Associate editor: P. Foster

Targeting oxidant-dependent mechanisms for the treatment of COPD and its comorbidities☆

CrossMark

Ivan Bernardo [a], Steven Bozinovski [a,b], Ross Vlahos [a,b,*]

[a] School of Health Sciences and Health Innovations Research Institute, RMIT University, Bundoora, VIC 3083, Australia
[b] Lung Health Research Centre, Department of Pharmacology & Therapeutics, The University of Melbourne, VIC 3010, Australia

ARTICLE INFO

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is an incurable global health burden and is characterised by progressive airflow limitation and loss of lung function. In addition to the pulmonary impact of the disease, COPD patients often develop comorbid diseases such as cardiovascular disease, skeletal muscle wasting, lung cancer and osteoporosis. One key feature of COPD, yet often underappreciated, is the contribution of oxidative stress in the onset and development of the disease. Patients experience an increased burden of oxidative stress due to the combined effects of excess reactive oxygen species (ROS) and nitrogen species (RNS) generation, antioxidant depletion and reduced antioxidant enzyme activity. Currently, there is a lack of effective treatments for COPD, and an even greater lack of research regarding interventions that treat both COPD and its comorbidities. Due to the involvement of oxidative stress in the pathogenesis of COPD and many of its comorbidities, a unique therapeutic opportunity arises where the treatment of a multitude of diseases may be possible with only one therapeutic target. In this review, oxidative stress and the roles of ROS/RNS in the context of COPD and comorbid cardiovascular disease, skeletal muscle wasting, lung cancer, and osteoporosis are discussed and the potential for therapeutic benefit of anti-oxidative treatment in these conditions is outlined. Because of the unique interplay between oxidative stress and these diseases, oxidative stress represents a novel target for the treatment of COPD and its comorbidities.

© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Contents

1. Introduction

An increased burden of oxidative stress is an important feature of the pathogenesis of chronic obstructive pulmonary disease (COPD) and its associated comorbid diseases (comorbidities). Current forms of therapy for COPD are largely ineffective and the development of effective treatments for COPD has been severely hampered as the mechanisms and mediators that drive the induction and progression of chronic inflammation, emphysema, altered lung function, defective lung immunity and many extrapulmonary comorbidities are still poorly understood. What is known is that four primary mechanisms have been implicated in the pathophysiological alterations observed in COPD: oxidative stress; inflammation; protease–antiprotease imbalance; and apoptosis (Barnes, 2013, 2014; Hillas, Nikolakopoulou, Hussain, & Vassilakopoulos, 2013). Although mostly underappreciated, oxidative stress has been recognized as a central component in the pathogenesis of COPD as it can trigger and further potentiate the other three mechanisms. In this review we focus on the role of oxidative stress in the pathogenesis of COPD and its comorbidities. Given its pivotal role in the onset and development of COPD, oxidative stress may be a novel target for the treatment of COPD and its comorbidities.

1.1. Chronic obstructive pulmonary disease overview

COPD represents an increasing global burden, afflicting over 600 million people and corresponding to approximately 5% of all deaths globally (Koul, 2013). It is currently the third leading cause of death (Lozano et al., 2012). It is well known that lower/middle income countries bear most of the burden of COPD with almost 90% of COPD-related deaths worldwide taking place in these countries (Lopez et al., 2006). Although the incidence rate of COPD is likely to escalate in both developed and developing countries, COPD poses a heavier burden on the Asia-Pacific and African regions where smoking is still widespread and gradually increasing (Chan-Yeung, Ait-Khaled, White, Ip, & Tan, 2004; Adeloye et al., 2015). Cigarette smoke is inarguably the biggest risk factor for COPD, with 90% of deaths from COPD directly attributable to smoking (Tashkin & Murray, 2009). Other risk factors include exposure to air pollutants and biomass fuels (Song, Christiani, XiaorongWang, & Ren, 2014). In addition, there is generally a long latency period between exposure to smoke and clinically-evident disease and as such, there is a high incidence of COPD in the older population and ex-smokers (Theander et al., 2014). This latency period generally lasts a number of years and by the time patients become symptomatic, the damage is already irreversible.

COPD is characterized by persistent airflow limitation and lung inflammation resulting in a progressive decline in lung function (Angelis et al., 2014). The main symptoms of the disease are chronic cough (smoker's cough), excessive mucus production and dyspnoea (particularly during exercise) (Theander et al., 2014). In addition to the pulmonary manifestation of COPD, many systemic manifestations occur in the form of comorbid diseases, such as skeletal muscle wasting, cardiac dysfunction, osteoporosis and lung cancer (Chatila, Thomashow, Minai, Criner, & Make, 2008; Barnes & Celli, 2009; Patel & Hurst, 2011). These comorbidities have been associated with increased oxidative stress and are known to affect, or are strong predictors of, the mortality of COPD patients independent of the decline in lung function (Schols, Slangen, Volovics, & Wouters, 1998; Marquis et al., 2002; Sin, Anthonisen, Soriano, & Agusti, 2006; Swallow et al., 2007).

Although COPD is a largely preventable disease; the numbers of diagnoses continue to increase resulting in ever increasing medical costs to patients, communities and governments (Lozano et al., 2012; GOLD, 2015). COPD patients generally have long hospital stays, require long-term treatments, and in addition to these medical costs, other "costs" such as an increase in days missed from work and limitations to quality of life, are important consequences of this disease (Rabe, 2007; Vestbo et al., 2013).

1.2. Acute exacerbations of chronic obstructive pulmonary disease

Patients with COPD often experience episodes of sudden worsening of symptoms, known as acute exacerbations (AECOPD). The Global initiative for chronic Obstructive Lung Disease (GOLD) defines an exacerbation as "a change in the patient's baseline dyspnoea, cough, and/or sputum and beyond normal day-to-day variations, that is acute in onset and may warrant a change in regular medication in a patient with underlying COPD". These exacerbations are considered to be part of the natural progression and chronicity of the disease and as COPD progresses, exacerbations become increasingly more frequent (Hurst & Wedzicha, 2009; Mackay & Hurst, 2013). Exacerbations result in a dramatic increase in lung inflammation and are associated with increased systemic inflammation compared to stable disease and as a result, there is only an increase in oxidative stress markers (Stanojkovic et al., 2011). This contributes to the worsening of symptoms and it has been shown that following hospitalisation for AECOPD, patients have increased mortality rates (Steer, Gibson, & Bourke, 2010).

AECOPD is often triggered by respiratory infections, such as those caused by the bacteria *Streptococcus pneumoniae* and *Influenza A virus*, or acute exposure to airborne irritants (Repine, Bast, & Lankhorst, 1997; Mackay & Hurst, 2013). There are also many non-aetiological risk factors that contribute to the frequency of exacerbations such as age, frequent past exacerbations and the presence of comorbid diseases (especially cardiovascular disease) (Laratta & van Eeden, 2014). The rates of exacerbations vary dramatically depending on the parameters used to define an exacerbation, however, rates of severe AECOPD measured by hospitalisations occur at an approximate rate of 0.5 to 3.6/person-year depending on the study cited (de Melo, Ernst, & Suissa, 2004; Seemungal, Hurst, & Wedzicha, 2009). The outcomes of AECOPD can vary from the return to near baseline spirometric parameters to respiratory failure and death. Exacerbations are the largest direct cost for the treatment of COPD due to the length of hospital stays and the frequency of the exacerbations per patient (Miravitlles, Murio, Guerrero, Gisbert, & EPOC, 2002; Miravitlles et al., 2004; Mackay & Hurst, 2013).

2. Oxidative stress in chronic obstructive pulmonary disease

2.1. What is oxidative stress?

Oxidative stress refers to the imbalance between the oxidant and antioxidant levels in favour of a pro-oxidant environment in cells and tissues (Kalyanaraman, 2013). An oxidant is a species that causes or promotes oxidation and an antioxidant is a molecule that inhibits either the formation of oxidants or inhibits oxidation itself. Oxidative stress arises from the inability of innate antioxidant mechanisms to neutralize oxidants generated endo- or exogenously resulting in an imbalance between oxidant and antioxidant factors. Consequentially, the oxidants predominate and chronic oxidative stress occurs, leading to the modification of lipids, proteins, and DNA (Rahman, 2005; Biswas, Hwang, Kirkham, & Rahman, 2013). The harmful modifications caused by oxidative stress are referred to as oxidative damage.

Oxidative stress can result from increased production of oxidants (in the form of free radicals/reactive oxygen and nitrogen species) or from diminished antioxidant levels or reduced antioxidant enzyme activity (Kalyanaraman, 2013). The depletion of dietary antioxidants (e.g., vitamins E, C, and D, flavonoids and carotenoids) and micronutrients (e.g., iron, copper, zinc, selenium) can also contribute to oxidative stress as they are needed for proper functioning of antioxidant enzymes (Delles, Xiong, True, Ao, & Dawson, 2014). In chronic inflammatory conditions, such as COPD, oxidative stress primarily results from the increased production of reactive oxygen species (ROS) from exposure to toxins (e.g., cigarette smoke, infection) and continuous activation of endogenous enzymes (e.g., NADPH oxidases) (Fig. 1).
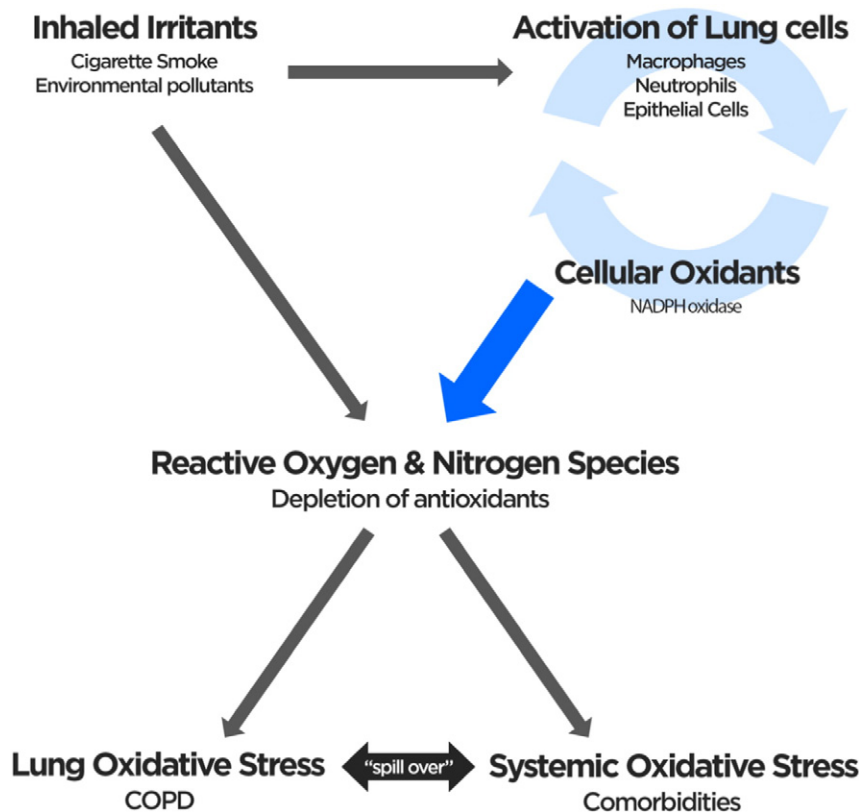
Fig. 1. Overview of oxidative stress in the pathogenesis of COPD. The generation of reactive oxidant species both exogenously and endogenously can lead to the onset and development of COPD and its comorbidities. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are found in cigarette smoke but in COPD the primary sources of ROS and RNS are from inflammatory cells including macrophages, neutrophils and epithelial cells. When activated by inhaled irritants (e.g., cigarette smoke), enzymatic (e.g., NADPH oxidase) generation of oxidants leads to the depletion of scavenging antioxidants (e.g., vitamin E), resulting in oxidative stress.

2.1.1. Chronic obstructive pulmonary disease and oxidative stress

The prolonged increase in oxidative stress is a major factor in potentiating both the airway and systemic inflammation in COPD and is known to play a key role in the onset and development of COPD and its comorbidities (Montuschi et al., 2000; Cavailles et al., 2013; Kirkham & Barnes, 2013). Direct damage occurs due to the oxidants found in cigarette smoke and from excessive levels of ROS and reactive nitrogen species (RNS) produced as a result of both pulmonary and systemic inflammation. An increase in ROS production in the airways is reflected by elevated levels of markers of oxidative stress (e.g., superoxide and malondialdehyde) in the airspaces, sputum, breath, lungs, and blood in patients with COPD (Rahman, 2005; Stanojkovic et al., 2011). These levels of oxidative stress markers are also dramatically increased during exacerbations of COPD (Antus, Harnasi, Drozdovszky, & Barta, 2014). Due to the nature of the oxidative burden and its consequences in the progression of COPD, this review is primarily focused on the role of ROS/RNS in the pathogenesis of COPD and its comorbidities, and the potential pharmacological targets related to ROS signalling.

2.1.2. Environmentally-derived reactive oxygen species

Each puff of cigarette smoke contains more than 10^{15-17} oxidant/free radical molecules and over 4700 highly reactive chemical compounds, such as aldehydes and quinones, which increases the oxidant burden in smokers (Church & Pryor, 1985; Nakayama, Church, & Pryor, 1989; Pryor & Stone, 1993; Rahman, 2005, 2012; Kirkham & Barnes, 2013). The nature of ROS found within cigarette smoke varies from short-lived oxidants, such as the superoxide radical ($O_2 \bullet^-$) and the nitric oxide radical (NO•), to long-lived organic radicals, such as semiquinones that can undergo redox cycling within the epithelial lining fluid of smokers for some considerable period of time (Nakayama et al., 1989;

Valavanidis, Vlachogianni, & Fiotakis, 2009a,2009b). Lung and systemic formation of protein carbonyls in response to cigarette smoke-derived lipid peroxides/carbonyls have also been implicated in the pathogenesis of COPD (Montuschi et al., 2000).

In addition to the release of oxidants, cigarette smoking is also associated with an increased amount of myeloperoxidase (MPO) in neutrophils, an oxidising factor that forms hypochlorous acid and converts tyrosine to tyrosyl radical (Bridges, Fu, & Rehm, 1985). Studies have shown a correlation between the content of MPO in neutrophils and the degree of pulmonary dysfunction observed in patients (Vaguliene, Zemaitis, Lavinskiene, Miliauskas, & Sakalauskas, 2013). In addition, studies suggest that neutrophil MPO-mediated oxidative stress plays a role in lung inflammation (Gernez, Tirouvanziam, & Chanez, 2010). It is also known that cigarette smoke can cause the activation of alveolar macrophages, which is observed in the bronchoalveolar lavage fluid (BALF) from the lungs of smokers and COPD patients but not present in non-smokers. The activation of macrophages contributes to the endogenous generation of ROS in the respiratory tract (Kirkham, Spooner, Ffoulkes-Jones, & Calvez, 2003; Barnes, 2004).

2.1.3. Cellular derived reactive oxygen species

Cellular-derived ROS is enzymatically produced by inflammatory and epithelial cells within the lung and/or systemically as part of an inflammatory-immune response towards a pathogen or irritant (Kim et al., 2013). Production of ROS by phagocytes can be enhanced by oxidants present in cigarette smoke leading to the release of inflammatory mediators (Rahman, 2012). Several sources for ROS production exist within a cell; however, the primary ROS generator is the enzyme NADPH oxidase (NOX), which comes in various isoforms: NOX-1, NOX-2, and NOX-4 (Selemidis, Sobey, Wingler, Schmidt, & Drummond, 2008; Drummond, Selemidis, Griendling, & Sobey, 2011).

In humans, NOX-1 and -2 are significant ROS-generators which are made up of an enzyme complex which is present in phagocytic and non-phagocytic cells including epithelial cells, macrophages, and skeletal muscles (Griffith et al., 2009; Barbieri & Sestili, 2012). Although NOX is found in many cell types, it is latent in neutrophils under normal circumstances (MacNee & Rahman, 2001). Once activated, neutrophils and macrophages can generate ROS via the NADPH oxidase system, leading to further augmentation of oxidative stress in the lungs of smokers and COPD patients (Fig. 2).

NOX-generated ROS have long been recognized to play key roles in the pathogenesis of a number of diverse chronic lung disorders that result in obstructive physiology, in particular asthma, cystic fibrosis, and emphysema (Griffith et al., 2009). Mice deficient in p47phox or NOX-2 exhibit increased cigarette smoke-induced lung inflammation and emphysema despite decreased ROS production compared with control mice (Yao et al., 2008). The lung responses in p47phox- and NOX2-null mice were associated with increased production of pro-inflammatory cytokines and chemokines via a TLR4–NF-kB pathway, indicating that NOX-2 may mediate anti-inflammatory functions by restraining TLR4 activation (Yao et al., 2008). However, another group reported that p47phox-null mice have less inflammation, IL-6, keratinocyte-derived chemokine, and monocyte chemoattractant protein-1 in lung-lavage specimens after cigarette-smoke exposure compared with WT mice (Gicquel et al., 2008). The differences observed by these groups may be due to variability in lung compartment sampling, cellular distributions, and chronicity of cigarette-smoke exposure.
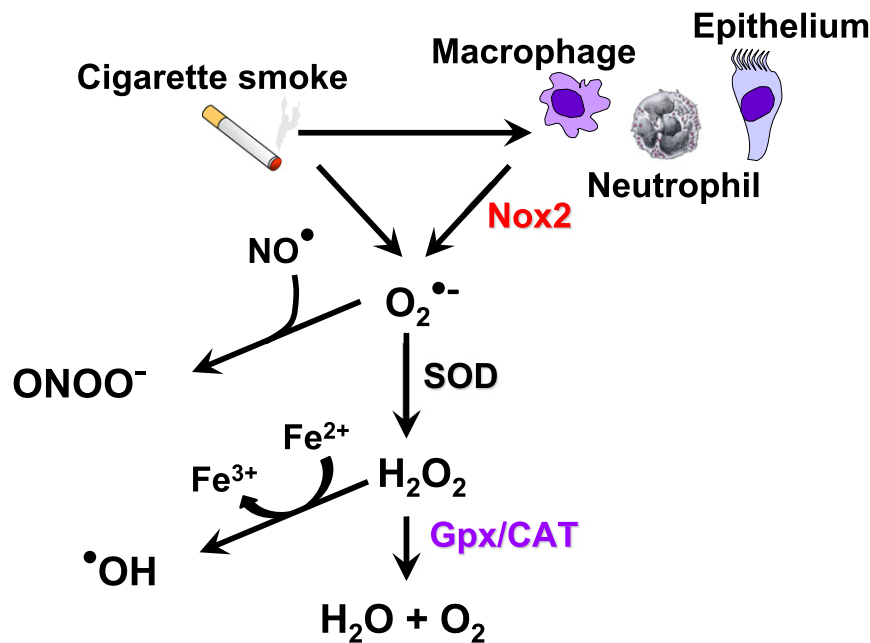
2.1.4. Reactive nitrogen species

Although increased production of ROS is the primary mechanism of oxidative stress in COPD/chronic lung diseases/acute lung diseases, there is compelling evidence to suggest that RNS also play a role in COPD (Ichinose, Sugiura, Yamagata, Koarai, & Shirato, 2000; Ichinose et al., 2003). Reactive nitrogen species include nitric oxide (NO•), a nitrogen free radical, and its derivative species such as peroxynitrite and nitrogen dioxide (Fig. 3). As with ROS, in addition to generation
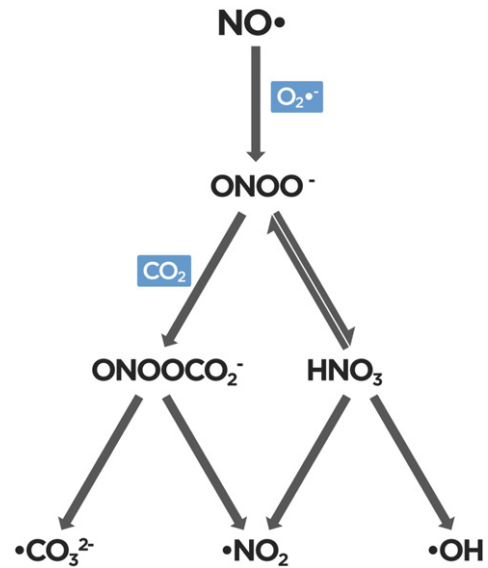
Fig. 3. The reactive nitrogen species cascade. In addition to its ability to cause direct damage, $ONOO^-$ can also react with carbon dioxide (CO_2) in vivo. The reaction between $ONOO^-$ and CO_2 occurs rapidly under physiological conditions, forming nitrosoperoxycarbonate ($ONOOCO_2^-$). $ONOOCO_2^-$ homolyzes (the molecule dissociates into 2 free radicals) to form the carbonate radical ($\bullet CO_3^{2-}$) and nitrogen dioxide ($\bullet NO_2$) (Kalyanaraman, 2013). It is these radicals ($\bullet CO_3^{2-}$ and $\bullet NO_2$) that are believed to cause peroxynitrite-related cellular damage. The conjugate acid of peroxynitrite, peroxynitrous acid (HNO_3) also homolyzes into $\bullet OH$ and $\bullet NO_2$ radicals, adding to the oxidative burden caused by peroxynitrite (Merenyi, Lind, Goldstein, & Czapski, 1998).

endogenously, RNS are present in cigarette smoke and air pollutants in the form of NO• and has many of the same harmful effects as ROS (Hasnis, Bar-Shai, Burbea, & Reznick, 2007). Endogenously, NO• is associated with a multitude of signalling pathways in mammalian physiological and pathological processes; however, in excess it too causes

Fig. 2. Cellular generation of reactive oxygen and nitrogen species in COPD. Cigarette smoke acts on inflammatory cells in the lung (e.g., macrophages, neutrophils, epithelium) where activation of NADPH oxidase 2 (Nox2) generates superoxide radicals. ($O_2^{\bullet-}$) which can then either react with nitric oxide (NO•) to form the reactive peroxynitrite molecule ($ONOO^-$) or be rapidly converted to hydrogen peroxide (H_2O_2) via the enzymatic activity of superoxide dismutase (SOD). In the presence of Fe^{2+}, H_2O_2 can be converted into the more damaging hydroxyl radical ($\bullet OH$) via the Fenton reaction. This reaction causes the oxidation of Fe^{2+} to Fe^{3+}, and in this oxidation state, the presence of iron can directly generate $\bullet OH$ from $O_2^{\bullet-}$. These iron reactions have increased importance in COPD as a higher concentration of iron has been reported in the lungs of smokers, thereby increasing the potential ROS burden (Gloire et al., 2006). The glutathione peroxidase (Gpx) family of enzymes, and catalase (CAT) are responsible for the conversion of H_2O_2 into harmless water and oxygen, which effectively reduces circulating ROS and thus reduces the oxidative burden (Vlahos & Bozinovski, 2013).

indiscriminate damage to surrounding tissues and can react with $O_2\bullet^-$ forming the even more harmful peroxynitrite radical ($ONOO^-$).

