Respiratory muscle dysfunction in animal models of hypoxic disease: antioxidant therapy goes from strength to strength

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Abstract: The striated muscles of breathing play a critical role in respiratory homeostasis governing blood oxygenation and pH regulation. Upper airway dilator and thoracic pump muscles retain a remarkable capacity for plasticity throughout life, both in health and disease states. Hypoxia, whatever the cause, is a potent driver of respiratory muscle remodeling with evidence of adaptive and maladaptive outcomes for system performance. The pattern, duration, and intensity of hypoxia are key determinants of respiratory muscle structural-, metabolic-, and functional responses and adaptation. Age and sex also influence respiratory muscle tolerance of hypoxia. Redox stress emerges as the principal protagonist driving respiratory muscle malady in rodent models of hypoxic disease. There is a growing body of evidence demonstrating that antioxidant intervention alleviates hypoxia-induced respiratory muscle dysfunction, and that N-acetyl cysteine, approved for use in humans, is highly effective in preventing hypoxia-induced respiratory muscle weakness and fatigue. We posit that oxygen homeostasis is a key driver of respiratory muscle form and function. Hypoxic stress is likely a major contributor to respiratory muscle malaise in diseases of the lungs and respiratory control network. Animal studies provide an evidence base in strong support of the need to explore adjunctive antioxidant therapies for muscle dysfunction in human respiratory disease.

Keywords: respiratory muscle, diaphragm, upper airway, hypoxia, antioxidants, N-acetyl-cysteine, OSA, COPD

Introduction

This review article focuses on the experimental evidence supporting a fundamentally important role for oxygen homeostasis in shaping respiratory muscle form and function. We consider studies employing rodent models of hypoxia relevant to human respiratory disease. Chronic intermittent hypoxia (CIH) is a dominant feature of human sleep-disordered breathing. Chronic sustained hypoxia (CSH) has relevance to environmental stress at high altitude and chronic lung diseases. We review studies exploring the mechanisms underpinning aberrant respiratory muscle remodeling following hypoxic exposure with a focus on studies employing antioxidant strategies to combat hypoxia-induced respiratory muscle dysfunction. We compare and discuss different respiratory muscles and hypoxic paradigms in various animal models. The emerging evidence strongly points to the need to explore the efficacy of adjunctive antioxidant therapies for respiratory muscle dysfunction in human respiratory disease.

Respiratory muscles: function, form, and plasticity

The respiratory muscles are the final effector organs of a complex neuromuscular control network that governs respiratory homeostasis ensuring adequate arterial blood...
gas and pH regulation. Neuromechanical coupling underpins ventilatory and nonventilatory functions such as protective airway clearance maneuvers and highly complex behaviors such as phonation. Activation of chest pump muscles, principally the diaphragm and intercostal muscles, generates dynamic cyclical changes in thoracic volume and pressure differentials sufficient to encourage airflow.\(^1\)\(^-\)\(^4\) This allows for effective gas exchange between the external environment and the alveoli, the highly vascular functional units of gas exchange in the lungs. Beyond the principal and accessory muscles of breathing that facilitate pulmonary ventilation across a range of activities are several other complementary muscles of the upper airways. These muscles serve a critical function in determining airway tone and caliber.\(^5\)\(^-\)\(^6\) These dilator and constrictor muscles regulate upper airways resistance, thus gating inspiratory and expiratory airflow, defending airway patency on a breath-by-breath basis.

