from the ARDSnet tidal volume study[2] demonstrated that the presence of a HCA at the time of randomization in patients who received high tidal volume ventilation was associated with an improved survival.[6] Consequently, hypercapnia, often for prolonged periods of time, has become a key component of contemporary ventilatory strategies in critically ill patients.

Paralleling these developments in the clinical setting, there is considerable experimental evidence that induced HCA, by the addition of carbon dioxide to the inspiratory limb of the ventilator circuit, may have a direct protective effect in a number of clinically relevant models of lung

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injury, independent of changes in tidal volume.[7] HCA has been demonstrated to attenuate ALI induced by free radicals,[8] pulmonary[9] and systemic ischemia-reperfusion,[10] pulmonary endotoxin instillation,[11] and excessive lung stretch.[12–14] The protective action of HCA seems due in part to its antiinflammatory effects, which include attenuation of neutrophil function,[15–17] reduction of free radical generation,[8] decreased oxidant-induced tissue damage,[11] and reduction in the levels of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1,[18] and IL-8.[16] Thus, the potential for deliberate induction of HCA to have therapeutic efficacy in critically ill patients is clear.[7,19,20]

Hypercapnia and Sepsis in the Critically Ill

Severe sepsis associated with multiple organ failure, whether pulmonary or systemic in origin, is the leading cause of death in critically ill adults and children.[21,22] The incidence of sepsis-induced critical illness is 150 per 100,000 person-years in the United States alone.[21] Evidence suggests that approximately 40% of patients with severe sepsis develop ARDS,[23] and it has been estimated that sepsis-associated ARDS has an incidence of 45–63 cases per 100,000 person-years.[24] Furthermore, infection frequently complicates critical illness due to other causes, with an infection prevalence of more than 44% reported in this population.[25] Pneumonia and lower respiratory tract infection account for two thirds of nosocomial infections acquired in the intensive care unit.[25] Sepsis-induced ARDS is associated with the highest mortality rates.[1,26,27]

Concerns have recently been raised regarding the safety of hypercapnia and/or acidosis in the context of sepsis.[19,28] The potent antiinflammatory effects of HCA underlie its protective effects in nonsepsis models of lung and systemic organ injury. However, immunocompetence is essential to an effective host response to microbial infection. Hypercapnia and/or acidosis may modulate the interaction between host and bacterial pathogen *via* several mechanisms. HCA results in broad based suppression of many events that contribute to microbial killing, which could be detrimental to the host by facilitating bacterial spread and replication. Of further concern, the recent findings that HCA slows resealing of stretch-induced cell membrane injuries[29] and inhibits repair of pulmonary epithelial wounds[30] raise the potential that it could reduce the barrier to access of bacteria from the lung to the bloodstream.

Effect of Hypercapnic Acidosis on Bacterial Proliferation

The effects of hypercapnia and its associated acidosis may be a combination of the effects of hypercapnia and acidosis *per se*. Optimal anaerobic *Escherichia coli* growth occurs at a carbon dioxide tension of 0.05 atm, which is similar to the P_{CO_2} in the gut, which is the usual environment in which *E. coli* live. The aerobic growth rate of *E. coli* was not inhibited by a P_{CO_2} of 0.2 atm but was inhibited at partial pressures of more than 0.6 atm.[31] *E. Coli* colony counts were halved when exposed to 350 atm of carbon dioxide for 20 min,[32] which is a massive dose. This inhibitory effect of high concentrations of carbon dioxide is mediated *via* a direct effect,[33] although the exact mechanism is unclear. It does seem that carbon dioxide has broadly similar effects within the various families of microorganisms, but the sensitivity to carbon dioxide varies across the families, that is, yeasts are quite resistant to the inhibitory effects of carbon dioxide, gram-positive organisms are somewhat less resistant, and gram-negative organisms are the most vulnerable.[34] However, it is important to recognize that the levels of carbon dioxide used in these studies to inhibit bacterial growth are extremely high in the context of human physiology.

Pugin *et al.*[35] have demonstrated that more clinically relevant degrees of metabolic acidosis can directly enhance bacterial proliferation *in vitro*. Cultured lung epithelial cells exposed to cyclic stretch similar to that seen with mechanical ventilation produced a lactic acidosis that markedly enhanced the growth of *E. coli*.[35] This was a direct effect of hydrogen ions, because direct acidification of the culture medium to a pH of 7.2 with hydrochloric acid enhanced *E. coli* growth. In contrast, alkalinizing the pH of conditioned media from stretched lung cells abolished the enhancement of *E. coli* growth effect. A range of gram-positive and gram-negative bacteria (including *E. coli*, *Proteus mirabilis*, *Serratia rubidaea*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*) isolated from patients with ventilator-associated pneumonia grew better in acidified media (fig. 1).[35] Interestingly, this effect was not seen with a methicillin-resistant *Staphylococcus aureus* strain, which seemed to grow best at an alkaline pH (fig. 1).[35]

The effects of HCA on bacterial proliferation at levels encountered in the context of permissive hypercapnia are unclear but may be a combination of the effects of the acidosis and of the hypercapnia. However, the inhibitory effects of carbon dioxide on bacterial growth have been demonstrated only at doses well beyond the clinically relevant range. In addition, the type of acidosis—respiratory *versus* metabolic—may have differential effects on bacterial growth. However, the demonstration that clinically relevant levels of metabolic acidosis enhance bacterial growth is of concern.

Effects of Hypercapnic Acidosis on the Immune Response

HCA modulates diverse components of the host immune response, including cytokine and chemokine signaling, neutrophil and macrophage function, complement activation, and the adaptive immune response. The effects of HCA on the immune response may predominantly be a function of the acidosis rather than the hypercapnia *per se*.[36] However, the type of acidosis, that is, the fact that this is a hypercapnic