2.1.4.1. Nitric oxide. For years, NO• had only been considered as a toxic, unstable free radical gas that was just a constituent of air pollutant and cigarette smoke. However, it is now known that NO• can be generated endogenously in several types of cells (Bredt, 1999). This implicated NO• in various physiological roles and pathways, including host defence, vascular regulation and neuronal communication (Kalyanaraman, 2013). NO• has been well characterized in human biology and is perhaps the most important endogenous vasoprotective molecule in addition to its role in cardiovascular function (Moncada & Higgs, 2006; Tang & Vanhoutte, 2009; Vanhoutte, Shimokawa, Tang, & Feletou, 2009; Feletou, Kohler, & Vanhoutte, 2012). NO• inhibits vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium, contributing to vascular homeostasis (Cooke, 2004; Moncada & Higgs, 2006; Tang & Vanhoutte, 2009; Vanhoutte et al., 2009; Feletou et al., 2012; Triggle et al., 2012). Individuals with hypertension, atherosclerosis and/or diabetes often show impaired NO• signalling, highlighting the importance of NO• with regards to comorbid cardiovascular disease (Huang, 2009) (see below).

Nitric oxide is generated by phagocytes and is biosynthesized endogenously from the amino acid L-arginine, oxygen, and NADPH by various nitric oxide synthase (NOS) enzymes (Palmer, Ashton, & Moncada, 1988). In addition to its signalling roles, NO• has both pro-oxidant and antioxidant activities; excessive NO• can cause direct oxidative damage, however, NO• can also scavenge circulating ROS (Joshi, Ponthier, & Lancaster, 1999; Wink et al., 2001). More importantly, $O_2\bullet^-$ can react directly with endothelium-derived NO• forming the harmful $ONOO^-$ molecule.

2.1.4.2. Peroxynitrite. Peroxynitrite is formed by the reaction between $O_2\bullet^-$ and NO• in vivo. The pairing of these 2 radicals results in $ONOO^-$ anion, which is not a free radical but is still a very potent oxidant. $ONOO^-$ is both an oxidising and nitrating agent and can thus damage a wide array of molecules, including DNA and proteins (Beckman, 1996; Szabo & Ohshima, 1997). The formation of $ONOO^-$ is of particular relevance to the comorbidities of COPD as its formation involves the consumption of NO•, reducing the bioavailability of NO• for physiological processes (see Section 3.2.3).

2.1.5. Depletion of antioxidants

ROS/RNS generated exogenously or endogenously, whether circulating or in pulmonary vasculature, are scavenged by blood antioxidants and antioxidant enzymes. Accordingly, the ability to protect against the deleterious effects of oxidative stress depends greatly on the antioxidant capacity of the blood and the tissues (Rahman, Morrison, Donaldson, & MacNee, 1996; Rahal et al., 2014). Studies have shown that in addition to the increased levels of circulating oxidants, there is also an observed decrease in systemic antioxidant capacity in smokers and patients with COPD (Rahman, Swarska, Henry, Stolk, & MacNee, 2000). This is due to the saturation of lung antioxidants, plasma antioxidants and protein sulfhydryls by the excessive amounts of circulating ROS released by neutrophils and macrophages (Rahman et al., 2000). The saturation of many of these antioxidants, such as uric acid, glutathione (GSH), vitamin E, and ascorbate is also associated with the severity of COPD exacerbations (Rahman, Skwarska, & MacNee, 1997). Cigarette smoke, the main aetiological risk factor for COPD, has also been shown to irreversibly modify glutathione to glutathione conjugates in the airway epithelium resulting in antioxidant deficiency and injurious lung response (van der Toorn et al., 2007). Cigarette smoking also inhibits the protective expression of the Nrf2/antioxidant response element pathway in peripheral mononuclear cells of smokers, favouring a pro-inflammatory state (Garbin et al., 2009). In addition to the antioxidant saturation, studies have also shown a decrease in anti-oxidative enzyme function in COPD

patients; specifically reduced SOD and Gpx activity (Kurys, Kurys, Kuzniar, & Kieszko, 2001).

There is evidence to suggest that the anti-oxidant enzyme glutathione peroxidase-1 (Gpx-1) may have a role in regulating the inflammatory response to cigarette smoke exposure. Elevated levels of H_2O_2 are measured in the exhaled breath condensate of COPD patients, particularly during exacerbations (Dekhuijzen et al., 1996). There is upregulation of Gpx-1 gene expression in the lungs of smokers (Bentley, Emrani, & Cassano, 2008) and depletion of Gpx activity in COPD patients and smokers (Santos et al., 2004; Kluchova, Petrasova, Joppa, Dorkova, & Tkacova, 2007; Vibhuti, Arif, Deepak, Singh, & Qadar Pasha, 2007). With respect to reduced Gpx activity in COPD patients and smokers, erythrocyte Gpx activity was significantly lower in patients with severe COPD compared with patients with moderate COPD and there is a direct relationship between systemic Gpx activity and FEV_1 (Kluchova et al., 2007). In addition, Gpx activity was decreased in plasma from COPD patients and oxidative stress correlates with both lung function and body mass index in COPD (Vibhuti et al., 2007). Moreover, Gpx activity was decreased in total blood from smokers and ex-smokers (Santos et al., 2004). However, these studies did not identify the isoform of Gpx that was involved in reduced activity of Gpx.

2.2. Oxidative modifications

Oxidative stress causes a wide array of physiological and pathological consequences not necessarily limited to just COPD patients. In COPD, increased oxidative stress can cause cell damage, cell necrosis, apoptosis, autophagy, remodelling of extracellular matrix and blood vessels, endothelial dysfunction, inactivation of antiproteases, premature cellular senescence, elevated mucus secretion, steroid resistance, unfolded protein response, cell proliferation, epigenetic changes, and autoimmunity (Rahman, 2005; Rahman & Kinnula, 2012; Kirkham & Barnes, 2013). Oxidative modifications of DNA, proteins and lipids all contribute to the pathophysiology of the disease.

2.2.1. DNA

Unlike the other ROS and RNS which do not react with DNA bases or deoxyribose, the hydroxyl radical and peroxynitrite can react with both purine (adenine & guanine) and pyrimidine (cytosine & thymine), forming specific products (Cooke, Evans, Dizdaroglu, & Lunec, 2003). This oxidative damage can lead to mutation of DNA bases caused by AT–GC transition and GC-AT transversion and, if left unrepaired, can result in changes in protein gene expression (Kalyanaraman, 2013). In addition to this, ROS/RNS-induced DNA damage also involves single- or double-stranded DNA breaks and DNA cross-links, DNA damage inducing either arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability (Naito, Suematsu, & Yoshikawa, 2011).

2.2.2. Protein

Oxidative modifications to proteins caused by ROS/RNS include protein fragmentation, oxidation of amino acids, the formation of carbonyls, dityrosine and nitrated and chlorinated tyrosines (Berlett & Stadtman, 1997; Grimsrud, Xie, Griffin, & Bernlohr, 2008). In COPD and other inflammatory diseases, elevated levels of nitrated, chlorinated and brominated tyrosines have been detected in the tissues of patients (Kim, Mofarrahi & Hussain, 2008). These oxidative changes to proteins can result in various functional consequences, such as inhibition of enzymatic and binding activities, increased susceptibility to aggregation and proteolysis, modifications in uptake by cells, and altered immunogenicity (Shacter, 2000).

2.2.3. Lipids

In the presence of ROS/RNS and oxygen, lipids undergo a chain oxidation reaction leading to peroxidation. This chain reaction is initiated by oxidants, primarily the hydroxyl radical and leads to the formation

$$LH + \bullet OH$$

$$L \bullet$$

LH   O$_2$

$$LOO \bullet$$

antioxidant

$$LOOH$$

(termination)
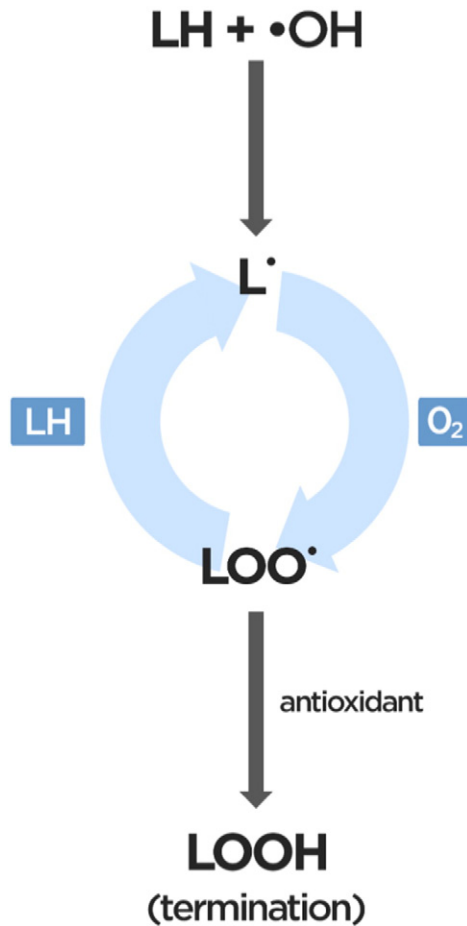
Fig. 4. Oxidants (•OH) abstract the allylic hydrogen from lipid molecules (LH) forming the carbon-centred lipid radical. This radical then undergoes molecular rearrangement to form a more stable form of the carbon-centred radical (L•). In the presence of oxygen, L• rapidly forms a lipid peroxy radical (LOO•). This new radical can then abstract an allylic hydrogen from another lipid molecule generating a new L•. This process can be terminated by the donation of a hydrogen atom from vitamin E to the LOO• species forming a vitamin E radical. The vitamin E radical then reacts with another LOO• forming non-radical products and thus terminating the reaction.

of lipid peroxide and lipid radicals which can reinitiate the chain reaction. This process continues indefinitely unless terminated by phenolic antioxidants, also known as chain-breaking antioxidants (e.g., vitamin E) (Kalyanaraman, 2013) (Fig. 4). This is especially important in comorbid cardiovascular disease where the formation of plaques in the arteries is initiated from lipid peroxidation.

2.3. Effects of oxidative stress

ROS and RNS normally play roles in cell signalling and homeostasis; however, in excess they become increasingly toxic. The ROS and RNS O$_2$•$^-$, ONOO$^-$, H$_2$O$_2$ and •OH, in addition to their indiscriminate oxidization of proteins, lipids and DNA (Taraseviciene-Stewart & Voelkel, 2012), can result in direct lung damage or induce an assortment of cellular responses. Additionally, these oxidants also influence the inflammatory responses in the lungs via various pathways such as the activation of transcription factor NF-κB, and transcription of pro-inflammatory mediator genes, further potentiating the inflammatory response in COPD (Gloire, Legrand-Poels, & Piette, 2006). This induces a pro-inflammatory state that drives the sustained production of more ROS, leading to a vicious cycle of oxidative stress and inflammation. The oxidative damage and modifications caused by ROS are known to precede the characteristic respiratory changes seen in COPD such as airway remodelling and emphysema (Barreiro et al., 2010). Recent studies

have also shown a decrease in glucocorticosteroid receptor expression as a result of prolonged oxidative stress indicating that in a pro-oxidant environment, such as in COPD patients, steroid therapy is of limited therapeutic benefit highlighting the need for new treatments (van Eeden & Sin, 2013).

2.3.1. Systemic oxidative stress

Even after smoke cessation, systemic oxidative stress still persists in COPD patients. This is reflected by an elevation in circulating ROS and a depletion of antioxidants. The increased oxidative stress is likely due to the persistent low grade systemic inflammation resulting in the production of ROS systemically (Foschino Barbaro, Carpagnano, Spanevello, Cagnazzo, & Barnes, 2007). This systemic oxidative stress has been implicated in various systemic manifestations of COPD, with many studies associating this increased burden of oxidative stress with skeletal muscle wasting and cardiovascular disease in COPD (Foschino Barbaro et al., 2007; Dalal, Shah, Lunacsek, & Hanania, 2011; Zampetaki, Dudek, & Mayr, 2013). In addition to the links between oxidative stress in COPD and comorbid diseases, increased extrapulmonary oxidative stress is also an independent risk factor of many comorbidities such as cardiovascular disease, osteoporosis and depressive disorders in patients without COPD (Sanchez-Rodriguez, Ruiz-Ramos, Correa-Munoz, & Mendoza-Nunez, 2007; Kawada, 2012; Michel, Pulschen, & Thome, 2012).

2.4. Oxidative stress in comorbidities of chronic obstructive pulmonary disease

A prominent feature of COPD is the presence of comorbid diseases (or comorbidities) in patients (Chatila et al., 2008; Patel & Hurst, 2011). These diseases can range from more "mild" conditions that affect quality of life such as osteoporosis and depression, to more fatal diseases such as ischaemic heart disease and cancer. COPD-related mortality is likely underestimated due to these comorbidities as their presence makes it difficult to determine the precise cause of death. Respiratory failure is considered to be the major cause of death in advanced COPD; however, comorbidities such as cardiovascular disease and lung cancer are also major causes of death. In early/mid stages of COPD, comorbidities are the leading causes of mortality (Sin et al., 2006). Comprehensive studies on the specific cause of deaths in COPD patients have found that rather than dying from progressive respiratory failure, many COPD patients die from a complex web of interconnected comorbidities (van Eeden & Sin, 2013). For the purpose of this review, comorbidities are defined as the presence of one or more distinct diseases or disorders that are not directly related to COPD or are not part of the natural history/progression of the disease (e.g., respiratory infections resulting in AECOPD). The underlying mechanisms resulting in these systemic manifestations in COPD are not fully understood; however, they have been linked to the persistent low-grade systemic inflammation and persistent oxidative stress in COPD as discussed earlier.

3. Cardiovascular disease (CVD)

3.1. Cardiovascular disease overview

CVD is a broad term used to describe any disease involving the heart and/or blood vessels. The presence of CVD is very common in COPD patients and is the leading cause of morbidity and mortality in younger patients and patients with mild-to-moderate COPD (Sin et al., 2006). Comorbid CVD can manifest itself in one or more various disorders such as angina, stroke, arrhythmias, hypertrophy of the heart, and myocardial infarction, and its presence greatly reduces the survivability of COPD patients (Dalal et al., 2011).

3.1.1. Cardiovascular disease and
chronic obstructive pulmonary disease links

It is well documented that COPD and CVD share many of the same risk factors, such as smoking, diet, and pre-existing hypertension. When considering these risk factors it is of significance that many, if not all, contribute to disease progression at least in part via oxidative stress. Multiple studies have shown links between COPD and classical cardiovascular risk factors such as family history of coronary heart disease and diabetes (Sidney et al., 2005; Bursi, Vassallo, Weston, Killian, & Roger, 2010; Terzano et al., 2010; Ford et al., 2012). Persistent low-grade systemic inflammation is present in both COPD and CVD and oxidative stress, which plays a major role in COPD, has also been implicated in CVD (Zampetaki et al., 2013). This indicates that the systemic inflammation and increased oxidative stress in COPD may lead to the onset and development of comorbid CVD.

In addition to this, acute inflammatory lung conditions, such as bacterial/viral infection or acute exposure to airborne irritants, are also associated with vascular dysfunction (Mills et al., 2005, 2007). Increased systemic inflammation, as a result of these lung conditions, is capable of destabilizing vulnerable plaques, inducing a prothrombotic state (Man, Van Eeden & Sin, 2012). COPD patients generally do not tolerate cardiac injury or intervention as well as healthy individuals. COPD patients with acute myocardial infarction have a five-year survival rate of 46% compared to 68% in those without COPD (Bursi et al., 2010).

3.1.2. Cardiovascular disease and acute
exacerbations of chronic obstructive pulmonary disease

Although many factors have been associated with poor outcomes from AECOPD, CVD is becoming increasingly recognized as a strong predictor of in-hospital mortality. Studies have shown that over 50% of patients hospitalized for AECOPD have a high prevalence of coexisting cardiovascular disease (Stefanelli et al., 2013). Comorbid cardiovascular disease is independently associated with increased risk of AECOPD (Masunaga et al., 2003). The severity of airway obstruction is a major predictor of AECOPD, and is also an independent risk factor for cardiovascular disease (Roberts et al., 2002; Mannino & Davis, 2006; Steer et al., 2010; Mannino, 2011). There is also mounting evidence associating a high frequency of acute CVD with acute respiratory illness, such as pneumonia or AECOPD. Studies have shown that in the general population, subjects with respiratory tract infections are more likely to experience a myocardial infarction within 2 weeks of infection (Meier, Jick, Derby, Vasilakis, & Jick, 1998; Corrales-Medina, Madjid, & Musher, 2010). A retrospective review examining 24 h mortality following AECOPD hospitalisation found that approximately 60% of deaths that occurred resulted from cardiovascular causes (Pastor et al., 2013).

3.1.3. Pulmonary hypertension, hypertrophy and heart failure

There are two principal pathological features in the pulmonary vasculature common to most forms of pulmonary hypertension: excessive vasoconstriction and remodelling of the pulmonary arteriolar wall, which primarily occur by a mechanism of smooth muscle proliferation within the medial layer (Demarco, Whaley-Connell, Sowers, Habibi, & Dellsperger, 2010). Because ROS may promote vasoconstriction, smooth muscle cell proliferation, and vascular remodelling, oxidative stress likely plays a critical role in many forms of pulmonary hypertension.

Multiple studies have shown that 25–70% of COPD patients have pulmonary hypertension, depending on the definition used (Rasubala, Yoshikawa, Nagata, Iijima, & Ohishi, 2003; Sekine et al., 2014). In addition to this, it is estimated that 25% of patients with moderate-to-severe COPD develop pulmonary hypertension within 6 years if they have no hypertension at baseline (Zhai, Yu, Wei, Su, & Christiani, 2014). The pathological changes implicated in the development of pulmonary hypertension can also be seen in tissue samples of COPD patients who do not have a diagnosis of pulmonary hypertension (Weitzenblum, 1984; Santos et al., 2002).

A major feature of COPD is emphysema, contributing to airway obstruction. The destruction of alveolar walls and enlargement of airspaces results in a reduction in gas exchange (van der Toorn et al., 2007). To compensate for this, blood pressure is increased to facilitate more pulmonary blood flow leading to pulmonary hypertension. This results in increased work in the right heart and over time this increased workload can lead to concentric hypertrophy of the right ventricle (RV) (Neofytou, Tzortzaki, Chatziantoniou, & Siafakas, 2012). This can lead to reduced ejection fraction, elevated end diastolic RV pressure, impedance of the right heart and, if left unchecked, can lead to heart failure. These changes can be detected in COPD patients, especially in end-stage COPD patients, using transthoracic echocardiography, which allows for the measurement of various parameters in the heart such as systolic and diastolic wall thickness, and chamber size. Both pulmonary hypertension and right heart failure are associated with an increase in morbidity and mortality in COPD patients, independent of the decline in lung function (Stone, Machan, Mazer, Casserly, & Klinger, 2011).