There is relatively tight structure–function coupling in respiratory muscles. The diaphragm is a thin, dome-shaped muscle with a mixed muscle fiber-type complement befitting the physiological roles of the muscle.\(^7\)\(^-\)\(^8\) Basal breathing requires rhythmic activation of the diaphragm at a low level of maximum force-generating capacity.\(^3\)\(^-\)\(^4\) The high oxidative capacity of the diaphragm muscle fibers\(^7\)\(^-\)\(^8\) confers considerable fatigue resistance, a useful trait for the most active striated muscle in the body. Fast glycolytic fibers are also present\(^7\)\(^-\)\(^8\) and are recruited for near-maximal activation during airway obstruction, sneezing, and exacerbations of chronic respiratory disease.\(^9\)\(^-\)\(^10\) In contrast, upper airway dilator muscles are characterized by a predominant expression of fast glycolytic fibers,\(^11\)\(^-\)\(^12\) which may be important in preventing/overcoming pharyngeal collapse and maintaining airway patency in addition to nonrespiratory functions in deglutition and speech.\(^5\)\(^-\)\(^6\) While the fast phenotype of upper airway muscles facilitates powerful contractions, it renders them more vulnerable to fatigue. Notwithstanding the essential function of respiratory muscles to sustain life, the pump and airway dilator striated muscles of breathing retain considerable capacity for structural and functional plasticity in response to changes in functional demands. This extends far beyond developmental periods wherein temporal fiber-type and functional changes are well recognized.\(^13\)\(^-\)\(^17\) Indeed, diaphragm and upper airway dilator muscles show remarkable capacity for plasticity, as impressive as that documented in limb skeletal muscles in response to a variety of stressors in health and disease.\(^18\)

Highly malleable respiratory muscles might confer advantage in allowing organisms to respond to physiological challenges (e.g., exercise, pregnancy, development, and aging); however, it is increasingly apparent that aberrant remodeling has the capacity to limit ventilatory performance and contribute substantively to patient morbidity and mortality. Diaphragm dysfunction is described in critically ill patients and related animal models,\(^19\)\(^-\)\(^22\) and in chronic respiratory conditions such as chronic obstructive pulmonary disease (COPD).\(^23\)\(^-\)\(^24\) Upper airway muscle dysfunction is implicated in the pathophysiology of obstructive sleep apnea syndrome (OSAS).\(^25\)\(^-\)\(^27\) Importantly, different paradigms of hypoxia feature in such respiratory diseases.

**Hypoxia is a consequence and cause of respiratory morbidity**

Hypoxia arises in most tissues when there is inadequate oxygenation to meet functional demands. In healthy individuals, hypoxia can manifest at higher altitudes (whole-body) and with physiological stress such as exercise (focal). Hypoxemia is a recognized key feature of respiratory dysfunction presenting as a consequence of impaired gas exchange in acute and chronic respiratory disease. Episodic hypoxemia is a cardinal feature of respiratory control disorders such as OSAS, a very common primary syndrome,\(^28\) which can also present in hypoxic patients with COPD, that is, overlap syndrome.\(^29\) Often underappreciated, hypoxemia also presents in neurodegenerative diseases such as amyotrophic lateral sclerosis,\(^30\) Parkinson’s disease,\(^31\) and Alzheimer’s disease,\(^32\) as well as in neuromuscular conditions such as muscular dystrophy,\(^33\) often culminating in end-stage respiratory failure.

More than merely a symptom of respiratory malady, hypoxia is a potent stimulus driving phenotypic change in many systems, including the cardiorespiratory control system. Empirically, hypoxia is a major driver of respiratory system plasticity (Table 1). This includes potential for adaptive or compensatory plasticity in respiratory muscle that serves to protect against the challenge of oxygen deficit as well as the contemporaneous capacity to drive aberrant remodeling, which can further exacerbate and perpetuate respiratory dysfunction.\(^27\)\(^-\)\(^34\)\(^-\)\(^35\)

Of importance, but beyond the scope of this review, there is tremendous scope for hypoxia-dependent signaling to alter neural regulation of respiratory control through actions on sensory, central integrative, and motor systems that could exacerbate or compensate for muscle-specific changes.\(^35\) Outcomes are dependent upon, inter alia, the context of hypoxic exposure (e.g., age, sex, co-stimuli, comorbidity), the explicit nature of the hypoxia stimulus (e.g., pattern, intensity, and...
duration of exposure), and the inherent or acquired capacity to tolerate and/or recover from hypoxic insult.