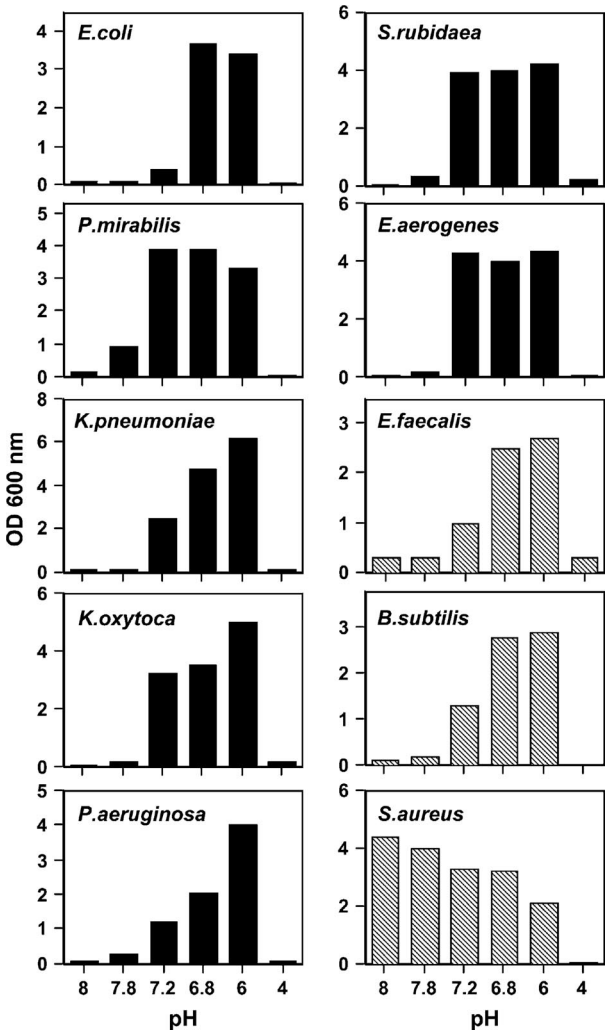

Fig. 1. Bacterial pathogens proliferate more rapidly in the setting of a metabolic acidosis. All bacterial strains tested, except for a methicillin-sensitive *Staphylococcus aureus* (*S. aureus*), had a marked growth advantage at moderately acidic pH levels (7.2–7.6) relevant to the clinical setting. Gram-negative bacteria are represented by *solid bars*, whereas gram-positive bacteria are represented by *hatched bars*. Reproduced with permission from Am J Resp Cell Mol Biol 2008; 38:362–70. *E. coli* = *Eschericia coli*; *P. mirabilis* = *Proteus mirabilis*; *K. pneumoniae* = *Klebsiella pneumoniae*; *K. oxytoca* = *Klebsiella oxytoca*; *P. aeruginosa* = *Pseudomonas aeruginosa*, *S. rubidaea* = *Serratia rubidaea*; *E. aerogenes* = *Enterobacter aerogenes*; *E. feacalis* = *Enterococcus feacalis*; *B. subtilis* = *Bacillus subtilis*.

rather than a metabolic acidosis seems to be important. As a specific example, although HCA inhibits neutrophil function *in vitro*,[37] metabolic acidosis seems to inhibit function in some studies,[38,39] while activating neutrophils in other studies.[40] Methodologic differences may explain the differences in these findings, because incubation of cells in strong acids may have caused injury.[40] Similarly, the infusion of hydrochloric acid into the bloodstream *in vivo* has been demonstrated to cause direct injury and activation of the inflammatory response.[41] For these reasons, we will focus on studies in which the effects of HCA have been determined and include studies of metabolic acidosis where these studies yielded important insights not otherwise available and/or where the acidosis was not generated using hyperosmolar strong acids.

Cytokine and Chemokine Production

HCA interferes with coordination of the immune response by reducing cytokine signaling between immune effector cells.[16,18,42] HCA reduces neutrophil[16] and macrophage[18,42] production of proinflammatory cytokines such as TNF-α, IL-1β, IL-8, and IL-6. HCA reduced endotoxin-stimulated macrophage release of TNF-α and IL-1β *in vitro*.[18,42] Peritoneal macrophages incubated under hypercapnic conditions demonstrated reduced endotoxin-stimulated TNF-α and IL-1β release. TNF-α inhibition was seen despite normal levels of TNF message, required more than 30 min of carbon dioxide exposure, and persisted after withdrawal of carbon dioxide.[42] This effect of HCA on cytokine production seems to be sustained. Intraperitoneal macrophages demonstrated impaired TNF-α production for up to 3 days after exposure to hypercapnia.[43,44] The mechanism underlying this inhibition of cytokine and chemokine production seems to be mediated at least in part *via* the inhibition of nuclear factor-κB, a pivotal transcriptional regulator in the setting of inflammation, injury, and repair.[45]

Complement Activation

The complement system is an essential component of the innate immune response, and its activation targets pathogens for phagocytosis or lysis. Acidosis, whether hypercapnic or metabolic, seems to activate the complement system. Emeis *et al.*[46] demonstrated that HCA and lactic and hydrochloric acid-induced acidosis all activate C3 and C5, two key components of the complement system. This activation of complement seems to be a direct effect of the acidosis *per se*.[46] The implications of HCA-mediated activation of complement, whether beneficial through enhanced complement-mediated bacterial killing or detrimental due to complement depletion or nonspecific actions of activated complement components, is not clear.

The Cellular Immune Response

Neutrophils and macrophages are important effectors of the innate immune response in the setting of bacterial infection. Neutrophils rapidly migrate from the bloodstream to areas of infection throughout the body. Once in contact with bacteria, neutrophils rapidly phagocytose them. Neutrophils contain granules in the cytoplasm, which contain a range of destructive enzymes, including elastases and proteases, nicotinamide adenine dinucleotide phosphate oxidase, which generates superoxide and hydrogen peroxide, and myeloperoxide, which generates hypochlorous acid. These granules rapidly fuse with phagosomes containing bacteria to lyse and destroy them. Reduction of neutrophil migration to infection sites is associated with a poor outcome in sepsis.[47]

Tissue macrophages and their blood-borne monocyte counterparts are of particular importance in coordinating the activation of the lymphocyte system in the setting of infec-

tion by presenting foreign antigen to the appropriate cells and secreting chemokines. Macrophages are activated by bacteria or by molecules such as endotoxin or complement components. Both monocytes and macrophages phagocytose and kill pathogens by similar mechanisms but at a slower rate than neutrophils.

HCA may impact on the cellular immune response *via* several mechanisms. HCA may indirectly impair the cellular immune response by impairing the cytokine signaling between immune effector cells necessary to the coordination of the response to pathogens. Direct effects on the cellular immune response include (1) reduced phagocyte migration, chemotaxis, and adhesion; (2) decreased phagocytic ability; (3) reduced oxidant-mediated bacterial killing; and (4) altered mechanisms of neutrophil death. These effects may be mediated at least in part by disruption of phagocyte intracellular pH regulation.