3.2. Oxidative stress in cardiovascular disease

The presence of oxidative stress in the form of increased ROS/RNS formation (e.g., $O_2 \bullet ^-$, $ONOO^-$) has been observed both clinically and in animal models of CVD (Schnabel & Blankenberg, 2007; Afanas'ev, 2011). Although implicated in CVD, the cause–effect relationship of oxidative stress with any of the different cardiovascular diseases has yet to be established. The increased generation of ROS due to impaired mitochondrial reduction of molecular oxygen, secretion of ROS by inflammatory cells, endothelial dysfunction, auto-oxidation of catecholamines, as well as exposure to radiation or air pollution can drive the oxidative stress in cardiac and vascular myocytes (Dhalla, Temsah, & Netticadan, 2000). In addition to the increased oxidant production, depression in the antioxidant reserve has also been implicated in CVD. These typically phenolic antioxidants (e.g., vitamin E) act as a protective mechanism in cardiac and vascular myocytes, and their reduced levels appear to be due to both the saturation by excess ROS/RNS and/or changes in gene expression. The harmful effects caused by ROS/RNS in cardiovascular tissues are mainly due to ability of the oxidant species to drive changes in subcellular organelles (e.g., mitochondria), reduce NO• bioavailability, and induce intracellular Ca^{2+}-overload (Dhalla et al., 2000).

3.2.1. Cardiovascular generation of
reactive oxygen species/reactive nitrogen species

As stated earlier, in addition to phagocytising cells, NOX-dependent ROS-generation has been observed in numerous non-phagocytising cells, though at a lower level. In the cardiovascular system these include vascular smooth muscle cells (VSMCs), endothelial cells, adventitial and cardiac fibroblasts and cardiomyocytes (Cave, Grieve, Johar, Zhang, & Shah, 2005). Normally, these cells continuously generate low levels of ROS even in the absence of external stimuli, and ROS derived from vascular NOX act as second messengers in VSMC signalling. Over long periods of time, vascular NOX complexes only produce low levels of $O_2 \bullet ^-$, with much of it generated intracellularly where it participates in cell signalling (compared to phagocyte $O_2 \bullet ^-$ which is generated extracellularly) (Fisher, 2009). When stimulated however, there is a significant increase in NOX-driven ROS production by these cells. It is important to recognize that both vascular and phagocytic NOX play an important role in superoxide production as phagocytes can infiltrate cardiovascular tissues and facilitate the functional and structural alterations observed in CVD (Matoba & Egashira, 2011).

3.2.2. Oxidative stress in the pathogenesis of cardiovascular disease

Damage to the endothelium is the initiating step in CVD. This damage can expose endothelial cells, along with the underlying cell layers, to the deleterious effects of the inflammatory process, which can ultimately lead to the formation of atherosclerotic lesions. Cellular oxidative stress caused by excess ROS/RNS production is considered to be

intrinsic to atherosclerotic lesion formation (Vogiatzi, Tousoulis, & Stefanadis, 2009). Exogenous factors contributing to oxidative stress such as smoking and comorbid diabetes also contribute to vascular oxidative stress and are strong risk factors for CVD (Fearon & Faux, 2009). Cigarette smoke has been associated with the down-regulation of key exogenous and endogenous antioxidants such as vitamin C (ascorbic acid), carotene, Gpx and SOD (Tsuchiya et al., 2002; Agnihotri et al., 2009). This can lead to dysfunction in endothelial cells, monocytes and VSMCs as well as mitochondrial damage. In addition to this, DNA damage can be caused by oxidised lipids and this may also contribute to the dysfunction of endothelial cells, VSMCs, T lymphocytes and macrophages (Madamanchi & Runge, 2007).

Oxidative stress has also been associated with the apoptosis or programmed cell death of cardio myocytes (Singal, Khaper, Palace, & Kumar, 1998). The loss of myocytes via apoptosis has been observed in the infarct regions of myocardium from patients that have suffered from heart failure or a myocardial infarction (MI) (Krijnen et al., 2002). Both in vitro studies and in vivo animal model studies found that apoptosis occurs in response to cardiovascular complications, such as MI, and chronic pressure overload (Fiorillo et al., 2005). The common factor in all of these conditions is the generation of oxidative stress and oxidative stress is known to play a role in the initiation of apoptosis (Nagata et al., 2003). Additionally, the apoptosis of myocytes is inhibited by antioxidants such as vitamin E and SOD, implicating ROS/ RNS in the pathological pathways of CVD (Kumar, Lou, & Singal, 2002). Although studies have implicated a role for ROS/RNS in CVD related apoptosis, the exact contribution of oxidative stress in the loss of myocardial function and heart failure remains to be established.

3.2.3. Nitric oxide in cardiovascular disease

The role of nitric oxide NO• in vascular homeostasis and signalling has been well characterized. As stated earlier, NO• plays a pivotal role in the maintenance of vascular tone and vasoreactivity. In contrast to this distinct role in cell physiology, NO• can also contribute to CVD pathology. Under certain conditions eNOS (the endothelial isoform of the nitric oxide synthase) becomes uncoupled from a NO• to an O_2-producing state (Montezano & Touyz, 2012). The excess O_2 produced can react with NO• resulting in the formation of excessive $ONOO^-$. This reaction reduces the bioavailability of NO•, leading to cardiovascular dysfunction and reduced endothelial vasoregulatory capacity (Kolluru, Bir, & Kevil, 2012). $ONOO^-$ has also been shown to promote the uncoupling of eNOS, propagating the increased $ONOO^-$ formation and decreased NO• bioavailability (Cassuto et al., 2014).

3.3. Current treatments and antioxidant therapies

The current treatments for comorbid CVD are the same treatments used for CVD independent of COPD, such as β receptor blockers, ACE inhibitors, or angiotensin receptor blockers. However, the benefit of these drugs in COPD patients with CVD is conflicting. Some studies have found that these treatments may be detrimental for COPD patients, worsening the pulmonary symptoms of the disease (Nojiri et al., 2014). However, others have shown that when given to COPD patients with and without comorbid COPD, these treatments have shown notable benefits with regards to airway symptoms (Huang et al., 2013). This may be due to the fact that both ACE inhibitors and angiotensin receptor blockers have displayed pleiotropic antioxidant effects in addition to their antihypertensive effects (Munger, 2011). Thus, this presents a unique opportunity for dual action therapy using CVD-specific treatments and antioxidants, and obviously warrants further research.

CVD and COPD also overlap with regards to their non-pharmacological management. Cardiovascular modifying factors such as physical activity and diet modification are reported to decrease hospital readmission for COPD (McLachlan, Hambly, Almsherqi, El Oakley, & McGuire, 2006). The recognition of the importance of oxidative stress in CVD has led to the fervent use of antioxidants in the treatment and prevention of the disease, however, the results of prospective, randomized clinical trials have been generally disappointing (Myung et al., 2013; Ye, Li, & Yuan, 2013). In contradiction, studies have shown that antioxidant therapy is beneficial in non-comorbid hypertension, atherosclerosis, ischaemic heart disease, cardiomyopathies and congestive heart failure (Dhalla et al., 2000). It should be noted that almost all antioxidant clinical studies only explored the effectiveness of traditional scavenging antioxidants such as vitamin E.

With regards to antioxidant treatments, the protective role of exercise in preventing oxidative stress is noteworthy. Acutely, it is known that exercise causes oxidative stress (Fisher-Wellman & Bloomer, 2009). However, exercise also leads to longer-term activation and enhanced synthesis of antioxidants and antioxidant enzymes (e.g., SOD, Gpx-1), as well as decreasing oxidant production (Gonzalez, Marquina, Rondon, Rodriguez-Malaver, & Reyes, 2008). This may explain the links between prolonged exercise and the beneficial effects to both COPD and CVD patients, and highlights the potential benefits of targeting oxidative stress in comorbid CVD.

Overall, it appears that targeting oxidative stress with antioxidant enzyme modifying treatments may have great potential in COPD and comorbid CVD by providing beneficial effects with regards to both the pulmonary and cardiovascular aspects.

4. Skeletal muscle wasting

4.1. Skeletal muscle wasting overview

Skeletal muscle wasting, also referred to as cachexia or skeletal muscle atrophy, occurs in approximately 20 to 40% of all COPD patients (Schols et al., 1998; Congleton, 1999; Maltais et al., 2014). Muscle wasting is characterized by a marked decrease in skeletal muscle mass, an increase in proportion of type I muscle fibres in the diaphragm and type 2 muscle fibres in the periphery, and associated with low exercise capacity and skeletal muscle weakness. Skeletal muscle wasting in COPD is a strong predictor of mortality, independent of decline in lung function (Schols et al., 1998; Marquis et al., 2002; Swallow et al., 2007). Although present in a large population of patients, the prevalence of muscle wasting can only be approximated as there are no simple techniques to measure muscle mass. Knowing this, the actual prevalence and extent of muscle wasting in the COPD population are likely underestimated. This is because all the data is extrapolated from body weight measurements and lean body mass, an index of muscle mass, may be reduced despite the preservation of total body weight. This is further supported by the fact that patients have a proportionally greater reduction in thigh muscle cross-sectional area compared to reduction in body weight (Marquis et al., 2002). This indicates that a preferential loss of muscle tissue exists in emaciated patients with COPD (Kim et al., 2008).

Skeletal muscle wasting, although not a directly fatal condition, reduces health-related quality of life and decreases survivability for COPD patients. The main feature of comorbid skeletal muscle wasting is a reduction in fat free mass (FFM) and is associated with weaker peripheral muscles, impaired functional status, as well as poor health-related quality of life (Debigare, Cote, & Maltais, 2001; Mathur, Brooks, & Carvalho, 2014). In COPD, the reduction in muscle mass is proportional to that of the reduction in strength indicating that the remaining contractile apparatus may be functionally preserved. However, in patients chronically treated with glucocorticosteroids, it is possible for the loss of strength to be disproportional to the reduction in muscle mass.

Deteriorations in FFM have also been described following acute exacerbations. These COPD patients also experience a reduction in exercise capabilities, with skeletal muscle alterations contributing to limitations in exercise in addition to pulmonary dysfunction (Wust & Degens, 2007). It should be noted that the strength of the quadriceps is a key determinant of exercise tolerance in COPD. This is explained by the influence that muscle strength has on the perceived leg effort

required during exercise, which is considered to be the main limiting symptom in 40–45% of patients with COPD (Debigare & Maltais, 2008).

4.1.1. Mechanisms of skeletal muscle dysfunction

The pathophysiological interaction between COPD and alterations in skeletal muscle tissue is poorly understood and represents an important gap in knowledge of the disease. Skeletal muscle wasting in COPD is multifactorial in nature with several of these factors likely interacting. Many factors (e.g., inflammation, oxidative stress, and poor nutrition) can initiate or enhance alterations in skeletal muscle, such as change in fibre type phenotypic expression and regenerative defects in peripheral muscles of patients with COPD (Maltais et al., 2014). In this review, multiple risk factors will be briefly discussed along with a more in-depth discussion regarding the role of oxidative stress and ROS.

4.1.1.1. Smoking.
Cigarette smoking or exposure to other airborne irritants is unlikely to be the primary mechanism involved in skeletal muscle wasting in COPD, as seen in several studies where patients and control subjects were matched for smoking history (Maltais et al., 2014). Nevertheless, smoking does have some effect on muscle biology and it may predispose patients to the development of skeletal muscle dysfunction (De Paepe et al., 2008; Rinaldi et al., 2012). Smoking is also associated with skeletal muscle weakness in otherwise healthy individuals (Seymour et al., 2010; Barreiro et al., 2011; van den Borst et al., 2011).

4.1.1.2. Disuse.
Peripheral (or limb) muscle dysfunction in COPD has been partly attributed to a reduction in physical activity, or "deconditioning." In general, the disuse of muscle can lead to many of the features and alterations of skeletal muscle in COPD patients: muscle weakness, muscle atrophy, loss of type I fibres, decreased cross-sectional area of muscle fibres, reduced oxidative enzyme activity, reduced capillary-to-fibre ratio, early lactate release, reduced rate of phosphocreatine synthesis after exercise and altered redox status (Booth & Gollnick, 1983; Coyle, Martin, Bloomfield, Lowry, & Holloszy, 1985; Larsson & Ansved, 1985; Polkey & Moxham, 2006). In healthy adults

these changes are fully reversible in response to training and increased exercise; however, full recovery is unusual in COPD patients (Troosters, Gosselink, & Decramer, 2000; Polkey & Moxham, 2006, 2011; Man, Kemp, Moxham & Polkey, 2009).

4.1.1.3. Inflammation.
A low body mass index (BMI) has been linked to systemic inflammation in COPD patients. As seen in Fig. 5, inflammation plays a key role in the activation of protein breakdown in skeletal muscle. Inflammation results in the production of key cytokines, such as IL-8, that can generate an array of cellular responses. This includes the induction of the ubiquitin proteasome (UbP) system through the transcriptional activities of NF-κB and FOXOs, apoptosis, and macroautophagy, all of which have been linked to the muscle atrophy (Kandarian & Jackman, 2006). In COPD patients, there is a lack of evidence with regards to inflammation in skeletal muscle during stable disease (Gosker et al., 2003; Montes de Oca et al., 2005); however, increased inflammation is seen during periods of exacerbation (Spruit et al., 2003; Yende et al., 2006). Because of this, the role of inflammation as the key event for the development of skeletal muscle dysfunction in COPD is still widely debated.

4.1.1.4. Hypoxia.
In humans (Hoppeler et al., 1990) and animals (Magalhaes et al., 2005) muscle mass decreases under hypoxic conditions. COPD patients that have low arterial O_2 and reduced O_2 delivery tend to have lower body mass than those with normal levels of arterial O_2 and sufficient O_2 delivery (Semenza, 2009). Hypoxia may be a factor driving changes in limb muscle tissue as hypoxia can induce downstream effects leading to the activation of the UbP system and reduced myogenesis (Caron, Theriault, Pare, Maltais, & Debigare, 2009).

4.2. Oxidative stress in skeletal muscle wasting

In addition to chronic inflammation, hypoxia, cigarette smoke, sepsis and an increased cost of breathing cause the increased generation of oxidants in the lungs (e.g., H_2O_2, $O_2^{\bullet-}$, MDA) (Rahman, 2005). It is suggested that, in addition to inflammatory mediators, these oxidants can
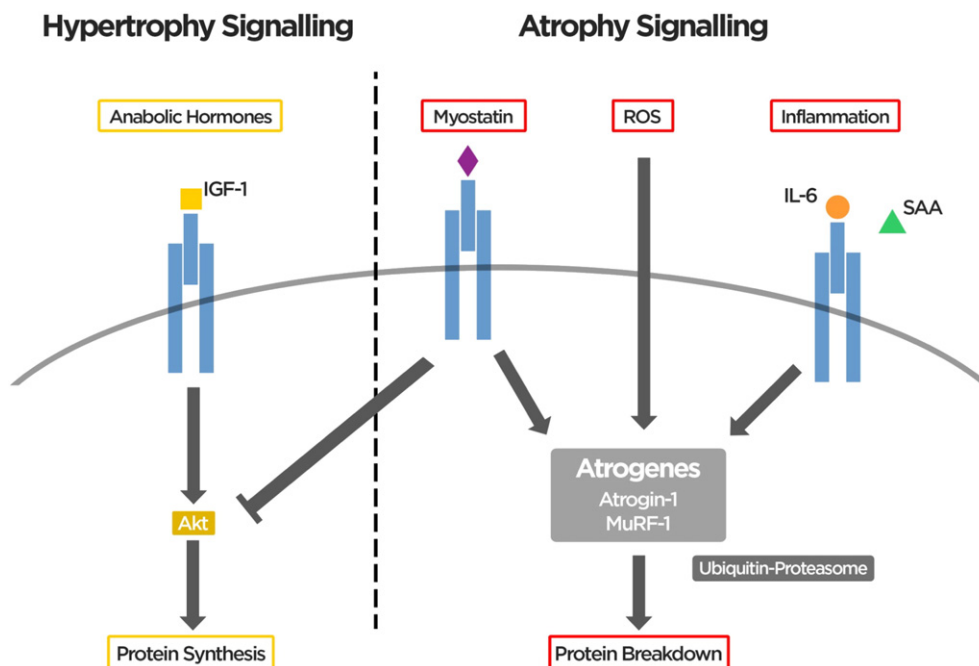
Fig. 5. Regulation of muscle mass relies on various hypertrophy and atrophy signalling pathways. In skeletal muscle wasting, there is an imbalance between these anabolic and catabolic processes resulting in enhanced muscle degradation as a result of protein breakdown. Myostatin (a negative regulator of muscle mass) can inhibit muscle growth. Inflammatory mediators and ROS can lead to the downstream activation of atrophagy-related genes (e.g., Atrogin-1, MuRF-1) resulting in enhanced protein degradation. Inflammation and oxidative stress have also been implicated in the activation of the ubiquitin–proteasome (UbP) system. The UbP system is the primary mechanism of the protein catabolism in mammalian skeletal muscle. Refer to Maltais et al. (2014) for an exhaustive overview of the signalling pathways involved.

spill into circulation, increasing the systemic oxidative stress burden of COPD patients (Zeng et al., 2013). In in vivo models of skeletal muscle wasting in COPD, increased oxidative stress has been observed (Rinaldi et al., 2012). This increase in oxidative stress can modify muscle proteins, reducing their integrity and enhancing their degradation (Aiken, Kaake, Wang, & Huang, 2011). Direct exposure to oxidative stress from environmentally-derived oxidants, or indirect exposure via cellular-derived ROS due to inflammation, can induce proteolysis. Acute bouts of physical exercise and acute exacerbations can also increase the level of oxidative stress (Couillard et al., 2003; Karadag, Karul, Cildag, Yilmaz, & Ozcan, 2008; Stanojkovic et al., 2011). It is suggested that oxidative stress may acutely affect skeletal muscle function by inhibiting the activity of the sodium/potassium pump, sarcoplasmic reticulum function, myosin ATPase and mitochondrial respiration (Wust & Degens, 2007). In addition to these acute effects, chronic oxidative stress also contributes to muscle wasting and dysfunction in both respiratory and peripheral muscles. The increased presence of ROS associated with COPD causes an increase in the expression of UbP components leading to increased protein breakdown in skeletal muscle (Fig. 6) (Maltais et al., 2014). There is evidence that COPD patients with muscle wasting experience a more severe abnormal oxidative stress response to submaximal and maximal exercises compared to non-muscle-wasted patients with COPD (Van Helvoort et al., 2006). Although most studies focus on the increased signalling of atrophy pathways as the major driving force of comorbid skeletal muscle wasting, it should be noted that decreases in hypertrophy signalling may also contribute greatly to the pathology of the disease. Further studies are required to determine the exact contribution of these various pathways to the disease.

### 4.3. Current treatments and antioxidant therapies

There are currently no effective drug treatments for skeletal muscle wasting in COPD. Pharmacological treatments that have been explored include anabolic steroids, growth hormone, other growth anabolic compounds and bioactive nutrients (e.g., ghrelin, creatine) and antioxidants. Most of these treatments have only shown little to modest benefits when treating skeletal muscle wasting and weakness in COPD. As with CVD, skeletal muscle wasting and COPD also overlap with regards to their non-pharmacological management. Currently, the most potent treatment for limb muscle dysfunction is exercise training, which is already a key component for the management of COPD (Maltais et al., 2014). Some treatments such as lifestyle modifications (diet and exercise) and anabolic steroids have shown improvement in patients; however, they do not reverse the progression of muscle loss and may only increase muscle growth without substantial improvements in strength or endurance (Casaburi et al., 2004; Hansen, Gualano, Bozinovski, Vlahos, & Anderson, 2006). Therefore, new treatments are needed as limiting muscle wasting in COPD will lead to improved quality of life and increased survivability of patients. Because of its role in the pathogenesis of comorbid skeletal muscle wasting, targeting oxidative stress may be able to slow the progression or onset of the disease, giving patients an improved quality of life whilst managing their other symptoms. Furthermore, it has been reported that increasing antioxidant potential can improve muscle performance whilst attenuating muscle fatigue (Maltais et al., 2014). Further pre-clinical and clinical studies are required to determine the effectiveness of targeting oxidative stress in comorbid skeletal muscle wasting.