### CIH modeling human respiratory disease causes respiratory muscle dysfunction

Several groups have independently observed upper airway dilator and diaphragm muscle weakness and/or fatigue following exposure to CIH in animal models modeling the CIH that occurs in human OSAS.15–17,26,39–46 CIH-induced upper airway muscle dysfunction does not require structural remodeling in the form of muscle fiber atrophy and/or fiber-type transitions,17,47 although fiber-type transitions including increased expression of MHC type 2B fast fibers have been described.37,39,40 This is of interest given that slow-to-fast fiber transitions are reported in human OSAS11,12,48 and that the relative area of fast fatigable fibers is increased in the English bulldog model of OSAS.49 The duration of CIH exposure is a key determinant of the respiratory muscle responses39,59 such that several weeks of exposure increases upper airway muscle fatigue,37,40–42,44,45 and impairs recovery from fatigue.36,37

Less is known about the effects of CIH on diaphragm muscle form and function. Short-term exposure to IH (hours to days) has been reported to have no deleterious effect on diaphragm structure and contractile function in rat.39,51 However, a subsequent study convincingly demonstrated that IH exposure of just 4 days in mice was sufficient to cause autophagy-associated atrophy of diaphragm, leading to weakness, which recovered in normoxia.52 Interestingly, Shortt et al52 demonstrated that rat diaphragm force and fatigue are affected in a manner dependent on the duration and intensity of the CIH exposure. Weakness and increased fatigue were observed following a 2-week exposure to IH (fractional of inspired oxygen concentration [FiO₂]=5% at nadir; 20 cycles per hour; 8 hours per day), with no muscle atrophy, but an increase in the relative area of MHC type 2B fast fibers and concomitant increase in the relative area of sarcoplasmic reticulum calcium ATPase 1-expressing fibers.50,53 This is consistent with the slow-to-fast transitions in rat airway dilator muscle.37,39 Longer durations of CIH (FiO₂=6%–8% at nadir; 120 cycles per hour; 8 hours per day for 5 weeks) are associated with increased fatigue and impaired recovery from fatigue in rat diaphragm.54 Moreover, exposure to CIH exacerbates diaphragm muscle weakness in mdx dystrophic mice,55 although it remains to be determined if there is altered susceptibility to CIH stress in mdx respiratory muscle. This is important to determine given that sleep-disordered breathing is a feature of muscular dystrophy.56

Furthermore, there is growing evidence to suggest that the effects of exposure to CIH on respiratory muscle performance may be age- and sex dependent. Upper airway dilator muscle weakness is observed following CIH exposure (FiO₂=5% at nadir; 20 cycles per hour; 8 hours per day) in young adult male rats,56 but not in middle-aged male rats,57 consistent with the findings of others who reported no structural or functional change in the upper airway muscles following CIH exposure in old-aged rats.57 In contrast, increased susceptibility to upper airway muscle weakness following CIH (FiO₂=5% at

### Table 1 Effects of hypoxic exposure on respiratory muscle form and function

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Intermittent hypoxia</th>
<th>Sustained hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease; environment</td>
<td>Sleep-disordered breathing</td>
<td>Lung diseases; altitude</td>
</tr>
<tr>
<td>Muscle force</td>
<td>Weakness15,17,26,36,37,39,44,45</td>
<td>Unchanged7,72,73; weakness3,72,73,77</td>
</tr>
<tr>
<td>Muscle endurance</td>
<td>Mixed effects: unchanged15,17,46,47; increased50; decreased40,42,44,45,50</td>
<td>Mixed effects: unchanged44,47; increased; decreased44,73,77</td>
</tr>
<tr>
<td>Muscle CSA</td>
<td>Unchanged46,52; decreased17,42,47,52</td>
<td>Unchanged or decreased: muscle specific7,14,72,75</td>
</tr>
<tr>
<td>Muscle fibers</td>
<td>Mixed effects: no change17,47,52; increased density of type 17; increased density of type 237,39,40,53</td>
<td>Increased density of type 17; slow-to-fast80</td>
</tr>
<tr>
<td>Duration-dependent</td>
<td>Weeks &gt; days50</td>
<td>Weeks &gt; days7,75,77</td>
</tr>
<tr>
<td>Acute effects</td>
<td>Yes (hours-to-days)17,47,52</td>
<td>Yes (hours)39,79,75</td>
</tr>
<tr>
<td>Intensity-dependent</td>
<td>Yes75</td>
<td>Yes (limited data)3,72,73</td>
</tr>
<tr>
<td>Age-dependent</td>
<td>Young &gt; adult &gt; old17,46,47,52,55</td>
<td>Young &gt; adult17</td>
</tr>
<tr>
<td>Sex-dependent</td>
<td>Males &gt; females46; OVX &gt; control40,59</td>
<td>Unknown: sex difference in acute hypoxia81</td>
</tr>
<tr>
<td>Redox-dependent</td>
<td>Markers of oxidative stress50,52,88; protein oxidation52; dysfunction without protein oxidation or lipid peroxidation15,50</td>
<td>Time-dependent protein oxidation75,77</td>
</tr>
<tr>
<td>HIF-1α stabilization</td>
<td>Mixed41,42,44,48,82</td>
<td>Muscle-specific5,57,77</td>
</tr>
<tr>
<td>Mitochondrial remodeling</td>
<td>Autophagy42; dysfunction5,81,87</td>
<td>Decreased density; increased efficiency21,89</td>
</tr>
<tr>
<td>Metabolic remodeling</td>
<td>Mixed14,17,46,57,82</td>
<td>Extensive75,77</td>
</tr>
<tr>
<td>Hypoxic tolerance</td>
<td>Muscle specific: unchanged46,47; improved15</td>
<td>Improved76</td>
</tr>
</tbody>
</table>

