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REVIEW

Oxidative stress, respiratory muscle dysfunction, and potential therapeutics in chronic obstructive pulmonary disease

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Abstract **Chronic obstructive pulmonary disease (COPD) is a highly relevant disorder that induces respiratory muscle dysfunction. One prevalent symptom of COPD is resistive breathing which causes respiratory muscle to significantly increase the magnitude of contractions, resulting in reactive oxygen species (ROS) formation and oxidative stress. Through cellular signaling cascades, ROS activate molecules such as mitogen-activated protein kinases and nuclear factor-κB. These signaling molecules stimulate the release of cytokines which in turn cause damage to the diaphragm, involving sarcomeric disruptions. In response to COPD induced fatigue, the diaphragm undergoes a beneficial fiber-type shift to type I muscle fibers, which are more resistant to hypoxia than type II fibers. The lung hyperinflation that occurs in COPD also causes intercostal muscle dysfunction, thereby exacerbating COPD symptoms. In addition, COPD is known to have a connection with heart failure, diabetes, and aging, further decreasing respiratory function. Currently, there is no cure for this disorder. Nevertheless, various potential therapeutic strategies focusing on respiratory muscle have been identified including respiratory muscle training, β_2-agonist therapy, and lung volume reduction surgery. In this review, we will outline the role of COPD, oxidative stress, and related complications in respiratory muscle dysfunction.**

Keywords COPD, diaphragm, ROS, cytokine, respiratory therapy

Introduction of COPD and its effect on the diaphragm

Chronic obstructive pulmonary disease (COPD) is defined by the progressive airflow obstruction of the peripheral airways and displays symptoms including lung inflammation, mucus hypersecretion, and emphysema (Groneberg and Chung, 2004). According to a 2012 report by the World Health Organization, approximately one person dies of COPD every ten seconds. It is also anticipated to become the world's third leading cause of death by 2020 (Groneberg and Chung, 2004). Besides its devastating morbidity rates, COPD places a substantial burden on the economy. Estimated direct and indirect costs of COPD in the United States are $29.5 billion and $20.4 billion, respectively (Roisin and Vestbo, 2011). Risk levels of COPD are measured by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) on a numeric scale: GOLD 1 identifies mild COPD symptoms, GOLD 2 moderate, GOLD 3 severe, and GOLD 4 very severe. Noxious inhalants such as tobacco smoke and sulfur dioxide are factors that can prompt COPD development and progressive damage to the respiratory system (Groneberg and Chung, 2004). Due to the lack of oxygen and the subsequent overexertion on the respiratory system, reactive oxygen species (ROS) formation is constantly triggered (Mohanraj et al., 1998; Reid, 2001). Excessive ROS are primarily responsible for degradation of intracellular proteins, rupturing of cellular membranes, intracellular Ca^{2+} overload, cellular necrosis, and apoptosis (Zuo et al., 2011b).

The most critical respiratory muscle, the diaphragm (Ottenheijm et al., 2005), as well as the intercostal muscles, experience the adverse effects of ROS similar to other skeletal muscles. Hypercapnic respiratory failure often accompanies COPD due to the declination of force per contraction of inspiratory muscle and the increase of intracellular CO_2 (Begin and Grassino, 1991; Loring et al., 2009). The work load placed on respiratory muscles produces a greater

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negative pleural pressure in order to inflate the lung (De Troyer and Wilson, 2009). Thus, COPD patients experience static hyperinflation of the lung due to emphysematous changes; additionally, expiration is prolonged as a result of expiratory flow limitation and many patients develop dynamic pulmonary hyperinflation (Dal Vecchio et al., 1990; Haluszka et al., 1990). This increase of the end-expiratory lung volume significantly exerts breathing loads on COPD patients (Loring et al., 2009). Consequently, long-term respiratory muscle stresses have required some patients to use ventilators. Noninvasive mechanical ventilation (NIV) has consistently proven to be successful in moderate COPD cases by decreasing respiratory rate and reducing severity of breathlessness in patients (Meyer and Hill, 1994; Brochard et al., 1995; Roisin and Vestbo, 2011). However, critical COPD cases may require invasive mechanical ventilation (Roisin and Vestbo, 2011).