5. Lung cancer

5.1. Lung cancer overview

Lung cancer is one of the more fatal comorbidities of COPD. It is currently the most frequently diagnosed cancer worldwide and is the number one cause of cancer-related deaths in males (Jemal et al., 2011). Lung cancer accounts for up to 13% of all cancer diagnoses worldwide, accounting for more than one million deaths per year (Alberg &
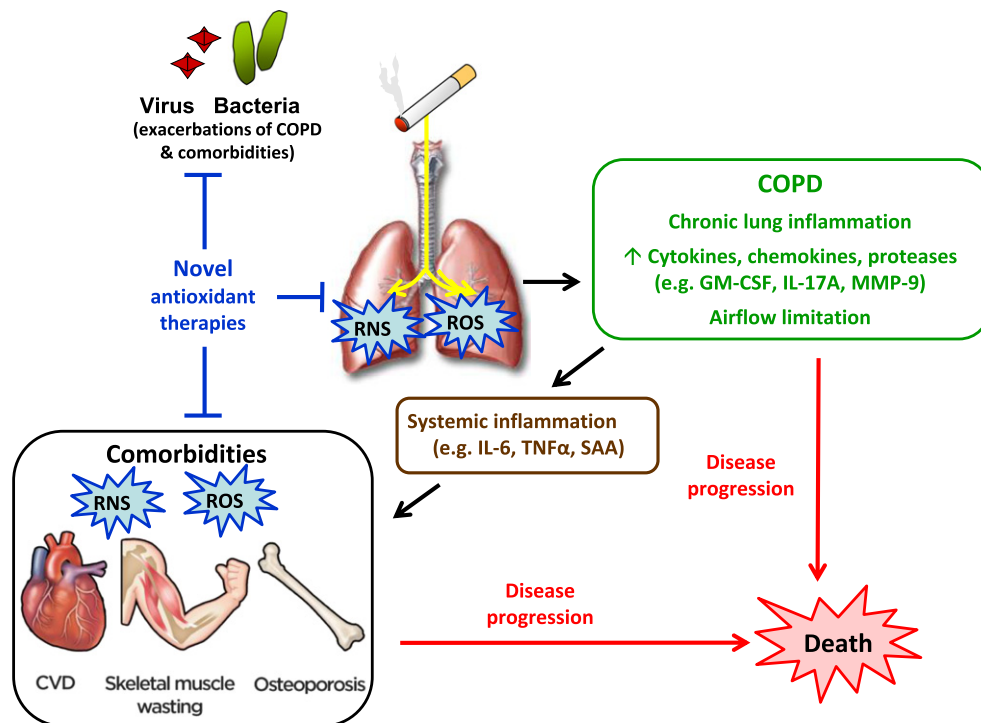
Fig. 6. Points of therapeutic intervention in COPD and its comorbidities. Oxidative stress indiscriminately damages a number of tissues and, as such, has been implicated in the onset and development of multiple diseases regardless of COPD diagnosis. Using novel antioxidant therapies it may be possible to treat COPD, AECOPD and comorbidities of COPD. Inhibiting the generation of ROS and RNS with pharmacological compounds could reduce lung and systemic inflammation, reduce the severity and progression of COPD and comorbidities, improve the quality of life of patients, and increase their survival.

Nonemaker, 2008; WHO, 2013). Patients with COPD have a higher risk of developing lung cancer and due to the presence of comorbid lung cancer, the survival rate of these COPD patients is drastically low (Raviv, Hawkins, DeCamp, & Kalhan, 2011). It is estimated that up to 70% of lung cancer patients have co-existing COPD, and one study found that the most common cause of death among patients with airflow obstruction was lung cancer (Lung Health Study Research, 2000). It is also known that, whilst lung cancer survival in the general population is very low, COPD patients with comorbid lung cancer have an even lower rate of survival. In one study, lung cancer patients without COPD had a 26% 3 year survival after diagnosis versus 15% in lung cancer patients with COPD (Kiri, Soriano, Visick, & Fabbri, 2010).

5.1.1. Lung cancer and chronic obstructive pulmonary disease links

It is well established that exposure to airborne irritants, especially those in cigarette smoke, induces both diseases. Cigarette smoke, among other airborne irritants, is an independent risk factor of both COPD and lung cancer with approximately 80% and 90% of cases associated with cigarette smoking respectively (Fathy, Hamed, Youssif, Fawzy, & Ashour, 2014). Additionally, almost 1% of COPD patients develop lung cancer yearly, which may be associated with genetic susceptibility to particulates found in cigarette smoke (Sekine, Katsura, Koh, Hiroshima, & Fujisawa, 2012). COPD is also considered to be a risk factor for lung cancer independent of smoking status. It has also been suggested that even a small reduction in airflow significantly predicted lung cancer (Wilson et al., 2008). The Multiple Risk Factor Intervention Trial reported that a 10% reduction in lung function was associated with an almost 3-times greater risk of lung cancer compared to patients without airflow limitations, after adjusting for smoking (Shaten et al., 1997). It also indicated that the lag time between smoking cessation and the beneficial effects on lung cancer development may be as long as 20 years. A separate study found that the presence of COPD increases the risk of lung cancer by up to 4.5-fold (Wang, 2013). This indicates that COPD patients have a greater risk of developing lung cancer compared to those with normal pulmonary function regardless of smoker status. On the other hand, a study looking at the prevalence of COPD in lung cancer patients, independent of age, sex, or smoking history, determined that the prevalence of pre- or co-existing COPD in recently diagnosed lung cancer cases was six-times greater than in smokers without lung cancer (Young et al., 2009). The study found that approximately 50% of newly diagnosed lung cancer patients had COPD, whereas this number was only 8% in otherwise healthy smokers. This suggests that impaired lung function may be more important than age or smoking history as a predictor of lung cancer.

Whilst the association between lung cancer and COPD has always been of great interest, the details of their various molecular pathways involved and their clinical correlates have only begun to be established. Chronic inflammation is known to induce both COPD and lung cancer by means of the release of inflammatory mediators and the excess production of ROS/RNS (Lawless, O'Byrne, & Gray, 2009; Lee, Walser & Dubinett, 2009; Bozinovski et al., 2015). Inflammatory mediators may promote the growth of bronchoalveolar stem cells, activation of NF-κB and signal transducer and activation of transcription 3 (STAT3), which all play crucial roles in the development of lung cancer (Sekine et al., 2012). Chronic inflammation leads to increased ROS production, and increased oxidative stress has also been associated with the development of lung cancer (Barreiro et al., 2013).

5.2. Oxidative stress in lung cancer

The primary association between oxidative stress and the development of lung cancer is the oxidative modifications to DNA as a result of ROS/RNS (Cooke et al., 2003; Paz-Elizur et al., 2003). Oxidative damage to DNA by ROS and RNS has been implicated in carcinogenesis (Cooke et al., 2003). As stated earlier it is known that oxidant-induced DNA damage also involves single- or double-stranded DNA breaks and DNA cross-

links, DNA damage inducing either arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability, all of which have been linked to carcinogenesis (Dawane & Pandit, 2012). It should be noted that studies have found that excessive amounts of NO• increase apoptosis in some tumour cells, whereas reduced amounts of NO• can increase the vascularity of the tumour and protect the cells from apoptosis, particularly in lung cancer (Masri, 2010).

Due to the direct damage to DNA and the increased demand for DNA repair as a result of oxidative stress, more DNA mutations may occur. One of the most important oxidative modifications to DNA is the adduct of •OH on DNA nucleobases. The 8-hydroxy-2′-deoxyguanosine (8-OHdG) and/or its tautomeric 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) have been widely studied and proven important mutagenic adducts to DNA and are therefore key biomarkers of oxidative stress induced carcinogenesis (Valavanidis et al., 2009a,2009b). Mutations of 8-oxodG involve a GC→AT transversion which can modify protein transcription as stated earlier in the review. In addition to this, ROS and RNS may initiate carcinogenesis by attacking DNA nucleobases causing changes in oncogenes and tumour suppressor genes (Valavanidis, Vlachogianni, Fiotakis, & Loridas, 2013). Studies found that •OH can generate various DNA phenotypes with various metastatic potentials (Malins, Polissar, & Gunselman, 1996; Valavanidis et al., 2013). It is suggested that these different phenotypes likely contribute to the diverse physiological properties and heterogeneity characteristic of metastatic cell population (Malins et al., 1996).

In addition to direct damage to DNA, ROS and RNS can indirectly damage DNA via oxidative modifications to lipids. This occurs when ROS and/or RNS react with cellular membrane phospholipids generating lipoperoxide radicals (LOO•) and toxic aldehydes (malondialdehyde, MDA). This results in membrane permeability and microcirculation alteration and also causes the activation of nuclear factors leading to other pro-inflammatory agents (Valavanidis et al., 2013). MDA is a naturally occurring by-product of lipid peroxidation and is a known mutagenic and carcinogenic compound. MDA reacts with DNA to form multiple adducts with mutagenic potential (Marnett, 2000). Additionally, MDA itself has also been shown to be carcinogenic (Marnett & Tuttle, 1980).

As with patients with COPD and comorbid lung cancer, patients without co-existing COPD also experience an increase in systemic oxidative stress and inflammation (Barreiro et al., 2013). These processes can cause the activation of NF-κB, which has been associated with the development of comorbid lung cancer (Chen, Li, Bai, & Lin, 2011). NF-κB activation and the subsequent actions of inflammatory-related genes may play a central role in both COPD and lung cancer. Activation of NF-κB increases the release of inflammatory mediators that can induce COPD, whilst also inhibiting apoptosis, inducing proliferation and, accelerating cancer development (Karin, 2009). The release of these inflammatory mediators can activate STAT3 which is a transcription factor with various physiological functions. Continuous activation of the STAT3 signalling pathway can potentiate pulmonary inflammation and induce adenocarcinoma formation in the lung (Takata et al., 2012). Clinically, activation of STAT3 and its downstream genes serve as biomarkers of COPD and lung cancer diagnosis and prognosis (Qu et al., 2009).

As stated previously, oxidative stress can stimulate the recruitment of macrophages and neutrophils which release various cytokines and chemokines. Released matrix metalloproteinases and ROS promote inflammation, induce apoptosis, matrix degradation, and ineffective tissue repair, resulting in enlarged airspaces (emphysema) (Belvisi & Bottomley, 2003). Bronchoalveolar stem cells may attempt to repair and replace damaged alveolar cells however, in an inflammatory environment, they can enhance carcinogenesis (Sekine et al., 2012).

5.3. Current treatments and antioxidant therapies

Treatment for lung cancer, regardless of co-existing COPD, depends on the type and severity of the cancer with the main treatments being surgery, chemotherapy and radiotherapy in varying combinations

(Byers & Rudin, 2015). It is thought that chemotherapy may have suppressive effects against oxidative stress in patients that respond well to the treatment, compared to non-responders (Gupta et al., 2010). This phenomenon may be at least partially responsible for the improved survival among responders.

As with COPD, the most effective lung cancer prevention measure is smoking cessation. Because of the long lag time between smoke cessation and beneficial effects with regards to lung cancer development, new treatments for comorbid lung cancer should be primarily focused on prevention and early detection. Early detection, prevention and intervention are critical for both COPD and lung cancer as both diseases have high mortality rates and incur severe economic burdens worldwide. Due to the strong associations and interactions between lung cancer and COPD, novel therapies for the prevention of developing comorbid lung cancer should focus on targeting pathways implicated in both diseases. Inflammation and oxidative stress go hand-in-hand in the onset and development of both of these diseases and are therefore key candidates for preventative therapy of comorbid COPD. More research is required to determine the exact contribution of oxidative stress to the pathology of lung cancer and the effectiveness of antioxidant compounds in the disease.

6. Osteoporosis

6.1. Osteoporosis overview

Osteoporosis is a progressive, metabolic bone disease that is characterized by a decrease in bone mass and density which leads to a deterioration in bone strength and an increased risk of fracture (Kanis et al., 2013). Patients with osteoporosis experience reduced bone mineral density (BMD) and deterioration of bone microarchitecture (Kanis, 2002). Additionally, patients experience alterations to the amount and variety of proteins in bone as a result of the disease (Donoso, Pino, Seitz, Osses, & Rodriguez, 2015). Osteoporosis presents no actual symptoms; however the major consequence of osteoporosis is the increased risk of bone fractures as a result of the bone alterations. The key difference between normal bone fractures and osteoporotic fractures is that osteoporotic fractures occur in scenarios where healthy individuals would not normally break a bone. Therefore, these fractures are often regarded as fragility fractures. Generally, these fragility fractures occur in the vertebral column, ribs, hips and wrists (Adachi et al., 2003; Leslie et al., 2014).

As bone is a dynamic tissue, it continuously renews itself throughout life and this is accomplished via bone remodelling. This process is carried out by a functional and anatomic structure known as the basic multicellular unit. This structure requires the coordinated action of three major types of bone cells: osteoclasts, osteoblasts and osteocytes (Feng & McDonald, 2011). The interactions between these cells and multiple molecular mediators, including hormones, growth factors, and cytokines, drive the remodelling process (Mundy, 1993). Bone remodelling follows a time sequence that lasts approximately six months in humans and during this process, osteoclasts eliminate old and/or damaged bone which is then replaced by new bone formed by osteoblasts. Osteocytes are relatively inert cells, however, they are primarily responsible for the molecular synthesis and modification, and transduction of signals (akin to the nervous system) necessary to sustain mechanical loads (Compton & Lee, 2014). In order to prevent significant alterations to bone mass and/or mechanical strength following after each remodelling cycle, the process of bone formation and bone resorption is tightly regulated and maintained in healthy bone (Feng & McDonald, 2011). Any disturbance in the remodelling process can lead to the development of metabolic bone diseases. Oxidative stress is known to affect the remodelling process by altering the functions of both osteoclasts and osteoblasts and, as such, is associated with the pathogenesis of osteoporosis. Due to the overlap in risk factors of COPD and osteoporosis, patients with COPD have an increased incidence of osteoporosis (Jorgensen et al., 2007).

6.1.1. Osteoporosis and chronic obstructive pulmonary disease links

In COPD, patient health and treatment is primarily focused on the level of lung function and ability of oxygenation. Because of this, osteoporosis often goes undiagnosed in COPD even though COPD patients are at a higher risk of developing the disease. Osteoporosis, however, may be as equally debilitating as COPD, and can further contribute to the impaired respiratory function if the patient experiences vertebral compressions and loss of height as a result of bone degradation (Silverman, 1992; Yang et al., 2007; Masala et al., 2014). It is not clear why COPD patients often develop osteoporosis, but many of the risk factors for osteoporosis overlap with or are also associated with COPD. These risk factors include smoking, chronic inactivity, reduced weight, medication taken, poor nutrition, low antioxidant status and oxidative stress (Cielen, Maes, & Gayan-Ramirez, 2014). At a molecular level, the pathophysiology underlying the development of comorbid osteoporosis is largely unknown; however, the risk factors and causes of the disease have been well established.

Chronic treatment with glucocorticosteroids (GCS) is one of the more obvious causes of osteoporosis in COPD patients (Canalis, 2005). Although the use of GCS in the treatment of COPD is controversial due to their limited effectiveness and major side effects, they are still prescribed to patients with severe COPD and patients particularly prone to exacerbations. Most studies conclude that a beneficial effect from GCS is only observed when administered to the most severely affected patients with tendency to exacerbate (Falk, Minai, & Mosenifar, 2008; Leuppi et al., 2013; Price, Yawn, Brusselle, & Rossi, 2013). The treatment with GCS for airway impairment is associated with an increased occurrence of fractures (De Vries et al., 2007). The incidence of fractures, as a result of osteoporosis, contributes to the overall disability and mortality of patients with COPD, whilst adding to the economic burden of the disease. The increased incidence of osteoporosis is also seen in patients that are treated with GCs chronically due to other chronic diseases (McDonough, Curtis, & Saag, 2008). Although GCS use is a risk factor for the development of comorbid osteoporosis, it does not entirely account for the low bone mass and high prevalence of osteoporosis in COPD patients. In addition to chronic GCS use, inflammation has also been determined to be a major risk factor of comorbid osteoporosis.

Systemic inflammation as a consequence of COPD has been linked to the development of a myriad of comorbidities, including osteoporosis. One study showed that the increased arterial stiffness due to the severity of airway obstruction experienced by COPD patients is related to osteoporosis and various markers of systemic inflammation (Sabit et al., 2007). One such inflammatory marker is interleukin (IL)-6; a cytokine which may also contribute to the increased risk of other comorbidities. In addition to its pro-inflammatory effects, IL-6 is involved in regulation of bone turnover and has been implicated in osteoporosis development (Ishimi et al., 1990). Another study suggested that COPD is associated with increased insulin resistance (another metabolic disorder) and that this is associated with elevation of the proinflammatory marker tumour necrosis factor (TNF)-α receptor expression (Hotamisligil, 1999; Nieto-Vazquez et al., 2008). TNF-α is a documented stimulator of osteoclastic bone resorption and has been implicated in postmenopausal osteoporosis (Boyce et al., 2005). In one study examining patients awaiting pulmonary transplantation, of which 56% were COPD patients, there was a positive correlation between TNF-α receptor expression and bone turnover (Forli et al., 2008). Increased levels of the bone resorption marker 1-CTP were proportional to increased levels of TNF-α receptor II. This suggests a strong correlation between COPD induced inflammation and increased bone loss.

As stated earlier, inflammation in COPD is also implicated in skeletal muscle wasting leading to significant reductions in FFM. The increased enzyme catalytic activity in muscle wasting is also associated with bone loss. Significant changes in enzyme function, such as increased activity of alkaline phosphatase (ALP), have been identified in sera collected from osteoporosis patients (Leung, Fung, Sher, Li, & Lee, 1993). These changes have also been associated with increased bone metabolism,

and altered bone rearrangement in COPD (Jorgensen & Schwarz, 2008). MMP activity (primarily MMP-9 and MMP-12), which is implicated in parenchyma destruction, has also been linked to the development of osteoporosis in COPD patients (Bolton et al., 2009).

6.2. Oxidative stress in osteoporosis

In addition to inflammation, there have been a multitude of studies implicating oxidative stress with an increased rate of bone loss, thus making oxidative stress a risk factor for osteoporosis (Varanasi, Francis, Berger, Papiha, & Datta, 1999; Chavan, More, Mulgund, Saxena, & Sontakke, 2007; Stanojkovic et al., 2013; Cervellati et al., 2014). It is suggested that ageing and the consequential increase in ROS are responsible for bone loss (Almeida & O'Brien, 2013). Increased oxidative stress is associated with alterations in the activity and function of both osteoblast and osteoclast cells; the two major bone cells involved in the pathogenesis of osteoporosis.

6.2.1. Osteoblasts

The role of oxidative stress in osteoblasts has only been researched extensively in the past decade. As stated earlier, osteoblasts are responsible for replacing old or damaged bone with new bone. Studies found that osteoblasts can be induced to produce intracellular ROS ($O_2^{\bullet-}$ and H_2O_2) and the production of which can cause a decrease in ALP which can cause cell death, and this effect is partially inhibited by scavenging antioxidants (Darden, Ries, Wolf, Rodriguiz, & Key, 1996; Onyia et al., 1999). In high concentrations, ROS can damage osteoblast cells, preventing the normal growth and development of bone, and has been shown to induce osteoblast cell death (Lee, Lim, Lee & Yang, 2006). Additionally, H_2O_2 can modulate the activity of intracellular calcium in osteoblasts by increasing the amount of Ca^{2+} released from intracellular Ca^{2+} stores (Nam, Jung, Yoo, Ahn, & Suh, 2002).

6.2.2. Osteoclasts

Whilst still not well understood, the different mechanisms and pathways involved in the differentiation of osteoclasts and their ability to resorb bone are beginning to be elucidated. It is well known that ROS is involved in this process, but the extent of its contribution is still to be determined. Superoxide has been detected intracellularly in osteoclasts and at the osteoclast–bone interface, suggesting a contribution from superoxide in bone resorption (Key, Wolf, Gundberg, & Ries, 1994). It is known that osteoclastic superoxide is produced by NOX (Darden et al., 1996). H_2O_2 produced by endothelial cells associated with osteoclasts and the H_2O_2 produced by osteoclasts have been shown to increase osteoclastic activity and bone resorption, which may contribute to the increased breakdown of bone in osteoporosis (Bax et al., 1992). H_2O_2 has also been implicated in various other osteoclast functions such as osteoclast motility, differentiation of osteoclast precursors and the regulation of osteoclast formation (Steinbeck, Kim, Trudeau, Hauschka, & Karnovsky, 1998). The reaction of H_2O_2 with tartrate-resistant acid phosphatase found on the surface of osteoclasts is responsible for the degradation of collagen and other proteins (Bull, Murray, Thomas, Fraser, & Nelson, 2002). Additionally, antioxidants play a role in osteoclast activity. Osteoclasts innately possess the antioxidant enzyme SOD in the plasma membrane (Steinbeck, Appel, Verhoeven, & Karnovsky, 1994). Studies found that after treating osteoclast cells with antioxidant enzymes such as SOD and catalase, ROS production is inhibited indicating that antioxidant therapy may be beneficial for patients with osteoporosis (Lee, Kim & Jang, 2014).

6.3. Current treatments and antioxidant therapies

In addition to diet supplementation with calcium and vitamin D, there is also a wide range of pharmaceuticals available for the treatment of osteoporosis. The current anti-resorptive treatments prescribed to osteoporosis patients include a number of bisphosphonates which inhibit

bone resorption (MacLean et al., 2008). These vary by way of administration and are taken orally either daily, weekly, monthly or intermittently. Many other drugs are available such as, but not limited to, calcitonin and strontium renalate, both of which increase bone formation by stimulating osteoblasts, whilst reducing bone resorption via inhibiting the activity of osteoclasts. Emerging research has shown beneficial effects of anabolic treatments in osteoporosis (Neer et al., 2001). Currently, the only available pure anabolic drugs are parathyroid hormone mimetics. The use of fully human monoclonal antibodies has also been approved for osteoporosis and a number of drugs are being tested clinically for osteoporotic treatment and prevention (Lewiecki, 2011; Padhi, Jang, Stouch, Fang, & Posvar, 2011). However, unsurprisingly, there is a lack of interventional studies regarding the treatment of osteoporosis in patients with COPD.