Notes: Summary of the effects of chronic intermittent hypoxia and chronic sustained hypoxia on respiratory muscle form and function derived from studies in rodent models. *Inferred from reference 72 compared with reference 7. Abbreviations: CSA, cross-sectional area; HIF-1α, hypoxia inducible factor-1 alpha; OVX, ovariectomized.
nadir; 12 cycles per hour; 8 hours per day) is observed during early life compared with adult animals. Moreover, upper airway muscle dysfunction following neonatal exposure to CIH extends into young adulthood suggesting lasting effects of antecedent exposure to CIH during critical windows of development. Indeed, neonatal exposure to CIH primes increased susceptibility to subsequent hypoxic stress in later life. Unlike airway dilator muscle responses, exposure to CIH (FiO2=5% at nadir; 12 cycles per hour; 8 hours per day) has no discernible effect on diaphragm form or function, an outcome that may relate to intrinsic differences in upper airway and diaphragm muscle fiber type and metabolic programming during development.

That sex hormones may influence respiratory muscle responses to intermittent hypoxic stress is evinced by studies involving male vs. female comparison, ovarioctomized females, estrogen replacement therapy, and phytoestrogens. Female skeletal muscle is usually less fatigable than male, but differences are task specific and dependent upon contraction velocity and specific muscle groups, though of interest, sex differences can persist with aging. Estrogen-dependent signaling may confer protection in upper airway muscles in response to CIH, which may explain in part the recognized 2–3-fold lower incidence of OSAS in premenopausal women compared with men. A recent review of the role of sex in sleep apnea is provided by O’Halloran and Lewis.

The significance of these findings is that upper airway and diaphragm muscle dysfunction is reported in human OSAS, and myogenic mechanisms may contribute to the pathophysiology of the disorder. Although it is recognized that OSAS is a complex multifaceted condition, exposure to CIH because of recurrent pauses in ventilation is a major pathogenic factor driving multisystem morbidity, including aberrant function in the effector muscles of breathing. An understanding of the mechanisms by which CIH drives respiratory muscle dysfunction offers potential for the development of therapeutic strategies serving to protect respiratory muscle performance.

**CSH modeling human respiratory disease causes respiratory muscle dysfunction**

Exposure to sustained hypoxia is a feature of a altitude and respiratory disease. Exposure to hypobaric hypoxia (380 mmHg, equivalent to ~10% inspired oxygen) for 6 weeks during adulthood has differential effects on rat upper airway dilator and diaphragm muscle endurance, with evidence that diaphragm muscle endurance is preserved or enhanced, whereas upper airway dilator muscle endurance is preserved or decreased. Exposure to 10% inspired oxygen for 4 weeks in mice decreased diaphragm muscle fiber cross-sectional areas and improved mitochondrial aerobic efficiency, likely contributing to the maintenance of diaphragm endurance. CSH decreasing diaphragm muscle fiber cross-sectional areas in rat and mouse could be viewed as advantageous in decreasing oxygen diffusion distance. On the other hand, evidence points to consequential time-dependent and substantial loss of force-generating capacity, which is also consistent with decreased fiber cross-sectional areas. Physiological tradeoffs may be at play in response to hypoxia as rat diaphragm shows evidence of increased hypoxic tolerance compared with normoxic diaphragm following CSH and CIH exposure.