Phagocyte Intracellular pH Regulation. Maintenance of intracellular pH in the physiologic range (6.8–7.3) is necessary for normal immune function and also for other cellular functions, such as proliferation, differentiation, apoptosis, migration, cytoskeletal organization, and maintenance of cell volume.[48,49] Two main transport systems regulate intracellular pH in immune cells, namely the Na/H exchanger and the plasmalemmal V-type H ATPase.[48,49] Intracellular neutrophil pH decreases when neutrophils are activated.[17,50] Where milieu pH is normal, there tends to be a rapid recovery in neutrophil cytosol pH back toward neutral or even alkaline pH.[51] Decreases in pH and increases in carbon dioxide in the local milieu, as might be encountered with HCA, result in a rapid decrease in neutrophil cytosolic pH.[16,52] Studies of the effects of metabolic acidosis demonstrate the potential for intracellular acid loading to decrease neutrophil spreading,[52,53] motility and migration,[51] and chemotaxis.[54,55] HCA may, therefore, reduce neutrophil and macrophage recruitment to the site of a septic insult *via* this mechanism. Furthermore, the inhibition of carbon dioxide induced changes in neutrophil cytosol pH by acetazolamide[16] and buffering of intracellular pH abolish neutrophil inhibition.[36]

Phagocyte Migration, Chemotaxis, and Adhesion. Neutrophil margination within the bloodstream, adhesion to the capillary wall, and migration and recruitment to the site of injury is an important step in the host response.[56] The ability of cells to migrate is dependent on several well-defined processes, such as cytoskeleton remodeling, membrane recycling by endocytosis and exocytosis, detachment and reformation of focal adhesions mediated by integrins, and cell volume regulation. HCA has been demonstrated to inhibit the neutrophil expression of the chemokines, selectins, and intercellular adhesion molecules[16,45] that facilitate neutrophil binding to the endothelium and migration out of the vascular system. The potential for HCA to inhibit neutrophil chemotaxis and migration to the site of injury has been confirmed *in vivo*, where HCA inhibits pulmonary neutrophil infiltration in response to endotoxin instillation.[11]

Phagocytic Activity. Macrophages and neutrophils phagocytose bacteria and internalize them into the phagosomes. The phagosome subsequently fuses with endosomes and lysosomes containing lytic enzymes that digest the bacteria. Acidosis, whether hypercapnic or metabolic, impairs neutrophil and macrophage phagocytic ability. HCA has been demonstrated to decrease cytokine production by both stimulated and unstimulated macrophages.[18,42,43] Metabolic acidosis depresses macrophage phagocytosis,[57] slows internalization of bacteria, and decreases intracellular bacterial killing.[58] HCA has also been demonstrated to directly impair neutrophil phagocytosis *in vitro*.[37] This inhibitory effect seems to be a function of the acidosis, with buffering restoring neutrophil phagocytosis.[36]

Free Radical-induced Bacterial Killing. Neutrophil and macrophage production of free radicals, such as superoxide, hydrogen peroxide, and hypochlorous acid, produced during the "respiratory burst" in response to immune activation is a major mechanism by which phagocytes kill bacteria.[59] In these cells, the enzyme nicotinamide adenine dinucleotide phosphate oxidase, which produces superoxide in response to microbial invasion, is markedly pH sensitive and displays a pH optimum of 7.0–7.5.[60] Reduction of cytosolic pH impairs intracellular enzyme function and decreases free radical production. Macrophage superoxide radical release decreases linearly at an intracellular pH less than 6.8.[61] HCA inhibits the generation of oxidants such as superoxide by unstimulated neutrophils and by neutrophils stimulated with opsonized *E. coli* or with phorbol esters.[16] In contrast, hypocapnic alkalosis stimulated neutrophil oxidant generation.[16] Carbon dioxide modulation of neutrophil oxidant generation seems to be pH mediated, because inhibition in intracellular pH changes with acetazolamide attenuated this effect. HCA has also been demonstrated to reduce macrophage superoxide production.[62]

Mechanism of Neutrophil Death. Neutrophils have a short lifespan and tend to die within 48 h of release into the circulation, by undergoing programmed cell death, that is, apoptosis. Apoptosis seems to be a normal and appropriate fate of a neutrophil after phagocytic activity, whereas neutrophil death *via* necrosis causes release of intracellular contents, including harmful enzymes, which can cause tissue destruction. Neutrophils seem to have an increased probability of dying by necrosis after intracellular acidification during phagocytosis.[17] HCA may, therefore, increase the probability of neutrophil cell death occurring *via* necrosis rather than apoptosis.

The Adaptive Immune Response

Much of the focus of the effect of acidosis on the adaptive immune response has been on the implications for cancer, because the tumor microenvironment is characterized by poor vascularization, tissue hypoxia, and acidosis.[63] In a situation analogous to sepsis, acidosis in this setting may hamper the host immune response to tumor cells, potentially leading to increased tumor growth and spread. The cytotoxic

activity of human lymphokine activated killer cells[64] and natural killer cells[65] is diminished at acidic pH. Metabolic acidosis reduces lysis of various tumor cell lines by cytotoxic T lymphocytes[66] and also inhibits IL-2-stimulated lymphocyte proliferation.[67] In contrast, the motility of IL-2-stimulated lymphocytes seems to be stimulated in the presence of an acidified extracellular matrix.[68] Severe extracellular acidosis (pH 6.5) also seems to enhance the antigen-presenting capacity of dendritic cells.[69] The net effect of these contrasting actions of metabolic acidosis on the adaptive immune response is unclear. However, the demonstration that HCA enhances systemic tumor spread in a murine model[70] raises clear concerns regarding the potential for HCA to suppress cell-mediated immunity.

Effect of Hypercapnic Acidosis on Bacteria-induced Tissue Injury

The mechanisms by which bacterial infection produces lung and systemic organ injury are complex and involve contributions from the host response, injury due to bacterial exotoxins, endotoxins, and other components of the bacterial wall, and direct bacterial-induced injury caused by spread and tissue invasion. The host response to sepsis, while central to immunocompetence, may also play an important role in the pathogenesis of organ injury. In addition, the relative contributions of different processes involved in causing tissue injury may vary depending on the stage of the infective process.

HCA and the Host Response

The effects of hypercapnia in the context of infection may result in part from its modulation of the host immune response. As discussed earlier, HCA results in broad-based suppression of many events necessary for effective microbial killing. Central to the effects of HCA in the context of bacterial infection are its effects on the cellular immune system, particularly its effects on phagocytes, and on the coordination of the immune response. The phagocytic activity of neutrophils and macrophages is necessary for an effective host response to invading bacteria. However, the neutrophil armory of cytotoxic agents also has the potential to escape into the extracellular space and damage host tissue[71] and contribute to the development of lung injury. The protective effects of HCA in nonseptic injury models seems to be mediated at least in part *via* inhibition of neutrophil recruitment and/or neutrophil function.[11] These findings are consistent with previous observations that there is decreased severity of ALI in neutrophil depleted animal models,[72–74] and the finding that the severity of ALI worsens in neutropenic humans on recovery of the neutrophil count.[75] The potential deleterious effect of this HCA-induced alteration in neutrophil function in the presence of live bacterial sepsis is highlighted by the fact that defects in neutrophil function are associated with increased sepsis severity[76] and worsened outcome.[47]

Early versus *Prolonged Bacterial Infection*

The effects of HCA on bacterial injury may vary—from benefit to harm—depending on the stage of injury process, that is, depending on whether it is an early or established infection or whether the infection is prolonged. Early bacterial pneumonia is accompanied by a vigorous host inflammatory response. The antiinflammatory effects of HCA may reduce the magnitude of the host inflammatory response, thereby ameliorating host-induced tissue damage. HCA has also been demonstrated to reduce injury produced by bacterial endotoxin.[11] Therefore, in the setting of an early bacterial infection, HCA might reduce lung and systemic organ injury (fig. 2). In contrast, in late or prolonged bacterial infection, direct bacterial tissue invasion and spread may play a greater role. The immunosuppressive effects of HCA, particularly neutrophil inhibition, might impair bactericidal host responses. Overall, this would cause greater tissue damage in the setting of an established injury where a large bacterial load may exist. These effects would negate any protective effects of reduced host mediated tissue damage. This potentially deleterious effect of HCA has important clinical implications given that many critically ill patients already have well-established infections at presentation, and the fact that they may be exposed to prolonged hypercapnia in the context of protective lung ventilation strategies (fig. 2).