Antioxidant treatment for osteoporosis has proven to be quite effective. The beneficial effects of antioxidants with regards to bone health and osteoporosis have also been demonstrated epidemiologically and through clinical intervention (Rao, 2013). Bearing in mind the possible adverse effects of hormonal therapy and the increasing reports regarding the side effects of bisphosphonates in the management of osteoporosis, there is a high demand for complementary and/or alternative medicine for the prevention and treatment of osteoporosis. This is especially true for COPD patients who are inherently at a greater risk of developing the disease. Due to the damaging effects of ROS on bone growth and regulation, targeting oxidative stress in COPD patients may prove to be beneficial for both COPD and osteoporosis.

7. Novel antioxidant approaches for the treatment of chronic obstructive pulmonary disease

Besides never smoking, smoke cessation is the only effective method for preventing the onset and progression of COPD. The progression of airway inflammation, increased oxidative stress, and protease burden even months/years after cessation, and non-responsiveness to glucocorticosteroids have been documented as therapeutic challenges for the treatment of COPD (Kalyanaraman, 2013). Because of this, there is a lack of effective treatments for COPD and its comorbidities.

As stated throughout this review, tissue injury and inflammation as a result of oxidative stress are common to COPD and many of its comorbidities. A common theme throughout this review is the similarity and interplay between the pathological mechanisms of each condition and it is this similarity that provides a unique therapeutic opportunity (Fig. 6). Treatment with antioxidants may be able to progressively prevent and treat multiple diseases by suppressing the generation of ROS/RNS, neutralizing oxidants or both. Due to the overwhelming evidence implicating oxidative stress in the pathogenesis of COPD and its comorbidities, it is only logical to consider antioxidant intervention in this patient population. It is important that such interventions not only neutralize the excessive ROS and RNS generated, inhibit peroxidation of lipids, and the subsequent inflammatory response, but to also identify the source of these oxidants and inhibit their generation (Selemidis et al., 2008). Due to the imbalance in oxidants and antioxidants, this can be achieved by two approaches: increasing the endogenous antioxidant enzyme activity via enzyme modulators/mimetics, or by replenishing the depleted non-enzymatic defences through dietary or pharmacological means.

It should be noted that typical radical scavenging treatments, such as vitamin E and other dietary antioxidants, have shown minimal improvements in either COPD or the comorbidities outlined in this review. This may be due to the dose, route of administration or the specific antioxidant given and thus more research is required to determine the efficacy of these compounds with certainty. In addition, traditional antioxidants act in a "sacrificial manner," due to the fact that once they have scavenged a radical, they are essentially consumed.

Undoubtedly, the most damaging of all the ROS/RNS are the hydroxyl radical and peroxynitrite. These species are extremely reactive and

indiscriminate to the point that they basically react with the first substrate they come into contact with. Knowing this, the most effective form of protection would be to prevent their generation in the first place and this cannot be performed by traditional antioxidants (scavengers). Therefore, a pharmacological approach which inhibits the production of oxidant species is required. In addition to this, it may also prove beneficial to use combination therapy and treat patients with the pharmacological enzymatic antioxidants (e.g., NOX inhibitors) whilst also supplementing them with dietary antioxidants (e.g., vitamin E supplements).

7.1. Novel antioxidants

As shown on the ROS cascade (Fig. 2), there are 3 key enzymes that can be targeted in order to reduce oxidative stress; NOX, SOD, and Gpx/Cat. These enzymes can either be inhibited to reduce their activity (e.g., NOX inhibitor — apocynin) or amplified/mimicked to increase their activity (e.g., Gpx mimetics — ebselen). Outlined below are just some of the enzymatic compounds that may be of therapeutic benefit to COPD patients (also see Table 1).

7.1.1. NADPH oxidase inhibitors

As stated earlier, NOX-1 and -2 are the primary generators of $O_2\bullet^-$ and are responsible for the initiation of the ROS cascade (Fig. 2). NOX is a unique target in the sense that inhibiting the activity of the enzyme would not only reduce the generation of a single oxidant species but also reduce the generation of all other ROS and RNS. Inhibiting NOX would reduce $O_2\bullet^-$ production which would result in less available $O_2\bullet^-$ for the generation of H_2O_2 and $ONOO^-$, subsequently reducing $\bullet OH$ generation and increasing $\bullet NO$ bioavailability as a result.

7.1.1.1. Current studies.
There are several NOX inhibitors currently being studied, however the most common is apocynin, which is a NOX inhibitor that preferentially blocks NOX-2 at low doses. It inhibits NOX by preventing the assembly of the NOX enzyme subunits, resulting in the reduced formation of NOX complexes (Selemidis et al., 2008; Drummond et al., 2011). We have shown that apocynin reduces

Table 1
Various antioxidant compounds that have been studied in the context of COPD and/or its comorbidities. Pre-clinical and clinical tests are investigating, or have investigated, the effectiveness of intervention with various small-molecular weight antioxidant compounds that target oxidant generation, or quench inflammation/cigarette smoke-derived oxidants and aldehydes. Refer to Rahman and MacNee (2012) for an in-depth analysis of these various compounds.

Antioxidant compounds	Examples
Thiol compounds	N-acetyl-L-cysteine (NAC)
	Glutathione esters
	Fudosteine
	Thioredoxin
Inducers of glutathione biosynthesis	
Nrf2 activators	CDDO-imidazolide
	Sulforaphane
NOX, SOD, Gpx	
Mimetics	Salen compounds
	Metaloporphyrins
	Ebselen
Inhibitors	Apocynin
Lipid peroxidation/protein carbonylation inhibitors/blockers	Lazaroids/tirilazad
	Edaravone
Other antioxidants/enzymes and oxidant scavengers	Nitrone spin traps
	Peroxynitrite decomposition catalysts
	Porphyrins
Polyphenols, natural products, and dietary agents	Polyphenols/flavonoids/natural compounds
	(Apocynin, vitamin D, vitamin E, omega-3-fatty acid)

Source of the data: Rahman and MacNee (2012).

cigarette smoke-induced lung inflammation in mice (Bernardo et al., unpublished observations). Additionally, the inhibition of NOX-2 activity ameliorates influenza A virus-induced lung inflammation, indicating that pharmacologically targeting NOX-2 may also have therapeutic potential in seasonal and possibly pandemic influenza infection (Vlahos et al., 2011; Vlahos, Stambas, & Selemidis, 2012). Because of this, the possible therapeutic utility of NOX-2 inhibitors may extend to AECOPD. Clinically, COPD patients treated with apocynin had reduced H_2O_2 and NO_2^- in their exhaled breath concentrate compared to placebo control (Stefanska et al., 2012).

7.1.2. Superoxide dismutase mimetics

There are 3 human isoforms of SOD (1, 2, and 3), each of which can transform $O_2\bullet^-$ to H_2O_2. SOD3 (or extracellular SOD) is located in the extracellular matrix, the junctions of airway epithelial cells, the surface of airway smooth muscle, and the lining of blood vessels of the lung (Kinnula & Crapo, 2003). It should be noted that smokers and COPD patients have increased sputum levels of SOD3 as an adaptive response to the increased oxidative burden (Regan et al., 2011). Additionally, polymorphisms in the SOD3 gene have been associated with emphysema but not COPD susceptibility (Sorheim et al., 2010). It is possible to enhance the conversion of $O_2\bullet^-$ to H_2O_2 by introducing SOD mimetics. This would result in the same effects caused by NOX inhibition: reduced $O_2\bullet^-$, increased NO bioavailability, however, it would also increase H_2O_2 generated. Although there is increased H_2O_2 generation, the newest class of SOD mimetics (salen complexes — "salens") have also shown Catalase like activity, enhancing the neutralization of H_2O_2 in cells and decomposing $ONOO^-$ (Sharpe, Ollosson, Stewart, & Clark, 2002). Additionally, it has been suggested that SOD mimetics may be beneficial for the treatment of cancer in combination with traditional cancer therapies (Thomas & Sharifi, 2012).

7.1.2.1. Current studies.
Since the administration of exogenous SODs themselves has often proven to be problematic, a variety of innovative approaches are currently being explored, one of these being SOD mimetics. Multiple classes of SOD mimetics have been developed and each class has generally been effective in animal models of COPD. Administration of the SOD mimetic M40419 in rats treated with VEFG receptor blockers significantly decreased markers of oxidative stress in the lungs and prevented the development of emphysema (Tuder et al., 2003). In cigarette smoke-exposed rats, treatment with the SOD mimetic AEOL 10150 was found to significantly reduce BALF inflammation (Smith et al., 2002). It is known that the acute loss of SOD3 in adult mice causes death, whereas overexpression of SOD3 in animals exposed to hyperoxia reduces mortality (Folz, Abushamaa, & Suliman, 1999; Gongora et al., 2008). Additionally, administration of SOD mimetic significantly attenuated the elastase-induced emphysema in both wild-type and SOD3 knockout mice (Yao et al., 2010). This study found that SOD3 protected against oxidative fragmentation of extracellular matrix (ECM), resulting in the mitigation of lung inflammatory response and emphysema. Knowing this, it is possible that SOD3 may be more effective than other SOD isoforms for the management of COPD. Therefore, the development of pharmacological mimetics to replenish and augment SOD3 in the lung may have a therapeutic potential for the treatment of COPD/emphysema (Rahman, 2012).

7.1.3. Glutathione peroxidase mimetics

The Gpx family of enzymes, along with catalase, are responsible for the termination of the ROS cascade. There are 8 known isoforms of Gpx (1 to 8) but the most abundant isoform is Gpx-1, which is found in the cytoplasm of almost all mammalian cells and whose preferred substrate is H_2O_2 (Vlahos & Bozinovski, 2013). The main function of Gpx is the reduction of H_2O_2 to H_2O and O_2; however, Gpx is also known to reduce lipid peroxides to their corresponding alcohols. Additionally, it has been reported that Gpx-2 is a major cigarette smoke-inducible isoform found in the lung (Singh et al., 2006). As with SOD,

it is possible to increase the reduction of H_2O_2 by introducing Gpx (or catalase) mimetics. This would result in the termination of the ROS cascade by increasing conversion of H_2O_2 to H_2O and O_2, and subsequently reduced levels of •OH and decreased levels of lipid peroxides.

7.1.3.1. Current studies. There is currently a limited amount of studies exploring the effectiveness of Gpx mimetics in COPD. When exposed to cigarette smoke, Gpx-1 knockout mice exhibited increased BALF neutrophils, macrophages, proteolytic burden, whole lung IL-17A, and MIP1 mRNA compared with WT mice (Duong et al., 2010). When treated prophylactically with the Gpx-1 mimetic ebselen, the cigarette smoke-induced increases in BALF macrophages, neutrophils, proteolytic burden, and macrophage and neutrophil chemotactic factor gene expression were all inhibited in both the WT and knockout mice. In addition, ebselen inhibited established BALF inflammation when administered therapeutically, suggesting that Gpx-1 mimetics may have therapeutic utility in inflammatory lung diseases where cigarette smoke plays a role such as COPD (Vlahos & Bozinovski, 2013). In addition, we have shown that ebselen caused a reduction in influenza A virus-induced lung inflammation in mice, suggesting that targeting Gpx-1 may be of therapeutic benefit for AECOPD (Yatmaz et al., 2013).

Ebselen has also been shown to be protective *in vivo* in disease situations hallmarked by oxidative stress such as diabetes-associated atherosclerosis and cerebral ischaemia–reperfusion injury (Wong, Bozinovski, Hertzog, Hickey, & Crack, 2008). Additionally, ebselen has been used in clinical trials of acute ischaemic stroke and was found to improve the outcome of patients when administered within 24 h of stroke (Yamaguchi et al., 1998).

7.1.4. Nuclear factor erythroid 2-related factor 2 activators

In addition to the 3 enzymes in the ROS cascade, the DNA binding protein nuclear factor erythroid 2-related factor 2 (Nrf2) presents a potential target for reducing oxidative stress in COPD (Boutten, Goven, Artaud-Macari, Boczkowski, & Bonay, 2011). Nrf2 is found in the cytoplasm of many mammalian cells and is responsible for the regulation of various antioxidants and cytoprotective genes, acting as a "master switch" for these genes. In response to oxidative stress, Nrf2 translocates to the nucleus and binds to the antioxidant response element (ARE) of target genes, along with other binding factors and cofactors, resulting in the induction of stress response genes (Rahman, 2012). COPD patients have decreased levels of Nrf2 in the lungs, and may be linked to the reduced antioxidant capacity observed in patients (Nguyen, Nioi, & Pickett, 2009). In addition, Nrf2 may also help improve the clearance of bacteria by alveolar macrophages, which is particularly relevant to the prevention and treatment of AECOPD (Harvey et al., 2011).

7.1.4.1. Current studies. It is known that Nrf2 signalling is impaired in several chronic diseases, including COPD, cancer, and neurodegenerative diseases (Boutten et al., 2011; Bauer, Hill, & Alexander, 2013). The levels of Nrf2 protein are decreased in lungs of patients with COPD and one study found that Nrf2-dependent antioxidants was negatively associated with severity of COPD, suggesting that therapy directed towards enhancing Nrf2-regulated antioxidants may be a novel strategy for attenuating the effects of oxidative stress in the pathogenesis of COPD (Malhotra et al., 2008). Studies have shown increased susceptibility of Nrf2 knockout mice to CS- or elastase-induced pulmonary emphysema (Rangasamy et al., 2004; Iizuka et al., 2005; Ishii et al., 2005). Compared with WT littermates, the Nrf2 knockouts exhibited more pronounced inflammation and neutrophilic elastase activity in the BALF, enhanced alveolar expression of oxidative stress markers, increased numbers of apoptotic endothelial and alveolar type II epithelial cells, and decreased antioxidant and antiprotease gene expression in alveolar macrophages. Nrf2 deficiency also results in impaired alveolar type II cell growth and enhanced sensitivity to oxidants, thereby contributing to abnormal lung injury and repair (Boutten et al., 2011). Treatment with the Nrf2 activator CDDO-imidazolide was found to attenuate

cigarette smoke-induced emphysema and cardiac dysfunction in mice (Sussan et al., 2009). Nrf2 appears to protect against pulmonary hyperoxia/bleomycin injury and ovalbumin challenge in mice, presumably by upregulating the transcription of lung antioxidant defence enzymes. It was also found that treatment with Nrf2 activators may be able to restore glucocorticosteroid sensitivity in COPD patients (Malhotra et al., 2011). A clinical trial of the Nrf2 activators sulforaphane is currently in progress in patients with COPD (Barnes, 2013).

Activation of Nrf2 has also been observed to have anticancer effects. Several studies have found that Nrf2-activating compounds can prevent or suppress cancer in mouse models (Sporn & Liby, 2012).

8. Concluding remarks

COPD is a major incurable global health burden and is the 4th largest cause of death in the world. Exacerbations are a common occurrence in COPD patients and contribute mainly to morbidity, death and health-related quality of life. Comorbid diseases, in particular cardiovascular disease and skeletal muscle wasting, are also common in COPD and potentiate the morbidity of COPD, leading to increased hospitalisations, mortality and healthcare costs. Current treatments have limited efficacy and fail to modify the factors that initiate and drive the long-term progression of COPD, its exacerbations and its co-morbidities. In addition, no pharmacological treatment has been shown to reduce the risk of death in COPD in prospective clinical trials. It is now evident that increased oxidative stress within the local lung microenvironment is a major driving mechanism in the pathophysiology of COPD and that it may directly influence peripheral organ (e.g., heart, skeletal muscle, brain, bone) behaviour in a 'COPD-specific manner'. Therefore, targeting oxidant-dependent mechanisms that drive COPD and its co-morbidities may have great therapeutic potential.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

References

Adachi, J. D., Ioannidis, G., Pickard, L., Berger, C., Prior, J. C., Joseph, L., et al. (2003). The association between osteoporotic fractures and health-related quality of life as measured by the Health Utilities Index in the Canadian Multicentre Osteoporosis Study (CaMos). *Osteoporosis International 14*, 895–904.

Adeloye, D., Basquill, C., Papana, A., Chan, K. Y., Rudan, I., & Campbell, H. (2015). An estimate of the prevalence of COPD in Africa: a systematic analysis. *COPD 12*, 71–81.

Afanas'ev, I. (2011). ROS and RNS signaling in heart disorders: could antioxidant treatment be successful? *Oxidative Medicine and Cellular Longevity 2011*, 293769.

Agnihotri, R., Pandurang, P., Kamath, S. U., Goyal, R., Ballal, S., Shanbhogue, A. Y., et al. (2009). Association of cigarette smoking with superoxide dismutase enzyme levels in subjects with chronic periodontitis. *The Journal of Periodontology 80*, 657–662.

Aiken, C. T., Kaake, R. M., Wang, X., & Huang, L. (2011). Oxidative stress-mediated regulation of proteasome complexes. *Molecular & Cellular Proteomics 10*(R110), 006924.

Alberg, A. J., & Nonemaker, J. (2008). Who is at high risk for lung cancer? Population-level and individual-level perspectives. *Seminars in Respiratory and Critical Care Medicine 29*, 223–232.

Almeida, M., & O'Brien, C. A. (2013). Basic biology of skeletal aging: role of stress response pathways. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 68*, 1197–1208.

Angelis, N., Porpodis, K., Zarogoulidis, P., Spyratos, D., Kioumis, I., Papaiwannou, A., et al. (2014). Airway inflammation in chronic obstructive pulmonary disease. *J Thorac Dis 6*(Suppl. 1), S167–S172.

Antus, B., Harnasi, G., Drozdovszky, O., & Barta, I. (2014). Monitoring oxidative stress during chronic obstructive pulmonary disease exacerbations using malondialdehyde. *Respirology 19*, 74–79.

Barbieri, E., & Sestili, P. (2012). Reactive oxygen species in skeletal muscle signaling. *Journal Signal Transduction 2012*, 982794.

Barnes, P. J. (2004). Mediators of chronic obstructive pulmonary disease. *Pharmacological Reviews 56*, 515–548.

Barnes, P. J. (2013). New anti-inflammatory targets for chronic obstructive pulmonary disease. *Nature Reviews Drug Discovery 12*, 543–559.

Barnes, P. J. (2014). Cellular and molecular mechanisms of chronic obstructive pulmonary disease. *Clinics in Chest Medicine 35* (71-+).

Barnes, P. J., & Celli, B. R. (2009). Systemic manifestations and comorbidities of COPD. *The European Respiratory Journal 33*, 1165–1185.

Barreiro, E., Fermoselle, C., Mateu-Jimenez, M., Sanchez-Font, A., Pijuan, L., Gea, J., et al. (2013). Oxidative stress and inflammation in the normal airways and blood of patients with lung cancer and COPD. *Free Radical Biology & Medicine 65*, 859–871.

Barreiro, E., Ferrer, D., Sanchez, F., Minguella, J., Marin-Corral, J., Martinez-Llorens, J., et al. (2011). Inflammatory cells and apoptosis in respiratory and limb muscles of patients with COPD. *Journal of Applied Physiology (1985) 111*, 808–817.

Barreiro, E., Peinado, V. I., Galdiz, J. B., Ferrer, E., Marin-Corral, J., Sanchez, F., et al. (2010). Cigarette smoke-induced oxidative stress: a role in chronic obstructive pulmonary disease skeletal muscle dysfunction. *American Journal of Respiratory and Critical Care Medicine 182*, 477–488.

Bauer, A. K., Hill, T., 3rd, & Alexander, C. M. (2013). The involvement of NRF2 in lung cancer. *Oxidative Medicine and Cellular Longevity 2013*, 746432.

Bax, B. E., Alam, A. S., Banerji, B., Bax, C. M., Bevis, P. J., Stevens, C. R., et al. (1992). Stimulation of osteoclastic bone resorption by hydrogen peroxide. *Biochemical and Biophysical Research Communications 183*, 1153–1158.

Beckman, J. S. (1996). Oxidative damage and tyrosine nitration from peroxynitrite. *Chemical Research in Toxicology 9*, 836–844.

Belvisi, M. G., & Bottomley, K. M. (2003). The role of matrix metalloproteinases (MMPs) in the pathophysiology of chronic obstructive pulmonary disease (COPD): a therapeutic role for inhibitors of MMPs? *Inflammation Research 52*, 95–100.

Bentley, A. R., Emrani, P., & Cassano, P. A. (2008). Genetic variation and gene expression in antioxidant related enzymes and risk of COPD: a systematic review. *Thorax 63*, 956–961.

Berlett, B. S., & Stadtman, E. R. (1997). Protein oxidation in aging, disease, and oxidative stress. *The Journal of Biological Chemistry 272*, 20313–20316.