Exposure of mice to 10% inspired oxygen for 6 weeks resulted in significant upper airway dilator and diaphragm weakness because of time-dependent progressive oxidation of proteins key to muscle contraction, metabolism, and cellular homeostasis. Remarkably, recent studies have demonstrated that just 6–8 hours of exposure to sustained hypoxia in mice is sufficient to cause upper airway dilator and diaphragm muscle weakness. Of interest, mdx dystrophic upper airway dilator muscle, which is weak compared with wild-type muscle, shows evidence of hypoxic tolerance suggesting a potential role for hypoxia-dependent signaling in mdx muscle dysfunction. This is interesting in the context of Duchenne muscular dystrophy given that hypoxemia is a feature of the debilitating neuromuscular disorder owing to respiratory insufficiency.

Exposure to sustained hypoxia for 60 days during early life had no effect on diaphragm myosin fiber complement, whereas exposure for 7–9 months caused a slow-to-fast myosin transition. Exposure to sustained hypoxia (450 mmHg, equivalent to ~12% inspired oxygen) for 7 days during neonatal development causes rat airway dilator muscle dysfunction that is age-dependent, waning with advancing age beyond the neonatal period, but persisting during normoxic recovery for several weeks if exposure to sustained hypoxia occurred in the first or second week of life. Sustained hypoxia did not affect muscle fiber-type distribution, nor the activity of key representative oxidative or glycolytic enzyme activities. Similar to observations following exposure to CIH during early life, sustained hypoxia for 7 days had no deleterious effect on rat diaphragm functional properties revealing muscle-specific effects of sustained hypoxic stress, with relative resilience in diaphragm muscle compared with airway dilator muscle to CSH during early development.
A recent study revealed sex differences intrinsic to respiratory muscle in response to severe hypoxia, but otherwise little is known concerning putative sex differences in respiratory muscle responses to CSH representing a significant gap in our knowledge worthy of further investigation.

Respiratory muscle remodeling in response to short-term hypoxic stress has relevance to respiratory conditions such as acute respiratory distress syndrome, particularly in the light of evidence that diaphragm weakness is a major predictor of patient morbidity and clinical outcome.

**Redox stress is a major driver of respiratory muscle dysfunction**

Altered redox signaling is implicated in both CSH- and CIH-induced respiratory muscle dysfunction albeit differentially. Williams et al reported that exposure to CIH caused a significant increase in upper airway muscle nicotinamide adenine dinucleotide phosphate (NADPH) oxidase expression, although airway dilator muscle weakness presents in the absence of overt muscle damage or oxidative stress, revealing that altered redox signaling is sufficient to drive dysfunction. It is clear that reactive oxygen species (ROS) inhibit upper airway dilator muscle function, although relatively modest oxidative stress is evoked in respiratory muscle during CIH exposure evidenced by decreased aconitase and glutathione reductase activities without protein oxidation or lipid peroxidation. This has led to speculation that CIH enhances NADPH oxidase superoxide production in myocellular microdomains, tonically suppressing force-generating capacity of respiratory muscles. This hypothesis is supported by the observation that acute antioxidant administration is capable of reversing CIH-induced airway dilator muscle weakness and that the phytoestrogen genistein may improve upper airway muscle function through antioxidant mechanisms after CIH insult. Thus, enhanced redox-dependent suppression of force may be sufficient to drive CIH-induced respiratory muscle weakness. Longer exposure to CIH increases endoplasmic reticulum stress and apoptotic signaling in upper airway muscles, and causes mitochondrial remodeling and dysfunction for which altered redox homeostasis could be causal and/or a consequence.

In mouse diaphragm, 4 days of IH exposure increased autophagy-dependent protein degradation causing muscle weakness with increased antioxidant gene expression. A modest CIH exposure over 2 weeks increased rat diaphragm pro-inflammatory cytokines and superoxide anion generation, and rat diaphragm dysfunction following CIH exposure is associated with modest attendant oxidative stress.