Hypercapnic Acidosis in Pulmonary Sepsis

Early Pneumonia

The effect of hypercapnia in early pneumonia seems to depend on the severity of the injury. HCA did not modulate the development of evolving lung injury induced by *E. coli* pneumonia of moderate severity.[77] However, in the setting of more severe evolving pneumonia, produced *via* a higher intrapulmonary *E. coli* inoculate, HCA reduced the severity of the lung injury compared with normocapnic conditions.[78] Of interest, the protective effects of HCA in evolving pneumonia-induced lung injury seem to be independent of any effect on neutrophil function.[78] Of importance, HCA did not increase pulmonary bacterial load in early pneumonia, allaying concerns regarding the potential for HCA to retard killing and/or increase proliferation of bacteria.[77,78]

Established Pneumonia

HCA also reduces the severity of the lung injury caused by a more established bacterial-induced pneumonia,[79] a model more closely analogous to that seen in the clinical setting. When HCA was introduced several hours after the induction of an *E. coli*-induced lung injury, it reduced the severity of subsequent lung damage.[79] Of importance, although these protective effects of HCA were greater in the presence of appropriate antibiotic therapy (fig. 3A), HCA also reduced pneumonia-induced injury in the absence of antibiotic therapy (fig. 3B). Importantly, there was no evidence to suggest that HCA increased bacterial load, as evidenced by similar levels of bacteria recovered from the lungs of animals exposed

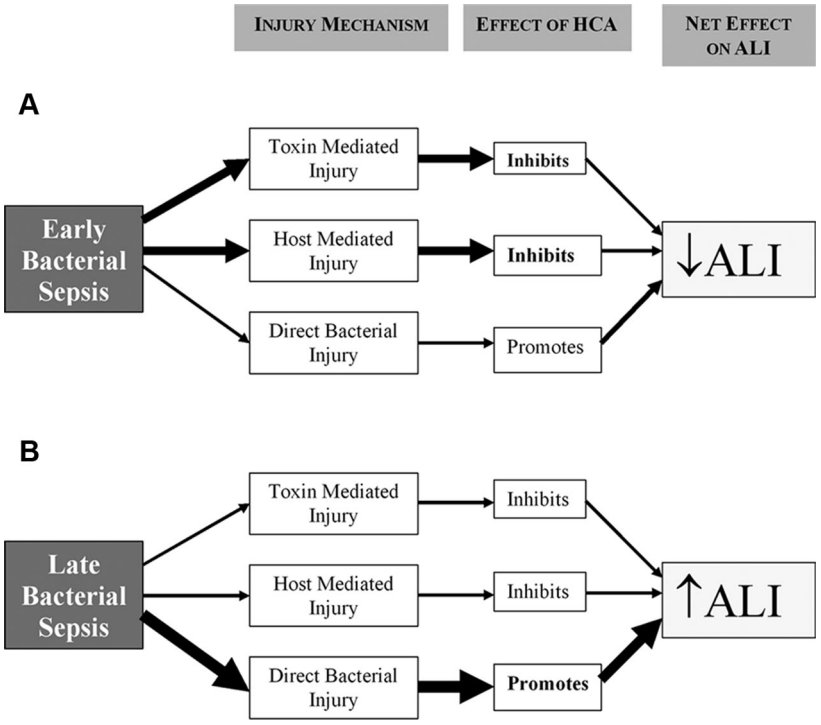

Fig. 2. Figure proposing potential mechanisms underlying the effects of hypercapnic acidosis in sepsis. (*A*) Early sepsis, in which hypercapnic acidosis may reduce the host inflammatory response and decrease the contribution of bacterial toxin–mediated injury to tissue injury and damage. This might result in an overall decrease in lung injury. (*B*) Late or prolonged bacterial sepsis, where a hypercapnic acidosis-mediated decrease in the host response to bacterial infection might result in unopposed bacterial proliferation, thereby increasing direct bacterial tissue invasion and injury and worsening lung injury. ALI = acute lung injury; HCA = hypercapnic acidosis.

to both HCA and normocapnia.[79] These findings again offer reassurance regarding the use of HCA in situations of established infection.

Prolonged Pneumonia

In contrast, hypercapnia increases the severity of lung injury caused by prolonged *E. coli* pneumonia.[37] Animals exposed to environmental hypercapnia for 48 h after intrapulmonary *E. coli* inoculation developed more severe lung damage, as evidenced by a greater reduction in pulmonary compliance, increased histologic injury, and greater alveolar neutrophil infiltration in comparison with similarly treated animals maintained under normocapnic conditions[37] (figs. 4A and B). Of particular concern, prolonged exposure to environmental hypercapnia increased bacterial load, as evidenced by increased levels of bacteria in the lungs of animals exposed to hypercapnia compared with normocapnia (fig. 4C). The mechanism underlying this effect seemed to involve the inhibition of neutrophil function, because neutrophils isolated from hypercapnic rats demonstrated impaired phagocytosis ability (fig. 4D).[37] Of importance to the clinical context, the use of appropriate antibiotic therapy abolished these deleterious effects of hypercapnia, reducing lung damage and lung bacterial load to levels comparable with that seen with normocapnia.

The deleterious effects of HCA in the setting of prolonged pneumonia are mediated, at least in part, *via* impairment of neutrophil phagocytosis.[37] In contrast, this inhibitory effect of HCA on neutrophils seems less important in the setting of more acute pneumonia,[78] and it can be abrogated in the setting of prolonged pneumonia by treatment with appropriate antibiotic therapy. However, these findings raise concerns regarding the safety of prolonged exposure to hypercapnia in critically ill patients with pneumonia.