Biswas, S., Hwang, J. W., Kirkham, P. A., & Rahman, I. (2013). Pharmacological and dietary antioxidant therapies for chronic obstructive pulmonary disease. *Current Medicinal Chemistry 20*, 1496–1530.

Bolton, C. E., Stone, M. D., Edwards, P. H., Duckers, J. M., Evans, W. D., & Shale, D. J. (2009). Circulating matrix metalloproteinase-9 and osteoporosis in patients with chronic obstructive pulmonary disease. *Chronic Respiratory Disease 6*, 81–87.

Booth, F. W., & Gollnick, P. D. (1983). Effects of disuse on the structure and function of skeletal muscle. *Medicine and Science in Sports and Exercise 15*, 415–420.

Boutten, A., Goven, D., Artaud-Macari, E., Boczkowski, J., & Bonay, M. (2011). NRF2 targeting: a promising therapeutic strategy in chronic obstructive pulmonary disease. *Trends in Molecular Medicine 17*, 363–371.

Boyce, B. F., Li, P., Yao, Z., Zhang, Q., Badell, I. R., Schwarz, E. M., et al. (2005). TNF-alpha and pathologic bone resorption. *The Keio Journal of Medicine 54*, 127–131.

Bozinovski, S., Vlahos, R., Anthony, D., McQualter, J., Anderson, G., Irving, L., et al. (2015). COPD and squamous cell lung cancer: aberrant inflammation and immunity is the common link. *British Journal of Pharmacology* (Epub ahead of print).

Bredt, D. S. (1999). Endogenous nitric oxide synthesis: biological functions and pathophysiology. *Free Radical Research 31*, 577–596.

Bridges, R. B., Fu, M. C., & Rehm, S. R. (1985). Increased neutrophil myeloperoxidase activity associated with cigarette smoking. *European Journal of Respiratory Diseases 67*, 84–93.

Bull, H., Murray, P. G., Thomas, D., Fraser, A. M., & Nelson, P. N. (2002). Acid phosphatases. *Molecular Pathology 55*, 65–72.

Bursi, F., Vassallo, R., Weston, S. A., Killian, J. M., & Roger, V. L. (2010). Chronic obstructive pulmonary disease after myocardial infarction in the community. *American Heart Journal 160*, 95–101.

Byers, L. A., & Rudin, C. M. (2015). Small cell lung cancer: where do we go from here? *Cancer 121*, 664–672.

Canalis, E. (2005). Mechanisms of glucocorticoid action in bone. *Current Osteoporosis Reports 3*, 98–102.

Caron, M. A., Theriault, M. E., Pare, M. E., Maltais, F., & Debigare, R. (2009). Hypoxia alters contractile protein homeostasis in L6 myotubes. *FEBS Letters 583*, 1528–1534.

Casaburi, R., Bhasin, S., Cosentino, L., Porszasz, J., Somfay, A., Lewis, M. I., et al. (2004). Effects of testosterone and resistance training in men with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine 170*, 870–878.

Cassuto, J., Dou, H., Czikora, I., Szabo, A., Patel, V. S., Kamath, V., et al. (2014). Peroxynitrite disrupts endothelial caveolae leading to eNOS uncoupling and diminished flow-mediated dilation in coronary arterioles of diabetic patients. *Diabetes 63*, 1381–1393.

Cavailles, A., Brinchault-Rabin, G., Dixmier, A., Goupil, F., Gut-Gobert, C., Marchand-Adam, S., et al. (2013). Comorbidities of COPD. *European Respiratory Review 22*, 454–475.

Cave, A., Grieve, D., Johar, S., Zhang, M., & Shah, A. M. (2005). NADPH oxidase-derived reactive oxygen species in cardiac pathophysiology. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 360*, 2327–2334.

Cervellati, C., Bonaccorsi, G., Cremonini, E., Romani, A., Fila, E., Castaldini, M. C., et al. (2014). Oxidative stress and bone resorption interplay as a possible trigger for postmenopausal osteoporosis. *Biomedical Research International 2014*, 569563.

Chan-Yeung, M., Ait-Khaled, N., White, N., Ip, M. S., & Tan, W. C. (2004). The burden and impact of COPD in Asia and Africa. *The International Journal of Tuberculosis and Lung Disease 8*, 2–14.

Chatila, W. M., Thomashow, B. M., Minai, O. A., Criner, G. J., & Make, B. J. (2008). Comorbidities in chronic obstructive pulmonary disease. *Proceedings of the American Thoracic Society 5*, 549–555.

Chavan, S. N., More, U., Mulgund, S., Saxena, V., & Sontakke, A. N. (2007). Effect of supplementation of vitamin C and E on oxidative stress in osteoporosis. *Indian Journal of Clinical Biochemistry 22*, 101–105.

Chen, W., Li, Z., Bai, L., & Lin, Y. (2011). NF-kappaB in lung cancer, a carcinogenesis mediator and a prevention and therapy target. *Frontiers in Bioscience (Landmark Ed) 16*, 1172–1185.

Church, D. F., & Pryor, W. A. (1985). Free-radical chemistry of cigarette smoke and its toxicological implications. *Environmental Health Perspectives 64*, 111–126.

Cielen, N., Maes, K., & Gayan-Ramirez, G. (2014). Musculoskeletal disorders in chronic obstructive pulmonary disease. *Biomedical Research International 2014*, 965764.

Compton, J. T., & Lee, F. Y. (2014). A review of osteocyte function and the emerging importance of sclerostin. *The Journal of Bone and Joint Surgery. American Volume 96*, 1659–1668.

Congleton, J. (1999). The pulmonary cachexia syndrome: aspects of energy balance. *The Proceedings of the Nutrition Society 58*, 321–328.

Cooke, J. P. (2004). The pivotal role of nitric oxide for vascular health. *The Canadian Journal of Cardiology 20*(Suppl. B), 7B–15B.

Cooke, M. S., Evans, M. D., Dizdaroglu, M., & Lunec, J. (2003). Oxidative DNA damage: mechanisms, mutation, and disease. *The FASEB Journal 17*, 1195–1214.

Corrales-Medina, V. F., Madjid, M., & Musher, D. M. (2010). Role of acute infection in triggering acute coronary syndromes. *The Lancet Infectious Diseases 10*, 83–92.

Couillard, A., Maltais, F., Saey, D., Debigare, R., Michaud, A., Koechlin, C., et al. (2003). Exercise-induced quadriceps oxidative stress and peripheral muscle dysfunction in patients with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine 167*, 1664–1669.

Coyle, E. F., Martin, W. H., 3rd, Bloomfield, S. A., Lowry, O. H., & Holloszy, J. O. (1985). Effects of detraining on responses to submaximal exercise. *Journal of Applied Physiology (1985) 59*, 853–859.

Dalal, A. A., Shah, M., Lunacsek, O., & Hanania, N. A. (2011). Clinical and economic burden of patients diagnosed with COPD with comorbid cardiovascular disease. *Respiratory Medicine 105*, 1516–1522.

Darden, A. G., Ries, W. L., Wolf, W. C., Rodriguiz, R. M., & Key, L. L., Jr. (1996). Osteoclastic superoxide production and bone resorption: stimulation and inhibition by modulators of NADPH oxidase. *Journal of Bone and Mineral Research 11*, 671–675.

Dawane, J. S., & Pandit, V. A. (2012). Understanding redox homeostasis and its role in cancer. *Journal of Clinical and Diagnostic Research 6*, 1796–1802.

de Melo, M. N., Ernst, P., & Suissa, S. (2004). Rates and patterns of chronic obstructive pulmonary disease exacerbations. *Canadian Respiratory Journal 11*, 559–564.

De Paepe, B., Brusselle, G. G., Maes, T., Creus, K. K., D'Hose, S., D'Haese, N., et al. (2008). TNF alpha receptor genotype influences smoking-induced muscle-fibre-type shift and atrophy in mice. *Acta Neuropathologica 115*, 675–681.

De Vries, F., Bracke, M., Leufkens, H. G., Lammers, J. W., Cooper, C., & Van Staa, T. P. (2007). Fracture risk with intermittent high-dose oral glucocorticoid therapy. *Arthritis and Rheumatism 56*, 208–214.

Debigare, R., Cote, C. H., & Maltais, F. (2001). Peripheral muscle wasting in chronic obstructive pulmonary disease. Clinical relevance and mechanisms. *American Journal of Respiratory and Critical Care Medicine 164*, 1712–1717.

Debigare, R., & Maltais, F. (2008). The major limitation to exercise performance in COPD is lower limb muscle dysfunction. *Journal of Applied Physiology (1985) 105*, 751–753 (discussion 755–757).

Dekhuijzen, P. N., Aben, K. K., Dekker, I., Aarts, L. P., Wielders, P. L., van Herwaarden, C. L., et al. (1996). Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine 154*, 813–816.

Delles, R. M., Xiong, Y. L., True, A. D., Ao, T., & Dawson, K. A. (2014). Dietary antioxidant supplementation enhances lipid and protein oxidative stability of chicken broiler meat through promotion of antioxidant enzyme activity. *Poultry Science 93*, 1561–1570.

Demarco, V. G., Whaley-Connell, A. T., Sowers, J. R., Habibi, J., & Dellsperger, K. C. (2010). Contribution of oxidative stress to pulmonary arterial hypertension. *World Journal of Cardiology 2*, 316–324.

Dhalla, N. S., Temsah, R. M., & Netticadan, T. (2000). Role of oxidative stress in cardiovascular diseases. *Journal of Hypertension 18*, 655–673.

Donoso, O., Pino, A. M., Seitz, G., Osses, N., & Rodriguez, J. P. (2015). Osteoporosis-associated alteration in the signalling status of BMP-2 in human MSCs under adipogenic conditions. *Journal of Cellular Biochemistry 116*, 1267–1277.

Drummond, G. R., Selemidis, S., Griendling, K. K., & Sobey, C. G. (2011). Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. *Nature Reviews. Drug Discovery 10*, 453–471.

Duong, C., Seow, H. J., Bozinovski, S., Crack, P. J., Anderson, G. P., & Vlahos, R. (2010). Glutathione peroxidase-1 protects against cigarette smoke-induced lung inflammation in mice. *American Journal of Physiology. Lung Cellular and Molecular Physiology 299*, L425–L433.

Falk, J. A., Minai, O. A., & Mosenifar, Z. (2008). Inhaled and systemic corticosteroids in chronic obstructive pulmonary disease. *Proceedings of the American Thoracic Society 5*, 506–512.

Fathy, M., Hamed, M., Youssif, O., Fawzy, N., & Ashour, W. (2014). Association between environmental tobacco smoke exposure and lung cancer susceptibility: modification by antioxidant enzyme genetic polymorphisms. *Molecular Diagnosis & Therapy 18*, 55–62.

Fearon, I. M., & Faux, S. P. (2009). Oxidative stress and cardiovascular disease: novel tools give (free) radical insight. *Journal of Molecular and Cellular Cardiology 47*, 372–381.

Feletou, M., Kohler, R., & Vanhoutte, P. M. (2012). Nitric oxide: orchestrator of endothelium-dependent responses. *Annals of Medicine 44*, 694–716.

Feng, X., & McDonald, J. M. (2011). Disorders of bone remodeling. *Annual Review of Pathology 6*, 121–145.

Fiorillo, C., Nediani, C., Ponziani, V., Giannini, L., Celli, A., Nassi, N., et al. (2005). Cardiac volume overload rapidly induces oxidative stress-mediated myocyte apoptosis and hypertrophy. *Biochimica et Biophysica Acta 1741*, 173–182.

Fisher, A. B. (2009). Redox signaling across cell membranes. *Antioxidants & Redox Signaling 11*, 1349–1356.

Fisher-Wellman, K., & Bloomer, R. J. (2009). Acute exercise and oxidative stress: a 30 year history. *Dynamics of Medicine 8*, 1.

Folz, R. J., Abushamaa, A. M., & Suliman, H. B. (1999). Extracellular superoxide dismutase in the airways of transgenic mice reduces inflammation and attenuates lung toxicity following hyperoxia. *The Journal of Clinical Investigation 103*, 1055–1066.

Ford, E. S., Wheaton, A. G., Mannino, D. M., Presley-Cantrell, L., Li, C., & Croft, J. B. (2012). Elevated cardiovascular risk among adults with obstructive and restrictive airway functioning in the United States: a cross-sectional study of the National Health and Nutrition Examination Survey from 2007–2010. *Respiratory Research 13*, 115.

Forli, L., Mellbye, O. J., Halse, J., Bjortuft, O., Vatn, M., & Boe, J. (2008). Cytokines, bone turn-over markers and weight change in candidates for lung transplantation. *Pulmonary Pharmacology & Therapeutics 21*, 188–195.

Foschino Barbaro, M. P., Carpagnano, G. E., Spanevello, A., Cagnazzo, M. G., & Barnes, P. J. (2007). Inflammation, oxidative stress and systemic effects in mild chronic obstructive pulmonary disease. *International Journal of Immunopathology and Pharmacology 20*, 753–763.

Garbin, U., Fratta Pasini, A., Stranieri, C., Cominacini, M., Pasini, A., Manfro, S., et al. (2009). Cigarette smoking blocks the protective expression of Nrf2/ARE pathway in peripheral mononuclear cells of young heavy smokers favouring inflammation. *PloS One 4* (e8225).

Gernez, Y., Tirouvanziam, R., & Chanez, P. (2010). Neutrophils in chronic inflammatory airway diseases: can we target them and how? *The European Respiratory Journal 35*, 467–469.

Gicquel, T., Victoni, T., Fautrel, A., Robert, S., Gleonnec, F., Guezingar, M., et al. (2008). Involvement of purinergic receptors and NOD-like receptor-family protein 3-inflammasome pathway in the adenosine triphosphate-induced cytokine release from macrophages. *Clinical and Experimental Pharmacology & Physiology 41*, 279–286.

Gloire, G., Legrand-Poels, S., & Piette, J. (2006). NF-kappaB activation by reactive oxygen species: fifteen years later. *Biochemical Pharmacology 72*, 1493–1505.

GOLD, G. I. f. C. O. P. D. (2015). *Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease: Updated 2015. In*.

Gongora, M. C., Lob, H. E., Landmesser, U., Guzik, T. J., Martin, W. D., Ozumi, K., et al. (2008). Loss of extracellular superoxide dismutase leads to acute lung damage in the presence of ambient air: a potential mechanism underlying adult respiratory distress syndrome. *The American Journal of Pathology 173*, 915–926.

Gonzalez, D., Marquina, R., Rondon, N., Rodriguez-Malaver, A. J., & Reyes, R. (2008). Effects of aerobic exercise on uric acid, total antioxidant activity, oxidative stress, and nitric oxide in human saliva. *Research in Sports Medicine 16*, 128–137.

Gosker, H. R., Kubat, B., Schaart, G., van der Vusse, G. J., Wouters, E. F., & Schols, A. M. (2003). Myopathological features in skeletal muscle of patients with chronic obstructive pulmonary disease. *The European Respiratory Journal 22*, 280–285.

Griffith, B., Pendyala, S., Hecker, L., Lee, P. J., Natarajan, V., & Thannickal, V. J. (2009). NOX enzymes and pulmonary disease. *Antioxidants & Redox Signaling 11*, 2505–2516.

Grimsrud, P. A., Xie, H., Griffin, T. J., & Bernlohr, D. A. (2008). Oxidative stress and covalent modification of protein with bioactive aldehydes. *The Journal of Biological Chemistry 283*, 21837–21841.

Gupta, A., Srivastava, S., Prasad, R., Natu, S. M., Mittal, B., Negi, M. P., et al. (2010). Oxidative stress in non-small cell lung cancer patients after chemotherapy: association with treatment response. *Respirology 15*, 349–356.

Hansen, M. J., Gualano, R. C., Bozinovski, S., Vlahos, R., & Anderson, G. P. (2006). Therapeutic prospects to treat skeletal muscle wasting in COPD (chronic obstructive lung disease). *Pharmacology & Therapeutics 109*, 162–172.

Harvey, C. J., Thimmulappa, R. K., Sethi, S., Kong, X., Yarmus, L., Brown, R. H., et al. (2011). Targeting Nrf2 signaling improves bacterial clearance by alveolar macrophages in patients with COPD and in a mouse model. *Science Translational Medicine 3*, 78ra32.

Hasnis, E., Bar-Shai, M., Burbea, Z., & Reznick, A. Z. (2007). Mechanisms underlying cigarette smoke-induced NF-kappaB activation in human lymphocytes: the role of reactive nitrogen species. *Journal of Physiology and Pharmacology 58*(Suppl. 5), 275–287.

Hillas, G., Nikolakopoulou, S., Hussain, S., & Vassilakopoulos, T. (2013). Antioxidants and mucolytics in COPD management: when (if ever) and in whom? *Current Drug Targets 14*, 225–234.

Hoppeler, H., Kleinert, E., Schlegel, C., Claassen, H., Howald, H., Kayar, S. R., et al. (1990). Morphological adaptations of human skeletal muscle to chronic hypoxia. *International Journal of Sports Medicine 11*(Suppl. 1), S3–S9.

Hotamisligil, G. S. (1999). Mechanisms of TNF-alpha-induced insulin resistance. *Experimental and Clinical Endocrinology & Diabetes 107*, 119–125.

Huang, P. L. (2009). eNOS, metabolic syndrome and cardiovascular disease. *Trends in Endocrinology and Metabolism 20*, 295–302.

Huang, D., Yang, L., Liu, Y., Zhou, Y., Guo, Y., Pan, M., et al. (2013). Functional polymorphisms in NFkappaB1/IkappaBalpha predict risks of chronic obstructive pulmonary disease and lung cancer in Chinese. *Human Genetics 132*, 451–460.

Hurst, J. R., & Wedzicha, J. A. (2009). Management and prevention of chronic obstructive pulmonary disease exacerbations: a state of the art review. *BMC Medicine 7*, 40.

Ichinose, M., Sugiura, H., Yamagata, S., Koarai, A., & Shirato, K. (2000). Increase in reactive nitrogen species production in chronic obstructive pulmonary disease airways. *American Journal of Respiratory and Critical Care Medicine 162*, 701–706.

Ichinose, M., Sugiura, H., Yamagata, S., Koarai, A., Tomaki, M., Ogawa, H., et al. (2003). Xanthine oxidase inhibition reduces reactive nitrogen species production in COPD airways. *The European Respiratory Journal 22*, 457–461.

Iizuka, T., Ishii, Y., Itoh, K., Kiwamoto, T., Kimura, T., Matsuno, Y., et al. (2005). Nrf2-deficient mice are highly susceptible to cigarette smoke-induced emphysema. *Genes to Cells 10*, 1113–1125.

Ishii, Y., Itoh, K., Morishima, Y., Kimura, T., Kiwamoto, T., Iizuka, T., et al. (2005). Transcription factor Nrf2 plays a pivotal role in protection against elastase-induced pulmonary inflammation and emphysema. *Journal of Immunology 175*, 6968–6975.

Ishimi, Y., Miyaura, C., Jin, C. H., Akatsu, T., Abe, E., Nakamura, Y., et al. (1990). IL-6 is produced by osteoblasts and induces bone resorption. *Journal of Immunology 145*, 3297–3303.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA: a Cancer Journal for Clinicians 61*, 69–90.

Jorgensen, N. R., & Schwarz, P. (2008). Osteoporosis in chronic obstructive pulmonary disease patients. *Current Opinion in Pulmonary Medicine 14*, 122–127.

Jorgensen, N. R., Schwarz, P., Holme, I., Henriksen, B. M., Petersen, L. J., & Backer, V. (2007). The prevalence of osteoporosis in patients with chronic obstructive pulmonary disease: a cross sectional study. *Respiratory Medicine 101*, 177–185.

Joshi, M. S., Ponthier, J. L., & Lancaster, J. R., Jr. (1999). Cellular antioxidant and pro-oxidant actions of nitric oxide. *Free Radical Biology & Medicine 27*, 1357–1366.

Kalyanaraman, B. (2013). Teaching the basics of redox biology to medical and graduate students: oxidants, antioxidants and disease mechanisms. *Redox Biology 1*, 244–257.

Kandarian, S. C., & Jackman, R. W. (2006). Intracellular signaling during skeletal muscle atrophy. *Muscle & Nerve 33*, 155–165.

Kanis, J. A. (2002). Diagnosis of osteoporosis and assessment of fracture risk. *Lancet 359*, 1929–1936.

Kanis, J. A., McCloskey, E. V., Johansson, H., Cooper, C., Rizzoli, R., Reginster, J. Y., et al. (2013). European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporosis International 24*, 23–57.

Karadag, F., Karul, A. B., Cildag, O., Yilmaz, M., & Ozcan, H. (2008). Biomarkers of systemic inflammation in stable and exacerbation phases of COPD. *Lung 186*, 403–409.

Karin, M. (2009). NF-kappaB as a critical link between inflammation and cancer. *Cold Spring Harbor Perspectives in Biology 1*, a000141.

Kawada, T. (2012). Oxidative stress markers and cardiovascular disease: advantage of using these factors in combination with lifestyle factors for cardiovascular risk assessment. *International Journal of Cardiology 157*, 119–120.