In stark contrast to CIH models, a pronounced time-dependent redox stress characterizes functional impairments in mouse upper airway and diaphragm muscles in response to CSH stress. Redox modulation of upper airway muscle metabolism and function, which appears hypoxia inducible factor-1 alpha (HIF-1α)-independent, gives rise to pronounced muscle weakness without proteolytic degradation, notwithstanding the substantial temporal increase in muscle protein carbonyl content, which, as revealed by two-dimensional proteomic analysis, ranges from mitochondria to the cross-bridges. Unlike diaphragm, fast fiber airway dilator muscle cross-sectional area is spared in CSH, and indeed hypertrophy has been reported in chronic hypoxic rat sternohyoid muscle, with evidence too of increased sterohyoid force-generating capacity in response to hypobaric hypoxia (450 mmHg, equivalent to ~12% inspired oxygen). More intense hypoxia over the same period (10% inspired oxygen for 6 weeks) does not affect rat upper airway muscle force, but causes airway dilator muscle weakness in mice, which appears related to direct redox modulation of the contractile apparatus.

There is pronounced diaphragm muscle protein carbonylation during exposure to CSH. In mouse diaphragm, CSH decreased mitochondrial density, inhibited mTOR-dependent protein synthesis, and promoted FOXO-3a and 20S proteasome activity. Pronounced metabolic remodeling occurs in CSH mouse diaphragm, which may be partly HIF-1α-dependent and thus redox-dependent, with altered mitochondrial respiration rates, decreased oxidative and glycolytic enzyme activities, and an apparent increased reliance on fatty acid metabolism. The redox-dependent plasticity confers early adaptive responses, which culminate in time-dependent diaphragm weakness, perhaps a tradeoff for cellular adaptive responses improving hypoxic tolerance. Lewis and O’Halloran have recently provided a review of redox-dependent adaptive and maladaptive responses of diaphragm muscle to CSH with relevance to respiratory muscle performance.

**Antioxidant intervention prevents hypoxia-dependent respiratory muscle dysfunction**

Antioxidant supplementation can ameliorate or prevent CIH-induced respiratory muscle dysfunction. The endogenous antioxidant, glutathione, appears pivotal to respiratory...
muscle responses to CIH. Pharmacological depletion of glutathione exacerbates CIH-induced airway dilator muscle fatigue, whereas N-acetyl cysteine (NAC) supplementation, which boosts endogenous glutathione levels, is the most effective intervention in preventing CIH-induced diaphragm weakness and fatigue, although this may also relate to general antioxidant effects of the supplement. Phytoestrogens are effective in preventing CIH-induced airway dilator muscle dysfunction with genistein demonstrating redox effects. There may be antioxidant effects of female sex hormones given that estrogen enhances cellular antioxidant defense. Antioxidant intervention is highly effective in preventing CSH-induced airway dilator and diaphragm dysfunction. Chronic antioxidant supplementation prevented respiratory muscle protein carbonylation following 6 weeks of sustained hypoxia in mice. NAC was especially effective in preventing respiratory muscle force decline following exposure to CSH. A pivotal role of ROS in driving maladaptive responses in chronic hypoxic mouse diaphragm is further revealed by antioxidant suppression of p38 MAP kinase, and likely subsequent suppression of FOXO-3a-dependent atrophy and proteolytic degradation. In this way it appears that NAC supplementation prevents CSH-induced diaphragm weakness. A comparison of the effects of antioxidants on muscle function in animal models of hypoxic stress is presented in Table 2. The data make a compelling argument in favor of exploration of the potential benefits of antioxidant intervention in human respiratory patients to limit or prevent respiratory muscle dysfunction arising from hypoxic stress, whatever the pattern of exposure (Figure 1).

### Activity-induced respiratory muscle plasticity: does muscle recruitment during hypoxic stress promote or mitigate respiratory muscle dysfunction?