Hypercapnic Acidosis in Systemic Sepsis

Evolving Systemic Sepsis

HCA reduces the severity of early septic shock and lung injury in the first 3 h after cecal ligation and puncture-induced fecal peritonitis.[80] HCA slowed the development of hypotension, preserved central venous oxygen saturations, and attenuated the increase in serum lactate when compared with normocapnia.[80] Central venous pressures did not change throughout the protocol, reducing the likelihood that differences in fluid volume status contributed to the hypotension in either group. HCA reduced the severity of lung injury, as evidenced by a reduction in the alveolar-arterial oxygen gradient, and reduced lung permeability, compared with normocapnia.[80] HCA reduced alveolar neutrophil infiltration but did not alter bronchoalveolar lavage IL-6 or TNF-α levels compared with normocapnia. There was no effect of HCA on bacterial load in the lung or in the bloodstream, whereas peritoneal fluid bacterial loads were also similar in both groups.

Established Systemic Sepsis

Wang *et al.*[81] demonstrated that HCA improved the hemodynamic profile in a manner comparable with that seen with dobutamine in an ovine model of established fecal peritonitis. Fecal peritonitis was induced in anesthetized, invasively monitored, mechanically ventilated female sheep. Two hours after fecal spillage, animals were randomized to HCA, dobutamine infusion or control conditions, and followed up until

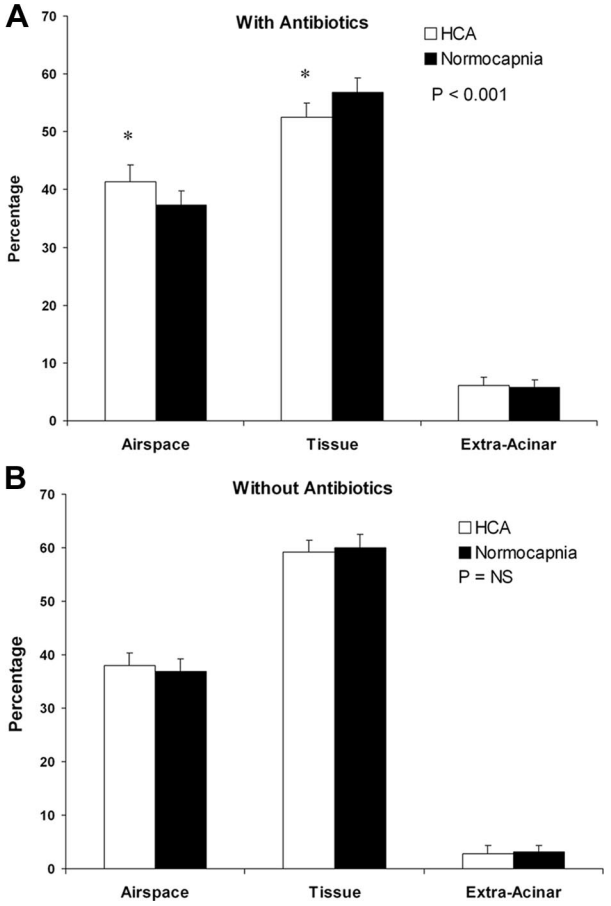

Fig. 3. (*A*) Hypercapnic acidosis reduces the severity of histologic lung injury induced by an established *Escherichia coli* pneumonia treated with appropriate antibiotic therapy. (*B*) Hypercapnic acidosis does not reduce or increase the severity of histologic lung injury induced by an untreated established *E. coli* pneumonia. Modified with permission from ANESTHESIOLOGY 2008; 109:837–48. HCA = hypercapnic acidosis; NS = not significant.

animal death. Both HCA and dobutamine increased heart rate, cardiac index, and oxygen delivery, and reduced lactate concentrations compared with normocapnic animals. HCA, but not dobutamine, also reduced certain indices of lung injury, as assessed by lung wet–dry ratio, alveolar-arterial oxygen partial pressure difference and shunt fraction.[81] However, HCA did not increase survival time compared with normocapnia in this setting.

Prolonged Systemic Sepsis

In contrast to the findings in prolonged pulmonary sepsis, environmental hypercapnia reduced the severity of prolonged systemic sepsis-induced lung damage.[80] Prolonged hypercapnia reduced histologic indices of lung injury in comparison with normocapnia. Of interest, hypercapnia did not reduce alveolar neutrophil infiltration or lung IL-6 or TNF-α concentrations. Furthermore, HCA did not alter survival from prolonged systemic sepsis. Reassuringly, there was no effect of HCA on bacterial load in the lung, the bloodstream or the peritoneal cavity.[80]

Intraperitoneal Hypercapnia in Systemic Sepsis

A growing body of literature attests to the beneficial effects of direct intraabdominal administration of carbon dioxide—by means of a pneumoperitoneum—further supporting the safety and efficacy of HCA in abdominal sepsis. Carbon dioxide pneumoperitoneum improved survival compared with helium pneumoperitoneum in animals subjected to combined laparotomy and endotoxemia injury.[82] Insufflation of carbon dioxide into the peritoneal cavity before laparotomy for endotoxin contamination increases animal survival.[83] Most recently, carbon dioxide pneumoperitoneum has been demonstrated to increase survival in both mice and rabbits with polymicrobial peritonitis induced by cecal ligation and puncture (fig. 5).[84,85] These protective effects of intraperitoneal carbon dioxide insufflation seem to be due to the immunomodulatory effects of HCA,[86] which include an IL-10-mediated down-regulation of TNF-α.[83] Importantly, these effects seem to be mediated by the localized peritoneal acidosis produced rather than by any systemic effect.[87,88]

Buffered Hypercapnia in Sepsis

The effects of hypercapnia in sepsis may be a function of the hypercapnia or the acidosis *per se*. As discussed, the effects of HCA on the immune response seem to be predominantly a function of the acidosis, rather than the hypercapnia *per se*, but the fact that the acidosis is hypercapnic rather than metabolic is of importance. The potential exists for hypercapnia to exert direct effects, independent of pH changes. A specific example is the binding of carbon dioxide to free amine groups on proteins to form carbamates, which can alter certain protein behavior or activity. The classic example is hemoglobin in which carbamino formation alters HbO_2 affinity. In addition, the potential for buffering of a HCA to modulate its effects in sepsis is also of importance.