Key, L. L., Jr., Wolf, W. C., Gundberg, C. M., & Ries, W. L. (1994). Superoxide and bone resorption. *Bone 15*, 431–436.

Kim, H. C., Mofarrahi, M., & Hussain, S. N. (2008). Skeletal muscle dysfunction in patients with chronic obstructive pulmonary disease. *International Journal of Chronic Obstructive Pulmonary Disease 3*, 637–658.

Kim, W. J., Oh, Y. M., Kim, T. H., Lee, J. H., Kim, E. K., Lee, J. H., et al. (2013). CHRNA3 variant for lung cancer is associated with chronic obstructive pulmonary disease in Korea. *Respiration 86*, 117–122.

Kinnula, V. L., & Crapo, J. D. (2003). Superoxide dismutases in the lung and human lung diseases. *American Journal of Respiratory and Critical Care Medicine 167*, 1600–1619.

Kiri, V. A., Soriano, J., Visick, G., & Fabbri, L. (2010). Recent trends in lung cancer and its association with COPD: an analysis using the UK GP Research Database. *Primary Care Respiratory Journal 19*, 57–61.

Kirkham, P. A., & Barnes, P. J. (2013). Oxidative stress in COPD. *Chest 144*, 266–273.

Kirkham, P. A., Spooner, G., Ffoulkes-Jones, C., & Calvez, R. (2003). Cigarette smoke triggers macrophage adhesion and activation: role of lipid peroxidation products and scavenger receptor. *Free Radical Biology & Medicine 35*, 697–710.

Kluchova, Z., Petrasova, D., Joppa, P., Dorkova, Z., & Tkacova, R. (2007). The association between oxidative stress and obstructive lung impairment in patients with COPD. *Physiological Research 56*, 51–56.

Kolluru, G. K., Bir, S. C., & Kevil, C. G. (2012). Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. *International Journal of Vascular Medicine 2012*, 918267.

Koul, P. A. (2013). Chronic obstructive pulmonary disease: Indian guidelines and the road ahead. *Lung India 30*, 175–177.

Krijnen, P. A., Nijmeijer, R., Meijer, C. J., Visser, C. A., Hack, C. E., & Niessen, H. W. (2002). Apoptosis in myocardial ischaemia and infarction. *Journal of Clinical Pathology 55*, 801–811.

Kumar, D., Lou, H., & Singal, P. K. (2002). Oxidative stress and apoptosis in heart dysfunction. *Herz 27*, 662–668.

Kurys, E., Kurys, P., Kuzniar, A., & Kieszko, R. (2001). Analysis of antioxidant enzyme activity and magnesium level in chronic obstructive pulmonary disease (COPD). *University of Marie Curie Sklodowska Medicine 56*, 261–266.

Laratta, C. R., & van Eeden, S. (2014). Acute exacerbation of chronic obstructive pulmonary disease: cardiovascular links. *Biomedical Research International 2014*, 528789.

Larsson, L., & Ansved, T. (1985). Effects of long-term physical training and detraining on enzyme histochemical and functional skeletal muscle characteristic in man. *Muscle & Nerve 8*, 714–722.

Lawless, M. W., O'Byrne, K. J., & Gray, S. G. (2009). Oxidative stress induced lung cancer and COPD: opportunities for epigenetic therapy. *Journal of Cellular and Molecular Medicine 13*, 2800–2821.

Lee, S. H., Kim, J. K., & Jang, H. D. (2014). Genistein inhibits osteoclastic differentiation of RAW 264.7 cells via regulation of ROS production and scavenging. *International Journal of Molecular Sciences 15*, 10605–10621.

Lee, D. H., Lim, B. S., Lee, Y. K., & Yang, H. C. (2006). Effects of hydrogen peroxide (H_2O_2) on alkaline phosphatase activity and matrix mineralization of odontoblast and osteoblast cell lines. *Cell Biology and Toxicology 22*, 39–46.

Lee, G., Walser, T. C., & Dubinett, S. M. (2009). Chronic inflammation, chronic obstructive pulmonary disease, and lung cancer. *Current Opinion in Pulmonary Medicine 15*, 303–307.

Leslie, W. D., Aubry-Rozier, B., Lix, L. M., Morin, S. N., Majumdar, S. R., & Hans, D. (2014). Spine bone texture assessed by trabecular bone score (TBS) predicts osteoporotic fractures in men: the Manitoba Bone Density Program. *Bone 67*, 10–14.

Leung, K. S., Fung, K. P., Sher, A. H., Li, C. K., & Lee, K. M. (1993). Plasma bone-specific alkaline phosphatase as an indicator of osteoblastic activity. *Journal of Bone and Joint Surgery (British) 75*, 288–292.

Leuppi, J. D., Schuetz, P., Bingisser, R., Bodmer, M., Briel, M., Drescher, T., et al. (2013). Short-term vs conventional glucocorticoid therapy in acute exacerbations of chronic

obstructive pulmonary disease: the REDUCE randomized clinical trial. *JAMA 309*, 2223–2231.

Lewiecki, E. M. (2011). Sclerostin: a novel target for intervention in the treatment of osteoporosis. *Discovery Medicine 12*, 263–273.

Lopez, A. D., Shibuya, K., Rao, C., Mathers, C. D., Hansell, A. L., Held, L. S., et al. (2006). Chronic obstructive pulmonary disease: current burden and future projections. *The European Respiratory Journal 27*, 397–412.

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., et al. (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet 380*, 2095–2128.

Lung Health Study Research Group (2000). Effect of inhaled triamcinolone on the decline in pulmonary function in chronic obstructive pulmonary disease. *The New England Journal of Medicine 343*, 1902–1909.

Mackay, A. J., & Hurst, J. R. (2013). COPD exacerbations: causes, prevention, and treatment. *Immunology and Allergy Clinics of North America 33*, 95–115.

MacLean, C., Newberry, S., Maglione, M., McMahon, M., Ranganath, V., Suttorp, M., et al. (2008). Systematic review: comparative effectiveness of treatments to prevent fractures in men and women with low bone density or osteoporosis. *Annals of Internal Medicine 148*, 197–213.

MacNee, W., & Rahman, I. (2001). Is oxidative stress central to the pathogenesis of chronic obstructive pulmonary disease? *Trends in Molecular Medicine 7*, 55–62.

Madamanchi, N. R., & Runge, M. S. (2007). Mitochondrial dysfunction in atherosclerosis. *Circulation Research 100*, 460–473.

Magalhaes, J., Ascensao, A., Soares, J. M., Ferreira, R., Neuparth, M. J., Oliveira, J., et al. (2005). Acute and chronic exposition of mice to severe hypoxia: the role of acclimatization against skeletal muscle oxidative stress. *International Journal of Sports Medicine 26*, 102–109.

Malhotra, D., Thimmulappa, R. K., Mercado, N., Ito, K., Kombairaju, P., Kumar, S., et al. (2011). Denitrosylation of HDAC2 by targeting Nrf2 restores glucocorticosteroid sensitivity in macrophages from COPD patients. *The Journal of Clinical Investigation 121*, 4289–4302.

Malhotra, D., Thimmulappa, R., Navas-Acien, A., Sandford, A., Elliott, M., Singh, A., et al. (2008). Expression of concern: decline in NRF2-regulated antioxidants in chronic obstructive pulmonary disease lungs due to loss of its positive regulator, DJ-1. *American Journal of Respiratory and Critical Care Medicine 178*, 592–604.

Malins, D. C., Polissar, N. L., & Gunselman, S. J. (1996). Progression of human breast cancers to the metastatic state is linked to hydroxyl radical-induced DNA damage. *Proceedings of the National Academy of Sciences of the United States of America 93*, 2557–2563.

Maltais, F., Decramer, M., Casaburi, R., Barreiro, E., Burelle, Y., Debigare, R., et al. (2014). An official American Thoracic Society/European Respiratory Society statement: update on limb muscle dysfunction in chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine 189*, e15–e62.

Man, W. D., Kemp, P., Moxham, J., & Polkey, M. I. (2009). Exercise and muscle dysfunction in COPD: implications for pulmonary rehabilitation. *Clinical Science (London, England) 117*, 281–291.

Man, S. F., Van Eeden, S., & Sin, D. D. (2012). Vascular risk in chronic obstructive pulmonary disease: role of inflammation and other mediators. *The Canadian Journal of Cardiology 28*, 653–661.

Mannino, D. M. (2011). The natural history of chronic obstructive pulmonary disease. *Pneumonologia i Alergologia Polska 79*, 139–143.

Mannino, D. M., & Davis, K. J. (2006). Lung function decline and outcomes in an elderly population. *Thorax 61*, 472–477.

Marnett, L. J. (2000). Oxyradicals and DNA damage. *Carcinogenesis 21*, 361–370.

Marnett, L. J., & Tuttle, M. A. (1980). Comparison of the mutagenicities of malondialdehyde and the side products formed during its chemical synthesis. *Cancer Research 40*, 276–282.

Marquis, K., Debigare, R., Lacasse, Y., LeBlanc, P., Jobin, J., Carrier, G., et al. (2002). Midthigh muscle cross-sectional area is a better predictor of mortality than body mass index in patients with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine 166*, 809–813.

Masala, S., Magrini, A., Taglieri, A., Nano, G., Chiaravalloti, A., Calabria, E., et al. (2014). Chronic obstructive pulmonary disease (COPD) patients with osteoporotic vertebral compression fractures (OVCFs): improvement of pulmonary function after percutaneous vertebroplasty (VTP). *European Radiology 24*, 1577–1585.

Masri, F. (2010). Role of nitric oxide and its metabolites as potential markers in lung cancer. *The Annals of Thoracic Surgery Medicine 5*, 123–127.

Masunaga, S., Takahashi, A., Ohnishi, K., Ohnishi, T., Suzuki, M., Nagata, K., et al. (2003). Effects of p53 status and wortmannin treatment on potentially lethal damage repair, with emphasis on the response of intratumor quiescent cells. *Radiation Medicine 21*, 120–127.

Mathur, S., Brooks, D., & Carvalho, C. R. (2014). Structural alterations of skeletal muscle in COPD. *Frontiers in Physiology 5*, 104.

Matoba, T., & Egashira, K. (2011). Anti-inflammatory gene therapy for cardiovascular disease. *Current Gene Therapy 11*, 442–446.

McDonough, A. K., Curtis, J. R., & Saag, K. G. (2008). The epidemiology of glucocorticoid-associated adverse events. *Current Opinion in Rheumatology 20*, 131–137.

McLachlan, C. S., Hambly, B., Almsherqi, Z., El Oakley, R., & McGuire, M. A. (2006). Transfer of mouse embryonic stem cells to sheep myocardium. *Lancet 367*, 301–302.

Meier, C. R., Jick, S. S., Derby, L. E., Vasilakis, C., & Jick, H. (1998). Acute respiratory-tract infections and risk of first-time acute myocardial infarction. *Lancet 351*, 1467–1471.

Merenyi, G., Lind, J., Goldstein, S., & Czapski, G. (1998). Peroxynitrous acid homolyzes into *OH and *NO₂ radicals. *Chemical Research in Toxicology 11*, 712–713.

Michel, T. M., Pulschen, D., & Thome, J. (2012). The role of oxidative stress in depressive disorders. *Current Pharmaceutical Design 18*, 5890–5899.

Mills, N. L., Tornqvist, H., Gonzalez, M. C., Vink, E., Robinson, S. D., Soderberg, S., et al. (2007). Ischemic and thrombotic effects of dilute diesel-exhaust inhalation in men with coronary heart disease. *The New England Journal of Medicine 357*, 1075–1082.

Mills, N. L., Tornqvist, H., Robinson, S. D., Gonzalez, M., Darnley, K., MacNee, W., et al. (2005). Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. *Circulation 112*, 3930–3936.

Miravitlles, M., Ferrer, M., Pont, A., Zalacain, R., Alvarez-Sala, J. L., Masa, F., et al. (2004). Effect of exacerbations on quality of life in patients with chronic obstructive pulmonary disease: a 2 year follow up study. *Thorax 59*, 387–395.

Miravitlles, M., Murio, C., Guerrero, T., Gisbert, R., & EPOC, D. S. G. D. s. a. y. F. e. l. (2002). Pharmacoeconomic evaluation of acute exacerbations of chronic bronchitis and COPD. *Chest 121*, 1449–1455.

Moncada, S., & Higgs, E. A. (2006). The discovery of nitric oxide and its role in vascular biology. *British Journal of Pharmacology 147*(Suppl. 1), S193–S201.

Montes de Oca, M., Torres, S. H., De Sanctis, J., Mata, A., Hernandez, N., & Talamo, C. (2005). Skeletal muscle inflammation and nitric oxide in patients with COPD. *The European Respiratory Journal 26*, 390–397.

Montezano, A. C., & Touyz, R. M. (2012). Reactive oxygen species and endothelial function—role of nitric oxide synthase uncoupling and Nox family nicotinamide adenine dinucleotide phosphate oxidases. *Basic & Clinical Pharmacology & Toxicology 110*, 87–94.

Montuschi, P., Collins, J. V., Ciabattoni, G., Lazzeri, N., Corradi, M., Kharitonov, S. A., et al. (2000). Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. *American Journal of Respiratory and Critical Care Medicine 162*, 1175–1177.

Mundy, G. R. (1993). Cytokines and growth factors in the regulation of bone remodeling. *Journal of Bone and Mineral Research 8*(Suppl. 2), S505–S510.

Munger, M. A. (2011). Use of angiotensin receptor blockers in cardiovascular protection: current evidence and future directions. *P T 36*, 22–40.

Myung, S. K., Ju, W., Cho, B., Oh, S. W., Park, S. M., Koo, B. K., et al. (2013). Efficacy of vitamin and antioxidant supplements in prevention of cardiovascular disease: systematic review and meta-analysis of randomised controlled trials. *BMJ 346*, f10.

Nagata, K., Obata, K., Odashima, M., Yamada, A., Somura, F., Nishizawa, T., et al. (2003). Nicorandil inhibits oxidative stress-induced apoptosis in cardiac myocytes through activation of mitochondrial ATP-sensitive potassium channels and a nitrate-like effect. *Journal of Molecular and Cellular Cardiology 35*, 1505–1512.

Naito, Y., Suematsu, M., & Yoshikawa, T. (2011). *Free Radical Biology in Digestive Diseases.* Karger Medical and Scientific Publishers.

Nakayama, T., Church, D. F., & Pryor, W. A. (1989). Quantitative analysis of the hydrogen peroxide formed in aqueous cigarette tar extracts. *Free Radical Biology & Medicine 7*, 9–15.

Nam, S. H., Jung, S. Y., Yoo, C. M., Ahn, E. H., & Suh, C. K. (2002). H₂O₂ enhances Ca²⁺ release from osteoblast internal stores. *Yonsei Medical Journal 43*, 229–235.

Neer, R. M., Arnaud, C. D., Zanchetta, J. R., Prince, R., Gaich, G. A., Reginster, J. Y., et al. (2001). Effect of parathyroid hormone (1–34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *The New England Journal of Medicine 344*, 1434–1441.

Neofytou, E., Tzortzaki, E. G., Chatziantoniou, A., & Siafakas, N. M. (2012). DNA damage due to oxidative stress in Chronic Obstructive Pulmonary Disease (COPD). *International Journal of Molecular Sciences 13*, 16853–16864.

Nguyen, T., Nioi, P., & Pickett, C. B. (2009). The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *The Journal of Biological Chemistry 284*, 13291–13295.

Nieto-Vazquez, I., Fernandez-Veledo, S., Kramer, D. K., Vila-Bedmar, R., Garcia-Guerra, L., & Lorenzo, M. (2008). Insulin resistance associated to obesity: the link TNF-alpha. *Archives of Physiology and Biochemistry 114*, 183–194.

Nojiri, T., Inoue, M., Yamamoto, K., Maeda, H., Takeuchi, Y., Nakagiri, T., et al. (2014). Inhaled tiotropium to prevent postoperative cardiopulmonary complications in patients with newly diagnosed chronic obstructive pulmonary disease requiring lung cancer surgery. *Surgery Today 44*, 285–290.

Onyia, J. E., Hale, L. V., Miles, R. R., Cain, R. L., Tu, Y., Hulman, J. F., et al. (1999). Molecular characterization of gene expression changes in ROS 17/2.8 cells cultured in diffusion chambers in vivo. *Calcified Tissue International 65*, 133–138.

Padhi, D., Jang, G., Stouch, B., Fang, L., & Posvar, E. (2011). Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. *Journal of Bone and Mineral Research 26*, 19–26.

Palmer, R. M., Ashton, D. S., & Moncada, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature 333*, 664–666.

Pastor, M. D., Nogal, A., Molina-Pinelo, S., Melendez, R., Romero-Romero, B., Mediano, M. D., et al. (2013). Identification of oxidative stress related proteins as biomarkers for lung cancer and chronic obstructive pulmonary disease in bronchoalveolar lavage. *International Journal of Molecular Sciences 14*, 3440–3455.

Patel, A. R., & Hurst, J. R. (2011). Extrapulmonary comorbidities in chronic obstructive pulmonary disease: state of the art. *Expert Review of Respiratory Medicine 5*, 647–662.

Paz-Elizur, T., Krupsky, M., Blumenstein, S., Elinger, D., Schechtman, E., & Livneh, Z. (2003). DNA repair activity for oxidative damage and risk of lung cancer. *Journal of the National Cancer Institute (1988) 95*, 1312–1319.

Polkey, M. I., & Moxham, J. (2006). Attacking the disease spiral in chronic obstructive pulmonary disease. *Clinical Medicine 6*, 190–196.

Polkey, M. I., & Moxham, J. (2011). Attacking the disease spiral in chronic obstructive pulmonary disease: an update. *Clinical Medicine 11*, 461–464.

Price, D., Yawn, B., Brusselle, G., & Rossi, A. (2013). Risk-to-benefit ratio of inhaled corticosteroids in patients with COPD. *Primary Care Respiratory Journal 22*, 92–100.

Pryor, W. A., & Stone, K. (1993). Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. *Annals of the New York Academy of Sciences 686*, 12–27 (discussion 27-18).

Qu, P., Roberts, J., Li, Y., Albrecht, M., Cummings, O. W., Eble, J. N., et al. (2009). Stat3 downstream genes serve as biomarkers in human lung carcinomas and chronic obstructive pulmonary disease. *Lung Cancer 63*, 341–347.

Rabe, K. F. (2007). Treating COPD—the TORCH trial, P values, and the Dodo. *The New England Journal of Medicine 356*, 851–854.

Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., et al. (2014). Oxidative stress, prooxidants, and antioxidants: the interplay. *Biomedical Research International 2014*, 761264.

Rahman, I. (2005). The role of oxidative stress in the pathogenesis of COPD: implications for therapy. *Treatments in Respiratory Medicine 4*, 175–200.

Rahman, I. (2012). Pharmacological antioxidant strategies as therapeutic interventions for COPD. *Biochimica et Biophysica Acta 1822*, 714–728.

Rahman, I., & Kinnula, V. L. (2012). Strategies to decrease ongoing oxidant burden in chronic obstructive pulmonary disease. *Expert Review of Clinical Pharmacology 5*, 293–309.

Rahman, I., & MacNee, W. (2012). Antioxidant pharmacological therapies for COPD. *Current Opinion in Pharmacology 12*, 256–265.

Rahman, I., Morrison, D., Donaldson, K., & MacNee, W. (1996). Systemic oxidative stress in asthma, COPD, and smokers. *American Journal of Respiratory and Critical Care Medicine 154*, 1055–1060.

Rahman, I., Skwarska, E., & MacNee, W. (1997). Attenuation of oxidant/antioxidant imbalance during treatment of exacerbations of chronic obstructive pulmonary disease. *Thorax 52*, 565–568.

Rahman, I., Swarska, E., Henry, M., Stolk, J., & MacNee, W. (2000). Is there any relationship between plasma antioxidant capacity and lung function in smokers and in patients with chronic obstructive pulmonary disease? *Thorax 55*, 189–193.

Rangasamy, T., Cho, C. Y., Thimmulappa, R. K., Zhen, L., Srisuma, S. S., Kensler, T. W., et al. (2004). Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. *The Journal of Clinical Investigation 114*, 1248–1259.

Rao, L. G., & Rao, V. M. (2013). *Oxidative Stress and Antioxidants in the Risk of Osteoporosis — Role of the Antioxidants Lycopene and Polyphenols.: InTech.*

Rasubala, L., Yoshikawa, H., Nagata, K., Iijima, T., & Ohishi, M. (2003). Platelet-derived growth factor and bone morphogenetic protein in the healing of mandibular fractures in rats. *The British Journal of Oral & Maxillofacial Surgery 41*, 173–178.

Raviv, S., Hawkins, K. A., DeCamp, M. M., Jr., & Kalhan, R. (2011). Lung cancer in chronic obstructive pulmonary disease: enhancing surgical options and outcomes. *American Journal of Respiratory and Critical Care Medicine 183*, 1138–1146.