This review presents a synopsis of the physiologic and anatomical changes that occur in patients with COPD as well as its current and potential therapeutic strategies.

Oxidative stress on the diaphragm

In skeletal muscle taken from amphibian models, *in vitro* studies have implied that production of ROS is amplified with intensified contractile activity (Zuo et al., 2011a). A small amount of ROS is necessary for optimum contractile function; on the contrary, excessive levels of ROS may cause a disturbance between the pro-oxidant and antioxidant equilibrium (Reid, 2001; Zuo et al., 2011b). This severely impairs normal skeletal and respiratory muscle function by contributing to a decreased maximal force of the diaphragm, causing lessened inspiratory pressure (Barreiro et al., 2005).

Previous research has determined that COPD diaphragms display escalated catalase activity and decreased lipid peroxidation (Wijnhoven et al., 2006). Catalase, one of the standard antioxidant enzymes, catalyzes the decomposition of hydrogen peroxide (ROS) to non-toxic water and oxygen. The enhanced enzymatic activity of catalase is a response to oxidative stress occurring in the diaphragm of COPD patients. This in turn results in decreased levels of lipid peroxidation due to augmented antioxidant capacity induced by ROS (Wijnhoven et al., 2006). From a clinical perspective, this intracellular adaptation may be favorable for the recovery of diaphragm function.

Cell signaling cascade in COPD induced IRB

Severe inspiratory resistive breathing (IRB) in COPD is intensified during oxidative stress and rapidly advances diaphragm damage regulated by cell signaling cascades (Sigala et al., 2011). In this case, breathing could potentially overexert the diaphragm muscle and ROS production may be continually triggered (Reid, 2001). As shown in Fig. 1, ROS initiate intracellular communication by activating mitogen-activated protein kinases (MAPKs) p-P38, extracellular-signal-regulated kinases (p-ERK 1/2), and nuclear factor-κB (NF-κB); in addition, the accumulation of these signaling molecules stimulate cytokine formation (Allen and Tresini, 2000; Kosmidou et al., 2002). These cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-2, and IL-1β, are believed to play a substantial role in skeletal muscle injury (Cannon and St Pierre, 1998; Tidball, 2005).

Diaphragm adaptation in COPD

COPD forces the diaphragm to overload itself which increases fatigue. In response to this oxidative stress, the diaphragm adapts its structural configuration. As a protective response that delays fatigue, fibers in COPD diaphragms shift to the more aerobic and fatigue resistant type I fibers. Thus, type I fibers are able to accommodate respiratory exhaustion triggered by COPD (Ottenheijm et al., 2008).

COPD diaphragms have also demonstrated a decreased number of type IIx fibers. Type IIx fibers are fast twitch fibers that function under anaerobic conditions; therefore, they are susceptible to more severe fatigue. It is worth noting that a healthy human diaphragm contains ~50% slow twitch type I fibers, ~30% intermediate type IIa fibers, and ~20% type IIx