Pulmonary Sepsis

Buffered hypercapnia, that is, hypercapnia in the presence of normal pH, seems to worsen lung injury induced by intrapulmonary bacterial instillation.[36] To avoid the confounding effects of the administration of exogenous acid and/or alkali, animals were first exposed to environmental hypercapnia until renal buffering had restored pH to the normal range. These animals were then subjected to intrapulmonary inoculation of *E. coli*, and the severity of lung injury produced during a 6-h period was compared with that seen in similarly inoculated animals exposed to normocapnia. Buffered hypercapnia significantly increased *E. coli*-induced lung injury when compared with normocapnic controls, as assessed by arterial oxygenation, lung compliance, proinflammatory pulmonary cytokine concentrations, and measurements of structural lung damage. Of interest, buffered hypercapnia did not reduce the phagocytic capacity of neutrophils and did not increase lung bacterial load. These findings contrast markedly with the protective effects of HCA in evolving bac-

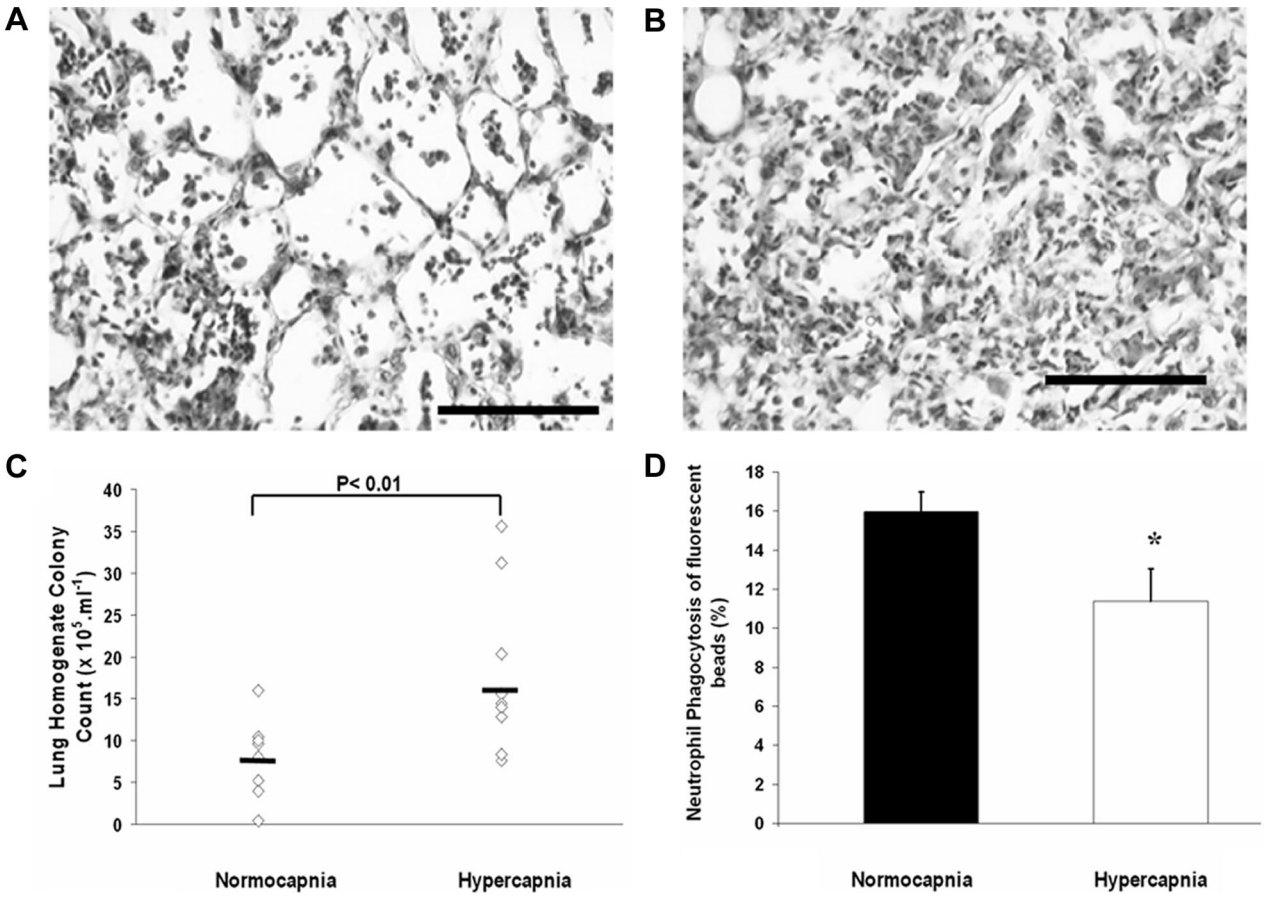

Fig. 4. Sustained hypercapnic acidosis worsens pneumonia-induced lung injury and increases bacterial load. (*A, B*) Photomicrographs of a section of lung tissue from a lung exposed to normocapnia and hypercapnia, respectively, 2 days after intratracheal infection with *Escherichia coli*. Animals exposed to environmental hypercapnia (inspired CO_2 5%) sustained a more severe lung injury. (*C*) *Scatterplot* demonstrating greater bacterial load in lungs from *E. coli*–infected groups exposed to hypercapnia compared to normocapnia. (*D*) Graph demonstrating that neutrophils from rats exposed to hypercapnia have a reduced ability to phagocytose fluorescent latex beads compared with neutrophils from normocapnic rats. *Scale bars* = 100 μm. * Significantly different from normocapnia. Modified with permission from Crit Care Med. 2008; 36:2128–35.

terial pneumonia-induced lung injury.[78] The effect of buffered hypercapnia in the setting of more established or prolonged pneumonia has not been determined.

Systemic Sepsis

Buffered hypercapnia exerts differential effects compared with that seen with HCA in the setting of systemic sepsis. In these experiments, animals were again exposed to environmental hypercapnia until renal buffering had restored pH to the normal range. These animals were then subjected to cecal ligation and puncture, and the severity of hemodynamic compromise and lung injury was assessed over a 6-h period. Buffered hypercapnia attenuated the hemodynamic effects of evolving systemic sepsis induced by cecal ligation and puncture.[89] The extent of attenuation of shock was comparable with, if not greater than that seen with HCA.[89] However, buffered hypercapnia did not protect the lung from systemic sepsis-induced lung injury. Reassuringly, there was no evidence to suggest that buffered hypercapnia worsened the degree of lung injury compared with normocapnia, and buffered hypercapnia did not increase the bacterial load in the lungs or the bloodstream.[89]

Summary and Conclusions

Hypercapnia constitutes an important component of protective lung ventilatory strategies. However, the generally beneficial effects of HCA in the setting of experimental nonseptic inflammatory injury contrast with a more complex spectrum of effects in the setting of live bacterial infection. Hypercapnia and/or acidosis exert diverse—and potentially conflicting—effects on the immune response, both humoral and cellular. Overall, HCA seems to suppress the immune response, although the net effect of its multiple actions seems to vary depending on the site of infection and also on whether the acidosis produced by the hypercapnia is buffered or not. HCA seems to protect the lung from injury induced by evolving or more established lung and systemic bacterial sepsis in relevant preclinical models. In contrast, the effects of HCA in prolonged untreated bacterial sepsis seem to differ depending on the source of the infection, with the immuno-