It is important to acknowledge that respiratory muscle activation is likely a critical co-factor, in addition to direct hypoxic stress, driving phenotypic change in muscle in response to exposure to hypoxia. It is well appreciated that respiratory muscle recruitment is altered in respiratory disease states. In OSAS, periodic occlusion of the upper airway during

| Table 2 Effects of antioxidants on hypoxic respiratory muscle form and function |
|------------------------|-------------------------|-------------------------|
| Phenotype              | Intermittent hypoxia     | Sustained hypoxia        |
| Muscle force           | UA muscle improvement – Tempol<sup>46</sup> | UA muscle improvement – NAC<sup>77</sup> |
|                       | Diaphragm muscle improvement – NAC<sup>50</sup> | *UA muscle no change – Tempol<sup>77</sup> |
| Muscle endurance       | UA muscle improvement – Genistein<sup>42</sup> | *UA muscle no change – Tempol<sup>77</sup> |
|                       | UA muscle improvement – NAC<sup>38</sup> | *UA muscle no change – NAC<sup>77</sup> |
|                       | Diaphragm muscle improvement – NAC<sup>50</sup> | Diaphragm muscle improvement – NAC<sup>75</sup> |
|                       | Diaphragm muscle improvement – Tempol<sup>50</sup> | Diaphragm muscle no change – Tempol<sup>75</sup> |
|                       | Diaphragm muscle improvement – Apocynin<sup>50</sup> | – |
| Protein signaling      | UA muscle increased p-ERK1/2/ERK1/2 content – Genistein<sup>42</sup> | UA muscle unchanged phospho-JNK – Tempol<sup>77</sup> |
|                       | –                       | UA muscle unchanged phospho-JNK – NAC<sup>77</sup> |
|                       | –                       | Diaphragm muscle decreased phospho-p38 – NAC<sup>75</sup> |
| Antioxidant activity   | UA muscle increased SOD, GPx, and catalase – Genistein<sup>42</sup> | Diaphragm muscle decreased HIF-1α – NAC<sup>75</sup> |
|                       | –                       | Diaphragm muscle decreased HIF-1α – Tempol<sup>75</sup> |
| Mitochondria           | UA muscle decreased mitochondrial ROS – Genistein<sup>42</sup> | – |
| Oxidative stress       | UA muscle decreased MDA – Genistein<sup>42</sup> | UA muscle decreased carbonyl content – Tempol<sup>77</sup> |
|                       | Diaphragm muscle decreased GSSG:GSH – NAC<sup>50</sup> | UA muscle decreased carbonyl content – NAC<sup>77</sup> |
|                       | –                       | UA muscle increased muscle free thiol content – Tempol<sup>77</sup> |
|                       | –                       | UA muscle increased muscle free thiol content – NAC<sup>77</sup> |
|                       | –                       | Diaphragm muscle decreased carbonyl content – Tempol<sup>75</sup> |
|                       | –                       | Diaphragm muscle decreased carbonyl content – NAC<sup>75</sup> |
|                       | –                       | Diaphragm muscle increased free thiol content – Tempol<sup>75</sup> |
|                       | –                       | Diaphragm muscle increased free thiol content – NAC<sup>75</sup> |
|                       | –                       | Diaphragm muscle decreased free thiol content – NAC<sup>75</sup> |
|                       | –                       | – |
|                       | –                       | – |
|                       | –                       | – |

**Notes:** Summary of the effects of antioxidants on hypoxic respiratory muscle form and function derived from studies in rodent models. *Improved but not statistically significant.

**Abbreviations:** GPx, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide; HIF-1α, hypoxia inducible factor-1alpha; L-NNA, N<sup>ω</sup>-nitro-L-arginine (a competitive inhibitor of nitric oxide synthase); MDA, malondialdehyde; NAC, N-acetyl cysteine; p-ERK1/2, phosphorylated extracellular signal-related kinases; phospho-JNK, phosphorylated c-Jun N-terminal kinase; ROS, reactive oxygen species; SOD, superoxide dismutase; UA, upper airway.
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Hypoxic adaptation in respiratory muscle

Figure 1 Hypoxia-dependent respiratory muscle dysfunction.
Notes: Redox stress is pivotal to hypoxia-dependent respiratory muscle structural-, functional-, and metabolic remodeling. Antioxidant intervention interrupts the vicious feedback cycle that perpetuates respiratory muscle dysfunction. The potential benefits of antioxidants in ameliorating respiratory muscle dysfunction in human hypoxic disease warrant investigation.