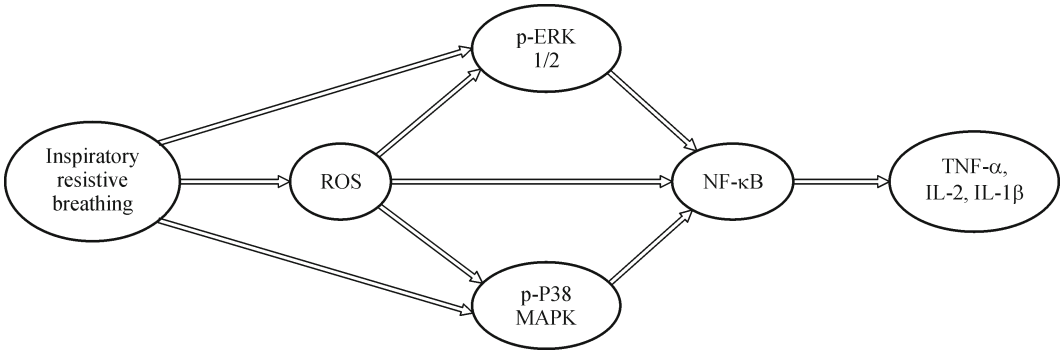

Figure 1 This schematic diagram outlines the proposed mechanism of TNF-α, IL-2, and IL-1β production in response to inspiratory resistive breathing.

fast twitch fibers (Mizuno, 1991; Levine et al., 1997; Mercadier et al., 1998). In contrast, a study on diaphragmatic adaptations by Levine and colleagues demonstrates the pronounced increase of type I and decrease of type IIx fibers in COPD diaphragms: 71±5% type I fibers, 21±3% type IIa fibers, and 8±3% type IIx fibers (Levine et al., 2002). The switch to slow twitch fibers is a positive adaptation of diaphragms affected by COPD.

Diaphragm metabolism and COPD

Skeletal muscles, including the diaphragm, require a vast amount of adenosine triphosphate (ATP) in order to generate force. To create this needed energy supply, the body relies on aerobic respiration which mainly occurs in the mitochondria (Wouters, 2000). A person with COPD, however, suffers from an inadequate supply of oxygen, sarcomeric damage of the diaphragm (Klimathianaki et al., 2011), and a reduction in the number of alveoli (Kang et al., 2008). To combat these hypoxic stresses, patients affected with mild COPD have undergone a shift in enzymatic activity in the diaphragm. A reduction in available oxygen due to COPD ultimately reduces the amount of glycolytic enzymes such as hexokinase (HK) and lactate dehydrogenase (LDH) which aid in glycolytic reactions in the cytosol of respiratory muscle cells (Sanchez et al., 1984). Furthermore, it increases the amount of oxidative enzymes that include L(+) 3-hydroacyl-CoA-dehydrogenase (HADH) and citrate synthase (CS) to utilize as much oxygen as possible (Doucet et al., 2004). CS activity, more specifically, is increased thereby initiating additional reactions in the citric acid cycle involving acetate and oxaloacetate which promote further ATP production (Ottenheijm et al., 2008). Moreover, a long-term deficiency in the oxygen supply of severe COPD patients has been found to induce diaphragmatic mitochondrial changes including enhanced coupling of oxidation to phosphorylation and heightened maximal mitochondrial respiration (Ribera et al., 2003).

Diaphragm injury in COPD

Numerous studies have identified that exertion can significantly injure muscle tissue (Friden et al., 1983; Armstrong, 1990), which is associated with morphological irregularities such as degeneration of the cytoplasm, disruption of cell membranous structures, and myofibril disorder (Orozco-Levi et al., 2001). Overloaded human diaphragms in COPD sustained greater sarcomeric damage compared to control, and this observation was proportionate to the severity of the condition (Klimathianaki et al., 2011). However, individuals without COPD displayed similar sarcomere alteration at a lower level (Orozco-Levi et al., 2001; Klimathianaki et al., 2011). There is also evidence of COPD induced inhibition of mitochondrial electron transport chain in respiratory muscles resulting in ROS formation and further muscular/sarcomeric damage (Puente-Maestu et al., 2009).

Earlier studies have revealed the interplay between extensive injury and collagen accumulation in overloaded diaphragms due to COPD. Examining the amount of collagen in these muscles may indicate increased exertion-induced muscle damage. More explicitly, muscle injury due to chronic exertion is associated with abnormal myofibers and the build-up of intramuscular collagen (Scott et al., 2006). Post mortem animal subjects with COPD consistently displayed larger collagen cross sectional areas compared to the control group, suggesting enhanced atrophy and extensive repair processes (Stauber et al., 2000; Willems and Stauber, 2001).