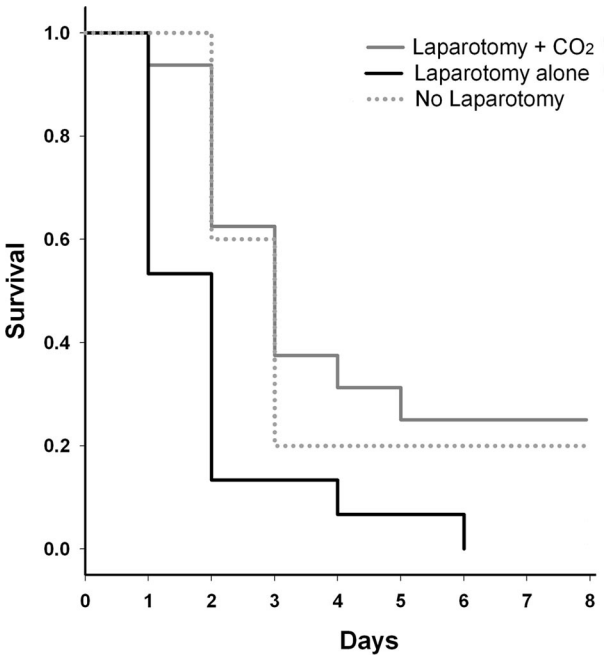

Fig. 5. Insufflation of carbon dioxide (CO_2) into the peritoneal cavity improves survival after cecal ligation and puncture-induced systemic sepsis. Animals were first subjected to cecal ligation and puncture. Four hours later, animals underwent a laparotomy and induction of a carbon dioxide pneumoperitoneum (laparotomy + carbon dioxide), laparotomy alone, or no laparotomy, and survival was determined over the following 8 days. Modified with permission from Eur J Pediatr Surg 2008; 18:171–5.

suppressive effects of HCA worsening lung injury in the setting of prolonged pneumonia. This deleterious effect is abrogated by effective antibiotic therapy. In contrast, HCA reduced lung damage caused by prolonged systemic sepsis, again highlighting the potential importance of the source of infection. Buffering of HCA seems to confer little benefit in the setting of experimental sepsis and worsens pneumonia induced lung injury.

Taken together, recent experimental findings in relevant preclinical models provide some reassurance regarding the safety of hypercapnia in sepsis, particularly in evolving and established pneumonia and in the setting of abdominal sepsis. However, in the setting of prolonged pneumonia, the immunosuppressive effects of hypercapnia remain a concern. Although the use of ventilation strategies resulting in hypercapnia is clearly justified in patients with ALI/ARDS, care is warranted in the setting of sepsis. The finding that deleterious effects of hypercapnia in the setting of prolonged pneumonia are abrogated by appropriate antibiotic therapy is of importance. Clinicians should, therefore, carefully consider the use of early empiric antibiotic therapy in hypercapnic ALI/ARDS patients in whom sepsis is suspected. However, concerns persist, particularly where antibiotic cover may be suboptimal, or the bacteria are more resistant to antibiotic therapy. The finding that hypercapnia may increase septic lung injury in the setting of prolonged pneumonia is also of relevance to other patient groups such as patients with infective exacerbations of chronic obstructive airways disease. Finally, buffering of the acidosis induced by hypercapnia does not confer any benefit and may worsen injury in the setting of pneumonia.

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Effect of Ventilator-induced Lung Injury on Skeletal Muscle Oxidative Balance

IN this issue of ANESTHESIOLOGY, Marin-Corral *et al.*[1] report a reduction of several markers of oxidative and nitroxidative stress in the diaphragm and limb muscles of rats exposed to ventilator-induced lung injury (VILI). This finding initially seems counterintuitive because VILI is thought to induce a systemic inflammatory state,[2] which should lead to increased, rather than decreased, oxidative stress also in tissues other than the lung. In this editorial, we will briefly review the basic biochemistry of the oxidative markers measured by Marin-Corral *et al.* to provide some context to these observations, highlight the main results of this study, and define a framework for future investigations of the skeletal muscle effects associated with VILI.

Oxidative Markers

Reactive oxygen species (ROS) are produced physiologically during cellular respiration. Although 95% of oxygen is reduced to H_2O and CO_2 during oxidative phosphorylation, 5% is reduced to superoxide anion radical ($O_2^{\cdot-}$) by capturing a single electron from the mitochondrial transport chain (fig. 1). $O_2^{\cdot-}$ is reduced to hydrogen peroxide (H_2O_2) both chemically and by enzymes such as superoxide dismutase (SOD), and H_2O_2 is converted to hydroxyl radicals ($^{\cdot}OH$). ROS radicals, and in particular $^{\cdot}OH$, oxidize proteins, lipids, and nucleic acids, altering the structure and function of these entities.

ROS production increases significantly above physiologic levels during inflammatory states. In their study, Marin-Corral *et al.* measured two end products of ROS-mediated oxidation: protein carbonyls[3,4] and malondialdehyde (MDA)-protein adducts.[5] Protein carbonyls are formed by several mechanisms including oxidation of primary (serine) or secondary (treonine) alcohol amino acid residues. MDA, instead, is a product of peroxidation of polyunsaturated fatty acids by ROS. It is highly reactive and binds covalently to proteins by alkylating several amino acid residues. Studies *in vitro* have shown that the levels of MDA correlate with those of MDA-protein adducts.[5]

Reactive nitrogen species (RNS) include nitric oxide ($^{\cdot}NO$) and its oxidation products with ROS. RNS can lead to both nitrosation (R-N=O) and nitration (R-NO_2) of amino acid residues (R). Tyrosine nitration has been recognized as a major posttranslational protein modification in cardiovascular[6] and respiratory[7] diseases and is often used as a biomarker of "nitroxidative stress."[8] The two main pathways for tyrosine nitration are as follows[6,8] (fig. 1): (1) reaction of $^{\cdot}NO$ with $O_2^{\cdot-}$ to generate peroxynitrite anion ($ONOO^-$), a highly oxidizing and nitrating compound that reacts with CO_2 to yield nitrogen dioxide ($NO_2^{\cdot}$) and carbonate radical ($CO_3^{\cdot-}$), which oxidizes tyrosine to tyrosyl radical. Tyrosyl radical is then nitrated by $NO_2^{\cdot}$ to yield 3-nitrotyrosine; (2) reaction of nitrite (NO_2^-, generated by oxidation of $^{\cdot}NO$ with molecular oxygen) with hemeperoxidases (*e.g.*, myeloperoxidase) and H_2O_2 to yield tyrosyl radical and $NO_2^{\cdot}$. As in the first pathway, $NO_2^{\cdot}$ then adds to the tyrosyl radical to generate 3-nitrotyrosine. This second pathway seems to be the main venue for tyrosine nitration *in vivo*, especially in heme-rich tissues such as skeletal muscle.[9]

ROS and RNS pathways are intimately interwoven as $O_2^{\cdot-}$ and H_2O_2 play a crucial role in the protein oxidation that forms the basis for tyrosine nitration.