Hypoxia,98–101 with implications for respiratory sensations and hyperinflation can further disadvantage the diaphragm because of altered length–tension relationships.93,94 These multifaceted, complex changes make it extremely difficult to assess the independent influences of various stressors on respiratory muscle performance in disease.95

With regard to animal models of hypoxic disease, mechanical confounders are largely eliminated. However, it is important to recognize that hypoxic stress produces an integrated cardiorespiratory response that results in increased activation of the respiratory muscles and attendant increases in respiratory muscle blood flow to support hypoxic hyperventilation. Thus, hypoxia results in a multimodal stimulus at the level of the respiratory muscles. Intense exercise can cause diaphragm fatigue,96,97 which is exacerbated by acute hypoxia,98–101 with implications for respiratory sensations (dyspnea) in patients.102 As ROS are generated in exercising muscle,103,104 it is conceivable that increased respiratory muscle work, particularly in the face of accompanying hypoxic stress, is an additional stressor to respiratory muscle in chronic models of hypoxia. We reason that this may be especially likely in CSH, which necessitates a persistent hyperventilation during chronic exposure. By its nature, CIH presents an intermittent activation of the respiratory muscles, though persistent hyperventilation following exposure to CIH has also been described.105 In short, though beyond the scope of this review, we acknowledge that increased respiratory muscle work during hypoxic exposure may contribute additional redox stress in respiratory muscles.

Notwithstanding the potential for activity-induced aberrant redox remodeling in respiratory muscles, which could exacerbate respiratory muscle performance by enhancing muscle oxidative stress, it is also plausible that activity-induced plasticity in respiratory muscles could drive adaptive outcomes. It has been shown that aerobic exercise results in dramatic adaptive changes in the diaphragm proteome.106 Exercise leads to improved diaphragm107,108 and upper airway109 muscle endurance, resulting from increased aerobic capacity. Increased endurance7 and mitochondrial efficiency73,86 in diaphragm, which differs to responses in limb muscles,73,110,111 might relate to differences in activation patterns as well as intrinsic differences in muscle form. Thus, one could argue that activity-induced plasticity might limit the deleterious effects of hypoxic stress. It should be understood that this will vary within and between diseases. For example, exercise worsens dystrophic diaphragm function112 purportedly because of increased mechanical stress on fragile myofibers. Collectively, it is difficult to discern the dynamic interplay between hypoxia, muscle activity (exercise), and cumulative redox balance in muscle, but these complex interactions are likely pivotal determinants of respiratory muscle performance in chronic disease. An integrated portfolio of stimuli must be borne in mind when considering the effects of hypoxia on respiratory muscle physiology (Figure 1), which likely result in dynamic temporospatial PO₂ gradients in muscle.

Conclusion and perspective
Despite the clinical relevance, there is a relative paucity of information on hypoxic adaptation in respiratory muscles. Recent studies in animal models illustrate that hypoxia, modeling human respiratory diseases, is a potent stimulus provoking plastic changes in respiratory muscles with implications for physiological function (Table 1). Redox stress is pivotal to hypoxic-dependent respiratory muscle dysfunction manifesting in pronounced weakness and fatigue, evidenced by redox remodeling (Table 1) and beneficial effects of antioxidant
supplementation (Table 2). The data reviewed herein suggest that greater appreciation of hypoxia-dependent remodeling in respiratory muscle is required to better inform mechanistic insight of respiratory disease and to shape interventional studies to halt or reverse disease progression. Hypoxia emerges as a likely major player in respiratory muscle remodeling in pulmonary diseases and respiratory control disorders. Antioxidants, in particular NAC, have proven highly effective in animal models of hypoxia, strengthening the case for their potential use in the treatment of respiratory morbidity in humans. The animal studies provide an evidence base in strong support of the need to explore adjunctive antioxidant therapies for muscle dysfunction in human respiratory disease. Beyond the relevance to primary respiratory diseases, we further suggest that hypoxia-dependent respiratory system maladaptation is an area worthy of careful consideration in neurodegenerative and neuromuscular conditions, both in terms of disease progression and interventional strategies.

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