Regan, E. A., Mazur, W., Meoni, E., Toljamo, T., Millar, J., Vuopala, K., et al. (2011). Smoking and COPD increase sputum levels of extracellular superoxide dismutase. *Free Radical Biology & Medicine 51*, 726–732.

Repine, J. E., Bast, A., & Lankhorst, I. (1997). Oxidative stress in chronic obstructive pulmonary disease. Oxidative Stress Study Group. *American Journal of Respiratory and Critical Care Medicine 156*, 341–357.

Rinaldi, M., Maes, K., De Vleeschauwer, S., Thomas, D., Verbeken, E. K., Decramer, M., et al. (2012). Long-term nose-only cigarette smoke exposure induces emphysema and mild skeletal muscle dysfunction in mice. *Disease Models & Mechanisms 5*, 333–341.

Roberts, C. M., Lowe, D., Bucknall, C. E., Ryland, I., Kelly, Y., & Pearson, M. G. (2002). Clinical audit indicators of outcome following admission to hospital with acute exacerbation of chronic obstructive pulmonary disease. *Thorax 57*, 137–141.

Sabit, R., Bolton, C. E., Edwards, P. H., Pettit, R. J., Evans, W. D., McEniery, C. M., et al. (2007). Arterial stiffness and osteoporosis in chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine 175*, 1259–1265.

Sanchez-Rodriguez, M. A., Ruiz-Ramos, M., Correa-Munoz, E., & Mendoza-Nunez, V. M. (2007). Oxidative stress as a risk factor for osteoporosis in elderly Mexicans as characterized by antioxidant enzymes. *BMC Musculoskeletal Disorders 8*, 124.

Santos, M. C., Oliveira, A. L., Viegas-Crespo, A. M., Vicente, L., Barreiros, A., Monteiro, P., et al. (2004). Systemic markers of the redox balance in chronic obstructive pulmonary disease. *Biomarkers 9*, 461–469.

Santos, S., Peinado, V. I., Ramirez, J., Melgosa, T., Roca, J., Rodriguez-Roisin, R., et al. (2002). Characterization of pulmonary vascular remodelling in smokers and patients with mild COPD. *The European Respiratory Journal 19*, 632–638.

Schnabel, R., & Blankenberg, S. (2007). Oxidative stress in cardiovascular disease: successful translation from bench to bedside? *Circulation 116*, 1338–1340.

Schols, A. M., Slangen, J., Volovics, L., & Wouters, E. F. (1998). Weight loss is a reversible factor in the prognosis of chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine 157*, 1791–1797.

Seemungal, T. A., Hurst, J. R., & Wedzicha, J. A. (2009). Exacerbation rate, health status and mortality in COPD—a review of potential interventions. *International Journal of Chronic Obstructive Pulmonary Disease 4*, 203–223.

Sekine, Y., Fujisawa, T., Suzuki, K., Tsutatani, S., Kubota, K., Ikegami, H., et al. (2014). Detection of chronic obstructive pulmonary disease in community-based annual lung cancer screening: Chiba Chronic Obstructive Pulmonary Disease Lung Cancer Screening Study Group. *Respirology 19*, 98–104.

Sekine, Y., Katsura, H., Koh, E., Hiroshima, K., & Fujisawa, T. (2012). Early detection of COPD is important for lung cancer surveillance. *The European Respiratory Journal 39*, 1230–1240.

Selemidis, S., Sobey, C. G., Wingler, K., Schmidt, H. H., & Drummond, G. R. (2008). NADPH oxidases in the vasculature: molecular features, roles in disease and pharmacological inhibition. *Pharmacology & Therapeutics 120*, 254–291.

Semenza, G. L. (2009). Regulation of oxygen homeostasis by hypoxia-inducible factor 1. *Physiology (Bethesda) 24*, 97–106.

Seymour, J. M., Spruit, M. A., Hopkinson, N. S., Natanek, S. A., Man, W. D., Jackson, A., et al. (2010). The prevalence of quadriceps weakness in COPD and the relationship with disease severity. *The European Respiratory Journal 36*, 81–88.

Shacter, E. (2000). Quantification and significance of protein oxidation in biological samples. *Drug Metabolism Reviews 32*, 307–326.

Sharpe, M. A., Ollosson, R., Stewart, V. C., & Clark, J. B. (2002). Oxidation of nitric oxide by oxomanganese–salen complexes: a new mechanism for cellular protection by superoxide dismutase/catalase mimetics. *The Biochemical Journal 366*, 97–107.

Shaten, B. J., Kuller, L. H., Kjelsberg, M. O., Stamler, J., Ockene, J. K., Cutler, J. A., et al. (1997). Lung cancer mortality after 16 years in MRFIT participants in intervention and usual-care groups. Multiple Risk Factor Intervention Trial. *Annals of Epidemiology 7*, 125–136.

Sidney, S., Sorel, M., Quesenberry, C. P., Jr., DeLuise, C., Lanes, S., & Eisner, M. D. (2005). COPD and incident cardiovascular disease hospitalizations and mortality: Kaiser Permanente Medical Care Program. *Chest 128*, 2068–2075.

Silverman, S. L. (1992). The clinical consequences of vertebral compression fracture. *Bone 13*(Suppl. 2), S27–S31.

Sin, D. D., Anthonisen, N. R., Soriano, J. B., & Agusti, A. G. (2006). Mortality in COPD: role of comorbidities. *The European Respiratory Journal 28*, 1245–1257.

Singal, P. K., Khaper, N., Palace, V., & Kumar, D. (1998). The role of oxidative stress in the genesis of heart disease. *Cardiovascular Research 40*, 426–432.

Singh, A., Rangasamy, T., Thimmulappa, R. K., Lee, H., Osburn, W. O., Brigelius-Flohe, R., et al. (2006). Glutathione peroxidase 2, the major cigarette smoke-inducible isoform of GPX in lungs, is regulated by Nrf2. *American Journal of Respiratory Cell and Molecular Biology 35*, 639–650.

Smith, K. R., Uyeminami, D. L., Kodavanti, U. P., Crapo, J. D., Chang, L. Y., & Pinkerton, K. E. (2002). Inhibition of tobacco smoke-induced lung inflammation by a catalytic antioxidant. *Free Radical Biology & Medicine 33*, 1106–1114.

Song, Q., Christiani, D. C., XiaorongWang, & Ren, J. (2014). The global contribution of outdoor air pollution to the incidence, prevalence, mortality and hospital admission for chronic obstructive pulmonary disease: a systematic review and meta-analysis. *International Journal of Environmental Research and Public Health 11*, 11822–11832.

Sorheim, I. C., DeMeo, D. L., Washko, G., Litonjua, A., Sparrow, D., Bowler, R., et al. (2010). Polymorphisms in the superoxide dismutase-3 gene are associated with emphysema in COPD. *COPD 7*, 262–268.

Sporn, M. B., & Liby, K. T. (2012). NRF2 and cancer: the good, the bad and the importance of context. *Nature Reviews. Cancer 12*, 564–571.

Spruit, M. A., Gosselink, R., Troosters, T., Kasran, A., Gayan-Ramirez, G., Bogaerts, P., et al. (2003). Muscle force during an acute exacerbation in hospitalised patients with COPD and its relationship with CXCL8 and IGF-I. *Thorax 58*, 752–756.

Stanojkovic, I., Kotur-Stevuljevic, J., Milenkovic, B., Spasic, S., Vujic, T., Stefanovic, A., et al. (2011). Pulmonary function, oxidative stress and inflammatory markers in severe COPD exacerbation. *Respiratory Medicine 105*(Suppl. 1), S31–S37.

Stanojkovic, I., Kotur-Stevuljevic, J., Spasic, S., Milenkovic, B., Vujic, T., Stefanovic, A., et al. (2013). Relationship between bone resorption, oxidative stress and inflammation in severe COPD exacerbation. *Clinical Biochemistry 46*, 1678–1682.

Steer, J., Gibson, G. J., & Bourke, S. C. (2010). Predicting outcomes following hospitalization for acute exacerbations of COPD. *QJM 103*, 817–829.

Stefanelli, F., Meoli, I., Cobuccio, R., Curcio, C., Amore, D., Casazza, D., et al. (2013). High-intensity training and cardiopulmonary exercise testing in patients with chronic obstructive pulmonary disease and non-small-cell lung cancer undergoing lobectomy. *European Journal of Cardio-Thoracic Surgery 44*, e260–e265.

Stefanska, J., Sarniak, A., Wlodarczyk, A., Sokolowska, M., Doniec, Z., Bialasiewicz, P., et al. (2012). Hydrogen peroxide and nitrite reduction in exhaled breath condensate of COPD patients. *Pulmonary Pharmacology & Therapeutics 25*, 343–348.

Steinbeck, M. J., Appel, W. H., Jr., Verhoeven, A. J., & Karnovsky, M. J. (1994). NADPH-oxidase expression and in situ production of superoxide by osteoclasts actively resorbing bone. *The Journal of Cell Biology 126*, 765–772.

Steinbeck, M. J., Kim, J. K., Trudeau, M. J., Hauschka, P. V., & Karnovsky, M. J. (1998). Involvement of hydrogen peroxide in the differentiation of clonal HD-11EM cells into osteoclast-like cells. *Journal of Cellular Physiology 176*, 574–587.

Stone, A. C., Machan, J. T., Mazer, J., Casserly, B., & Klinger, J. R. (2011). Echocardiographic evidence of pulmonary hypertension is associated with increased 1-year mortality in patients admitted with chronic obstructive pulmonary disease. *Lung 189*, 207–212.

Sussan, T. E., Rangasamy, T., Blake, D. J., Malhotra, D., El-Haddad, H., Bedja, D., et al. (2009). Targeting Nrf2 with the triterpenoid CDDO-imidazolide attenuates cigarette smoke-induced emphysema and cardiac dysfunction in mice. *Proceedings of the National Academy of Sciences of the United States of America 106*, 250–255.

Swallow, E. B., Reyes, D., Hopkinson, N. S., Man, W. D., Porcher, R., Cetti, E. J., et al. (2007). Quadriceps strength predicts mortality in patients with moderate to severe chronic obstructive pulmonary disease. *Thorax 62*, 115–120.

Szabo, C., & Ohshima, H. (1997). DNA damage induced by peroxynitrite: subsequent biological effects. *Nitric Oxide 1*, 373–385.

Takata, S., Takigawa, N., Segawa, Y., Kubo, T., Ohashi, K., Kozuki, T., et al. (2012). STAT3 expression in activating EGFR-driven adenocarcinoma of the lung. *Lung Cancer 75*, 24–29.

Tang, E. H., & Vanhoutte, P. M. (2009). Prostanoids and reactive oxygen species: team players in endothelium-dependent contractions. *Pharmacology & Therapeutics 122*, 140–149.

Taraseviciene-Stewart, L., & Voelkel, N. F. (2012). Oxidative stress-induced antibodies to carbonyl-modified protein correlate with severity of chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine 185*, 1026 (author reply 1026–1027).

Tashkin, D. P., & Murray, R. P. (2009). Smoking cessation in chronic obstructive pulmonary disease. *Respiratory Medicine 103*, 963–974.

Terzano, C., Conti, V., Di Stefano, F., Petroianni, A., Ceccarelli, D., Graziani, E., et al. (2010). Comorbidity, hospitalization, and mortality in COPD: results from a longitudinal study. *Lung 188*, 321–329.

Theander, K., Hasselgren, M., Luhr, K., Eckerblad, J., Unosson, M., & Karlsson, I. (2014). Symptoms and impact of symptoms on function and health in patients with chronic obstructive pulmonary disease and chronic heart failure in primary health care. *International Journal of Chronic Obstructive Pulmonary Disease 9*, 785–794.

Thomas, R., & Sharifi, N. (2012). SOD mimetics: a novel class of androgen receptor inhibitors that suppresses castration-resistant growth of prostate cancer. *Molecular Cancer Therapeutics 11*, 87–97.

Triggle, C. R., Samuel, S. M., Ravishankar, S., Marei, I., Arunachalam, G., & Ding, H. (2012). The endothelium: influencing vascular smooth muscle in many ways. *Canadian Journal of Physiology and Pharmacology 90*, 713–738.

Troosters, T., Gosselink, R., & Decramer, M. (2000). Short- and long-term effects of outpatient rehabilitation in patients with chronic obstructive pulmonary disease: a randomized trial. *The American Journal of Medicine 109*, 207–212.

Tsuchiya, M., Asada, A., Kasahara, E., Sato, E. F., Shindo, M., & Inoue, M. (2002). Smoking a single cigarette rapidly reduces combined concentrations of nitrate and nitrite and concentrations of antioxidants in plasma. *Circulation 105*, 1155–1157.

Tuder, R. M., Zhen, L., Cho, C. Y., Taraseviciene-Stewart, L., Kasahara, Y., Salvemini, D., et al. (2003). Oxidative stress and apoptosis interact and cause emphysema due to vascular endothelial growth factor receptor blockade. *American Journal of Respiratory Cell and Molecular Biology 29*, 88–97.

Vaguliene, N., Zemaitis, M., Lavinskiene, S., Miliauskas, S., & Sakalauskas, R. (2013). Local and systemic neutrophilic inflammation in patients with lung cancer and chronic obstructive pulmonary disease. *BMC Immunology 14*, 36.

Valavanidis, A., Vlachogianni, T., & Fiotakis, C. (2009a). 8-Hydroxy-2′-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. *Journal of Environmental Science and Health. Part C, Environmental Carcinogenesis & Ecotoxicology Reviews 27*, 120–139.

Valavanidis, A., Vlachogianni, T., & Fiotakis, K. (2009b). Tobacco smoke: involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles. *International Journal of Environmental Research and Public Health 6*, 445–462.

Valavanidis, A., Vlachogianni, T., Fiotakis, K., & Loridas, S. (2013). Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. *International Journal of Environmental Research and Public Health 10*, 3886–3907.

van den Borst, B., Koster, A., Yu, B., Gosker, H. R., Meibohm, B., Bauer, D. C., et al. (2011). Is age-related decline in lean mass and physical function accelerated by obstructive lung disease or smoking? *Thorax 66*, 961–969.

van der Toorn, M., Smit-de Vries, M. P., Slebos, D. J., de Bruin, H. G., Abello, N., van Oosterhout, A. J., et al. (2007). Cigarette smoke irreversibly modifies glutathione in airway epithelial cells. *American Journal of Physiology. Lung Cellular and Molecular Physiology 293*, L1156–L1162.

van Eeden, S. F., & Sin, D. D. (2013). Oxidative stress in chronic obstructive pulmonary disease: a lung and systemic process. *Canadian Respiratory Journal 20*, 27–29.

Van Helvoort, H. A., Heijdra, Y. F., Thijs, H. M., Vina, J., Wanten, G. J., & Dekhuijzen, P. N. (2006). Exercise-induced systemic effects in muscle-wasted patients with COPD. *Medicine and Science in Sports and Exercise 38*, 1543–1552.

Vanhoutte, P. M., Shimokawa, H., Tang, E. H., & Feletou, M. (2009). Endothelial dysfunction and vascular disease. *Acta Physiologica (Oxford, England) 196*, 193–222.

Varanasi, S. S., Francis, R. M., Berger, C. E., Papiha, S. S., & Datta, H. K. (1999). Mitochondrial DNA deletion associated oxidative stress and severe male osteoporosis. *Osteoporosis International 10*, 143–149.

Vestbo, J., Hurd, S. S., Agusti, A. G., Jones, P. W., Vogelmeier, C., Anzueto, A., et al. (2013). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *American Journal of Respiratory and Critical Care Medicine 187*, 347–365.

Vibhuti, A., Arif, E., Deepak, D., Singh, B., & Qadar Pasha, M. A. (2007). Correlation of oxidative status with BMI and lung function in COPD. *Clinical Biochemistry 40*, 958–963.

Vlahos, R., & Bozinovski, S. (2013). Glutathione peroxidase-1 as a novel therapeutic target for COPD. *Redox Report 18*, 142–149.

Vlahos, R., Stambas, J., Bozinovski, S., Broughton, B. R., Drummond, G. R., & Selemidis, S. (2011). Inhibition of Nox2 oxidase activity ameliorates influenza A virus-induced lung inflammation. *PLoS Pathogens 7* (e1001271).

Vlahos, R., Stambas, J., & Selemidis, S. (2012). Suppressing production of reactive oxygen species (ROS) for influenza A virus therapy. *Trends in Pharmacological Sciences 33*, 3–8.

Vogiatzi, G., Tousoulis, D., & Stefanadis, C. (2009). The role of oxidative stress in atherosclerosis. *Hellenic Journal of Cardiology 50*, 402–409.

Wang, Z. L. (2013). Association between chronic obstructive pulmonary disease and lung cancer: the missing link. *Chinese Medical Journal 126*, 154–165.

Weitzenblum, E. (1984). The structure and function of the pulmonary vasculature in mild chronic obstructive pulmonary disease. *The American Review of Respiratory Disease 129*, 1033.

WHO (2013). *Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11. vol. 2015*, . Lyon, France: International Agency for Research on Cancer.

Wilson, D. O., Weissfeld, J. L., Balkan, A., Schragin, J. G., Fuhrman, C. R., Fisher, S. N., et al. (2008). Association of radiographic emphysema and airflow obstruction with lung cancer. *American Journal of Respiratory and Critical Care Medicine 178*, 738–744.

Wink, D. A., Miranda, K. M., Espey, M. G., Pluta, R. M., Hewett, S. J., Colton, C., et al. (2001). Mechanisms of the antioxidant effects of nitric oxide. *Antioxidants & Redox Signaling 3*, 203–213.

Wong, C. H., Bozinovski, S., Hertzog, P. J., Hickey, M. J., & Crack, P. J. (2008). Absence of glutathione peroxidase-1 exacerbates cerebral ischemia–reperfusion injury by reducing post-ischemic microvascular perfusion. *Journal of Neurochemistry 107*, 241–252.

Wust, R.C., & Degens, H. (2007). Factors contributing to muscle wasting and dysfunction in COPD patients. *International Journal of Chronic Obstructive Pulmonary Disease 2*, 289–300.

Yamaguchi, T., Sano, K., Takakura, K., Saito, I., Shinohara, Y., Asano, T., et al. (1998). Ebselen in acute ischemic stroke: a placebo-controlled, double-blind clinical trial. Ebselen Study Group. *Stroke 29*, 12–17.

Yang, H. L., Zhao, L., Liu, J., Sanford, C. G., Jr., Chen, L., Tang, T., et al. (2007). Changes of pulmonary function for patients with osteoporotic vertebral compression fractures after kyphoplasty. *Journal of Spinal Disorders & Techniques 20*, 221–225.

Yao, H., Arunachalam, G., Hwang, J. W., Chung, S., Sundar, I. K., Kinnula, V. L., et al. (2010). Extracellular superoxide dismutase protects against pulmonary emphysema by attenuating oxidative fragmentation of ECM. *Proceedings of the National Academy of Sciences of the United States of America 107*, 15571–15576.

Yao, H., Edirisinghe, I., Yang, S. R., Rajendrasozhan, S., Kode, A., Caito, S., et al. (2008). Genetic ablation of NADPH oxidase enhances susceptibility to cigarette smoke-induced lung inflammation and emphysema in mice. *The American Journal of Pathology 172*, 1222–1237.

Yatmaz, S., Seow, H. J., Gualano, R. C., Wong, Z. X., Stambas, J., Selemidis, S., et al. (2013). Glutathione peroxidase-1 reduces influenza A virus-induced lung inflammation. *American Journal of Respiratory Cell and Molecular Biology 48*, 17–26.

Ye, Y., Li, J., & Yuan, Z. (2013). Effect of antioxidant vitamin supplementation on cardiovascular outcomes: a meta-analysis of randomized controlled trials. *PloS One 8* (e56803).

Yende, S., Waterer, G. W., Tolley, E. A., Newman, A. B., Bauer, D. C., Taaffe, D. R., et al. (2006). Inflammatory markers are associated with ventilatory limitation and muscle dysfunction in obstructive lung disease in well functioning elderly subjects. *Thorax 61*, 10–16.

Young, R. P., Hopkins, R. J., Christmas, T., Black, P. N., Metcalf, P., & Gamble, G. D. (2009). COPD prevalence is increased in lung cancer, independent of age, sex and smoking history. *The European Respiratory Journal 34*, 380–386.

Zampetaki, A., Dudek, K., & Mayr, M. (2013). Oxidative stress in atherosclerosis: the role of microRNAs in arterial remodeling. *Free Radical Biology & Medicine 64*, 69–77.

Zeng, M., Li, Y., Jiang, Y., Lu, G., Huang, X., & Guan, K. (2013). Local and systemic oxidative stress status in chronic obstructive pulmonary disease patients. *Canadian Respiratory Journal 20*, 35–41.

Zhai, R., Yu, X., Wei, Y., Su, L., & Christiani, D. C. (2014). Smoking and smoking cessation in relation to the development of co-existing non-small cell lung cancer with chronic obstructive pulmonary disease. *International Journal of Cancer 134*, 961–970.