Functioning as an internal ventilator, inspiratory muscle strength can impact the survival rate of COPD patients. A decline in myosin content was observed to be a general response to COPD (Testelmans et al., 2010). Myosin, the motor protein responsible for contraction, when substantially decreased, leads to limited force generation. This results in the attenuation of maximal inspiratory force, likely due to decreased calcium sensitivity at submaximal stimulation (Ottenheijm et al., 2008). Other deficiencies were also observed such as impaired cross-bridge cycling kinetics and fiber atrophy (Ottenheijm et al., 2008; Stubbings et al., 2008; Testelmans et al., 2010). It has been noted that structural changes in the titin molecule, a protein responsible for the passive elasticity of muscle, may weaken the muscle filaments and further contribute to fiber damage. Accordingly, oxidative stress and sarcomere disruption in COPD could potentially activate protein degradation, resulting in loss of contractile proteins and diaphragmatic force (Ottenheijm et al., 2007).

Intercostal muscle dysfunction in COPD

The intercostal muscles play a key role during respiratory functioning. With the exception of the parasternal intercostals, the internal intercostal muscles are a part of the main expiratory muscles (De Troyer et al., 2005). The external intercostals are categorized mainly as inspiratory muscles, yet some scholars believe that they are both inspiratory and expiratory (Gea and Barreiro, 2008). In patients with COPD, the intercostal muscles frequently function abnormally and compromised intercostal muscle performance worsens COPD symptoms. This is because the inspiratory intercostal muscles are responsible for displacing the rib cage cranially and outwardly for maximal pressure generation. COPD, however, results in lung inflation followed by a change in rib cage orientation. The increased lung volume adversely affects the maximal pressure generating capacity of the inspiratory intercostal muscles, impeding the pulmonary oxygen uptake

needed for normal physiologic functioning (De Troyer and Wilson, 2009). Interestingly, as COPD progresses there is a marked decrease in intercostal muscle mass even in an environment with adequate nutrition, which requires further investigation (Guerri et al., 2010).

COPD, diabetes, and diaphragm dysfunction

A study that involved nearly 100000 women validated a statistically significant increased risk for patients with COPD to develop type 2 diabetes (Rana et al., 2004). Thus, chronic inflammation is a risk factor that facilitates the development of diabetes. This is because the development of pro-inflammatory cytokines such as C-reactive proteins (CRP), interleukin (IL)-6, and tumor necrosis factor-alpha (TNF-α) directly induce insulin intolerance and type 2 diabetes (Mannino et al., 2003; Rana et al., 2004). Moreover, since ROS could also be generated by inflammatory cells including neutrophils and macrophages in COPD muscles (Mroz et al., 2006; Cavalcante and de Bruin, 2009; Barreiro et al., 2010), this oxidative stress causes the breakdown of diaphragm tissues (Eid et al., 2001; Pitsiou et al., 2002), resulting in fat gain (Mador, 2002; Schols, 2003; Rana et al., 2004), which in turn increases TNF-α levels to promote insulin resistance. This is a key step in understanding how these diseases share a common inflammatory pathway toward respiratory muscle dysfunction and diabetes.