Protection from ROS

The two main mechanisms of protection from ROS are SOD and catalase. SOD converts $O_2^{\cdot-}$ to H_2O_2, and catalase converts H_2O_2 to water. These metalloproteins act as antioxidants. In this study,[1] skeletal muscle and lung levels of catalase and the mitochondrial form of SOD (Mn-SOD) were measured to determine the level of protection from ROS.

VILI and Oxidative Stress

Because VILI has been postulated to initiate and propagate a systemic inflammatory response,[2] and ROS and RNS are critically important mediators of inflammatory states, one might expect that oxidative stress is increased during VILI. In the investigation by Marin-Corral *et al.*, inflammation occurred in the lungs of rats exposed to

◆ This Editorial View accompanies the following article: Marín-Corral J, Martínez-Caro L, Lorente JA, de Paula M, Pijuan L, Nin N, Gea J, Esteban A, Barreiro E: Redox balance and cellular inflammation in the diaphragm, limb muscles, and lungs of mechanically ventilated rats. ANESTHESIOLOGY 2010; 112:384–94.

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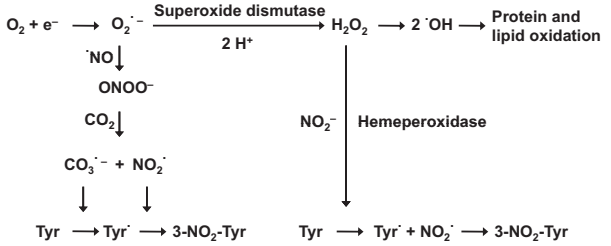

Fig. 1. Schematic representation of reactive oxygen and nitrogen species pathways leading to oxidation of organic substrates and tyrosine (Tyr) nitration.

VILI, because inflammatory cell infiltration and MDA-protein adducts increased in their lungs compared with nonmechanically ventilated controls. Moreover, protection from ROS seemed to decrease as Mn-SOD and catalase levels were lower than in the lungs of controls. However, instead of seeing evidence of increased oxidative stress in skeletal muscle, protein carbonyls, MDA-protein adducts, and protein tyrosine nitration decreased in skeletal muscle.

How can we explain these observations? One possibility is that, because of its hemodynamic effect, VILI impairs perfusion to peripheral tissues, including skeletal muscle, making them ischemic and limiting the amount of ROS that can form, akin to the ischemic phase of ischemia–reperfusion injury where the bulk of ROS is formed during the reperfusion rather than the ischemic phase. However, markers of oxidative stress as well as nitrotyrosine have been shown to increase within 40 min of renal ischemia even without reperfusion.[10] Furthermore, as Marin-Corral *et al.* point out, protein oxidation and nitration were also reduced in the moderate tidal volume group, which did not experience the hypotension and acidosis of the group receiving the higher tidal volume. Consequently, hypoperfusion is unlikely to be the sole explanation for these findings.

A more intriguing explanation is that the reduction in protein tyrosine nitration reflects an increase, rather than a decrease, in oxidative stress. As Bian *et al.*[9] elegantly showed, the relationship between H_2O_2 concentration and tyrosine nitration (and, to a lesser extent, also carbonyl formation) is biphasic: nitration increases steeply up to 0.5 mM H_2O_2 but decreases for higher concentrations of H_2O_2. This is probably because excess H_2O_2 causes suicide inactivation of peroxidases and degradation of heme in metalloproteins such as myoglobin.[9] Consequently, tyrosine nitration through the second pathway is expected to decrease at high concentrations of H_2O_2. Inactivation of peroxidase by excess H_2O_2 could also account for the apparently contradictory finding of decreased protein nitroxidation despite increased leukocyte infiltration in skeletal muscle, which would be expected to lead to increased tyrosine nitration by activation of the pathway by myeloperoxidase. In fact, all these biochemical assays measure reaction by-products of ROS and RNS, which may not necessarily correlate with the level of the reactive species themselves (see fig. 2B of Reference 9).

Finally, it is possible that oxidative and nitroxidative stress are simply not part of the early VILI-induced molecular changes in skeletal muscle, as the authors suggest.

Clinical Implications

What clinical inferences, if any, can we draw from this experiment? We know that loss of aeration in Acute Respiratory Distress Syndrome (ARDS) is highly heterogeneous[11] and the distribution of tidal volume uneven, such that very high regional stress and strain can develop in the ARDS lung even with clinically acceptable tidal volumes.[12] The value of studying the effect of these high levels of strain in normal lungs is to isolate the contribution of VILI to these biochemical phenomena while eliminating the confounding effect of other sources of lung injury. In this respect, studies in normal lungs provide "proof-of-concept" and are just as valuable as experiments in models of ARDS in which a second insult is imposed on the lung. It is quite possible that levels of strain comparable with those imposed on the whole lung in this study develop on a regional basis in ARDS. Thus, the results of this study suggest that VILI-induced skeletal muscle oxidative imbalance could contribute to muscle weakness and potentially to critical-illness myopathy in ARDS. If the observed decrease in protein oxidation was a marker of decreased ROS production, a reduction in muscle contractility could be expected because certain levels of ROS are required for optimal contractility.[13] If instead the decrease in protein oxidation and nitration was "paradoxically" a marker of increased ROS production, a reduction in contractility might be expected as a result of the inflammatory process associated with excessive ROS.

Future studies should clarify the relationship between levels of the primary noxious stimuli (*e.g.*, ROS or RNS), their reaction by-products (*e.g.*, protein carbonyls or nitrotyrosine), and the ensuing functional impairment (*e.g.*, reduced muscle contractility). How to interpret these associations and whether they have pathogenetic significance, however, require a focused assessment of the causal relation between the biomarker and the functional or structural abnormality. The big question that remains is whether the measured oxidative and nitroxidative protein changes induced by VILI affect skeletal muscle function and how this effect occurs.

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ANESTHESIOLOGY REFLECTIONS

The Lungmotor for Adults

In 1930 cartoonist and screenwriter Reuben L. "Rube" Goldberg (1883–1970) and his colleagues needed a resuscitating apparatus for a rescue scene in *Soup to Nuts*, the film debut of a trio now known as "The Three Stooges." The apparatus chosen for the comedy was America's adult version of Germany's Draeger Pulmotor—the "Lungmotor" manufactured by the Life Saving Devices Company. No laughing matter, one Lungmotor helped revive a mother and then her son from carbon monoxide poisoning just 35 miles from what is today's American Society of Anesthesiologists headquarters. Careful inspection of the example above (*courtesy of the Wood Library-Museum*) reveals the initial wording of the upside-down metal-punched hallmark of "THE LUNG MOTOR" on the apparatus' base.

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