COPD, heart failure, and diaphragm dysfunction

The comparable symptoms of heart failure and COPD as well as similar underlying causes such as aging and smoking, have suggested that there could be a possible linkage between these two distinct disorders (Mascarenhas et al., 2010). The occurrence of heart failure in COPD patients fluctuates between 7.2% and 20.9% (McCullough et al., 2003; Rutten et al., 2005; Sidney et al., 2005; Curkendall et al., 2006). Lung hyperinflation, one of the characteristic pulmonary deformities presented in COPD patients (Ferguson, 2006), was evaluated in order to determine whether it plays a critical role in reduced heart chamber size and cardiac dysfunction in COPD patients. A positive correlation between a decrease in heart contractility and an increase in the severity of COPD was identified (Watz et al., 2010). Importantly, given the similarities of these two disorders, to assess the connection between COPD and heart failure is challenging (Mascarenhas et al., 2010). Although currently there is a lack of strong evidence regarding shared molecular mechanism of pathology, the reduced chamber size of the heart and increased pulmonary hyperinflation is highly related regardless of different stages of COPD (Watz et al., 2010).

It is further suggested that cardiac and respiratory muscles are interconnected with the injurious effects of hypoxia induced by both COPD and cardiac dysfunction on the diaphragm. Exercise intolerance and dyspnea are two complications experienced by patients with COPD as well as chronic heart failure. The causes of these deficiencies could be a reduction in oxidative enzyme activity and mitochondrial abnormalities (Laoutaris et al., 2012). Accordingly, any malfunction of the diaphragmatic muscle can further decrease oxygen uptake capacity and impede cardiac efficiency.

COPD and muscle aging

Many patients display symptoms of COPD late in life due to the slow onset and progression of this disease (Ito and Barnes, 2009). With aging, alveoli become dilated which consequently decreases the surface area of gas exchange and contributes to a decreased static elastic recoil of the lung, an increased functional residual capacity, and an expanded residual volume (Janssens et al., 1999). As a result, the respiratory pump overexerts and damages itself in order to maintain the adequate oxygen levels necessary for function. COPD has been associated with accelerating this process. The forced expiratory volume in one second (FEV_1) of those with and without COPD was previously measured. By the age of ~60, COPD patients lost ~50 mL in FEV_1 as compared to non-COPD patients who lost only ~10 mL in FEV_1. This study determined that those who smoke, which contributes to the development of COPD, had more expiratory airflow loss as compared to nonsmokers (Rennard and Vestbo, 2008).

Moderate levels of ROS are naturally created by skeletal muscle and contribute to the aging process (Cutler, 2005; Reid et al., 1992). Moreover, ROS have been linked to several signaling cascades that involve transcription regulators such as NF-kB, MAPK, and phosphoinositide 3-kinase (PI3K) (Allen and Tresini, 2000; Son et al., 2012). The protein kinase PI3K is widely seen to be activated by aging because of the increased oxidative stress on the body over time (Ito and Barnes, 2009). As previously stated, rigorous exertion of skeletal muscle causes the level of ROS to elevate in COPD (Reid, 2001). Therefore, it is believed that COPD accelerates the skeletal muscle aging process by further stimulating oxidative stress thereby increasing the activation of signaling cascades that are specific to aging.

Respiratory therapies for COPD

Respiratory muscle training (RMT) is a valuable therapy for patients with COPD. It is proposed that the addition of resistance training and inspiratory muscle training to aerobic exercise is a safe and successful method of treatment (Laoutaris et al., 2012). When compared to the use of aerobic training, the combined therapy benefitted the quadriceps and

inspiratory muscle indices and the cardiopulmonary parameters while lowering dyspnea and improving patients' quality of life scores; this provides a new perspective to designated rehabilitation programs (Laoutaris et al., 2012).

One method of RMT is inspiratory muscle training (IMT), which has been investigated by both the American College of Chest Physicians and the American Association of Cardiovascular and Pulmonary Rehabilitation Committee (ACCP/AACVPR). This activity is used during pulmonary rehabilitation programs (ACCP/AACVPR evidence-based guidelines, 1997). Experimentation has shown that when a controlled load is placed on the functioning respiratory muscle, physical endurance could be prolonged (Lotters et al., 2002). The success of IMT may be due to an increased number of slow twitch type I muscle fibers (by 38%) and an enlarged size of type II fibers (by 21%) of intercostal muscles (Ramirez-Sarmiento et al., 2002). A typical procedure of respiratory training is 2 times per day at 30 min each, 3–5 days per week for 6 weeks, using training loads that exceed 30% of the maximal inspiratory pressure with repetition (Crisafulli et al., 2007). Overall benefits of RMT include an improvement to respiratory breathlessness and stamina (Covey et al., 2001).

Breathing treatments have been shown to be effective in treating patients with COPD. A study of the addition of nocturnal nasal positive-pressure ventilation to the long-term oxygen therapy led to substantial benefits in patients with hypercapnic COPD including improved daytime and nocturnal blood-gas value, sleep efficiency, and quality of life (Meecham Jones et al., 1995). Additional research on oxygen therapy in COPD demonstrated that by replacing nitrogen with less dense helium during exercise, breathlessness and expiratory flow resistance were significantly reduced (Laude et al., 2006). This is consistent with a recent study showing that heliox therapy effectively increases skeletal muscle function by improving oxygen delivery in COPD patients (Louvaris et al., 2012).

Beta-2-agonist therapies and other pharmaceutics for COPD

The use of β_2-agonists can effectively treat asthma and other pulmonary diseases. One noteworthy study evaluated the effect of Broxaterol, a synthesized β_2-agonist, in patients with COPD. Broxaterol, 1-(3-bromo-5-isoxzaolyl)-2-(*tert*-butyl amino) ethanol hydrochloride, was proven to significantly improve the endurance time of respiratory muscle during fatigue caused by COPD (Nava et al., 1992). Clenbuterol (CB), another β_2-adrenergic receptor agonist, can improve diaphragmatic functioning in animal models with emphysema (Van Der Heijden et al., 1998). CB increases total mass, maximal force and myofibrillar protein content in the senescent diaphragm. Thus, the aging effect is eliminated and diaphragm strength is returned to the levels of young adult animals (Smith et al., 2002). It is expected that β_2-agonists propose a promising therapy treatment for COPD patients.

Other pharmaceutical therapies have been identified. For example, administration of *N*-acetylcysteine (NAC), a powerful antioxidant, is relevant to enhanced diaphragm function and reduced lung inflammation (Shindoh et al., 1990; Moon et al., 2010). However, none of these medications have been successful in curing COPD patients (Anthonisen et al., 1994; Pauwels et al., 1999; Vestbo et al., 1999; Burge et al., 2000). Furthermore, GOLD identifies non-pharmacological treatments for COPD, such as vaccinations, rehabilitation, physical activity, and the cessation of smoking (Roisin and Vestbo, 2011). These efforts to manage COPD should be dependent on individual assessments of patients.

Lung volume reduction surgery for COPD

Lung volume reduction surgery (LVRS), one of the final options to treat COPD, is a procedure in which parts of the lung are removed in order to reduce hyperinflation (Cooper et al., 1995). Consequently, there is an increase in the elastic recoil pressure of the lung and therefore an improvement in the expiratory flow rate (Fessler and Permutt, 1998). Further investigations of LVRS delineated a novel improvement in the survival rate of patients with severe upper lobe emphysema and those with low post-rehabilitation exercise capacity (Naunheim et al., 2006). A further exploration of LVRS has determined that a significant lengthening of the diaphragm is attributed to the reduction in lung volume (Lando et al., 1999). Thus, LVRS diaphragm can substantially improve its strength, exercise endurance, and maximum voluntary ventilation (Lando et al., 1999).

Conclusions

In this review, we have discussed the most recent findings regarding respiratory muscle dysfunction in COPD and potential therapeutic treatments. Based on both clinical and scientific experiments, we have summarized the ongoing investigations of the molecular mechanisms of COPD as well as the resulting damage to respiratory muscles. Although there is no current cure for COPD, we have identified several promising therapies and provided a fundamental basis to understand the complex mechanism of COPD induced muscle injuries.

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