Available online at www.sciencedirect.com
SCIENCE DIRECT
Pharmacology & Therapeutics 110 (2006) 71 – 82
Pharmacology & Therapeutics
www.elsevier.com/locate/pharmthera

Associate editor: A. Trifilieff

Aerobic capacity, oxidant stress, and chronic obstructive pulmonary disease—A new take on an old hypothesis

Christopher S. Stevenson [a,*], Lauren Gerard Koch [b], Steven L. Britton [b]

[a] Novartis Institutes for BioMedical Research, Respiratory Disease Area, Wimblehurst Road, Horsham, West Sussex RH12 5AB, UK
[b] Physical Medicine and Rehabilitation, University of Michigan, Ann Arbor, MI 48109, USA

Abstract

Chronic obstructive pulmonary disease (COPD) is a smoking-related disorder that is a leading cause of death worldwide. It is associated with an accelerated rate of age-related decline in lung function due to the occurrence of destructive pathological changes such as emphysema, small airway remodeling, and mucus hypersecretion. Smokers are exposed to trillions of radicals and thousands of reactive chemicals and particles with every cigarette, thus oxidant stress is believed to be a central factor in the pathogenesis of COPD. The molecular activities of radicals, reactive oxygen, and nitrogen species can, over time, lead to a number of the detrimental changes in the lung. For instance, smoke can directly damage the mitochondrion, an organelle that has long been linked to age-related diseases associated with oxidant stress. Mitochondria are involved in a number of important cellular processes and are the largest source of endogenous reactive oxygen species (ROS) in the cell; therefore, any impairment of mitochondrial function can lead to greater oxidant damage, cellular dysfunction, and eventually to disease. Only a subset of smokers (15–50%) develops COPD, suggesting that there are polygenetic and/or environmental susceptibility factors involved in this complex disease. Here, we propose that the aerobic capacity for an individual may determine whether one is susceptible to developing COPD. Aerobic capacity is a polygenetic trait closely associated with mitochondrial function, and we suggest antioxidant defenses. Thus, those smokers who have the greatest aerobic capacity will be most resistant to the effects of chronic cigarette smoke exposure and be less likely to develop COPD.

Keywords: Chronic obstructive pulmonary disease (COPD); Oxidant stress; Mitochondria; Smoking; Aerobic capacity

Abbreviations: COPD, chronic obstructive pulmonary disease; GSEA, gene set enrichment analysis; HCR, high capacity runners; LCR, low capacity runners; MET, metabolic equivalents; MRC, mitochondrial respiratory chain; mtDNA, mitochondrial DNA; ROS, reactive oxygen species; RNS, reactive nitrogen species.

Contents

* Corresponding author. Fax: +44 1403 323 307.
E-mail address: Christopher.Stevenson@pharma.novartis.com (C.S. Stevenson).

0163-7258/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pharmthera.2005.09.006

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a collective group of lung conditions that result in a significant loss of lung function. It is the fourth most common cause of chronic morbidity and mortality in the United States. As many as 120,000 Americans died from this disease in 2002. Approximately 80–90% of COPD deaths are coupled with a history of smoking. In 2002, another 11.2 million Americans were diagnosed with the disease while 24 million others had evidence of impaired lung function, indicating that this disease is underdiagnosed. The costs for COPD to the United States last year were approximated at $37.2 billion. In the year 2020, COPD is projected to be the third leading cause of death worldwide (American Lung Association, 2005).

COPD is defined by the Global Initiative on Obstructive Lung Disease criteria as "airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases" (Pauwels et al., 2001). The pathological changes that contribute to the loss in lung function are heterogeneous in nature consisting of parenchymal destruction (described clinically as emphysema), remodeling and thickening of the airways (described clinically as small airways disease), and mucus hypersecretion (described clinically as chronic bronchitis). These changes occur at every level of the airways. In the central airways, the main pathological changes are enlargement of the mucus glands, goblet cell metaplasia, epithelial thickening (hypertrophy and hyperplasia), ciliary dysfunction, smooth muscle hypertrophy, and subepithelial inflammation (neutrophils, macrophages, and $CD8^+$ T-cells). The peripheral airways have many of these same features as well as subepithelial fibrosis/matrix deposition that contribute to the fixed airway obstruction associated with the disease. In the parenchyma, destruction of the respiratory bronchioles (centrilobular emphysema), alveolar walls, and capillary beds affect lung mechanics and gas exchange capabilities (Pauwels et al., 2001; Hogg, 2004). These morphological changes occur as focal lesions in the tissue and may vary in degree between affected patients (Hogg, 2004). There are also systemic consequences associated with smoking that are observed in COPD patients. The most notable involves significant skeletal muscle alterations affecting both the mobility of patients and their ability to respire properly (Wouters et al., 2002; Barreiro et al., 2005).

Cigarette smoke is a major source of particles, free radicals, and reactive chemicals and gases, all of which can produce an overwhelming oxidant burden on the lungs that are thought to play a central role in the pathogenesis of COPD. The molecular effect of these highly reactive molecules can explain a number of the pathologies observed in COPD patients—namely, inflammation, emphysema, airway fibrosis, mucus hypersecretion, and skeletal muscle wasting. However, only 15–50% of smokers are diagnosed with COPD; therefore, there must be genetic and/or environmental factors that play a role in determining susceptibility (Lundback et al., 2003). Because oxidant stress is believed by many to be central to the disease process, it has been suggested that differences in individual antioxidant defenses may hold the key to understanding COPD susceptibility, but no studies have demonstrated a clear link or plausible mechanism for this deficiency. We propose that smokers' aerobic capacity—that is, a complex, polygenetic trait closely associated with mitochondrial function and antioxidant capacity—may determine whether they are susceptible to developing COPD.

2. Hypothesis

Complex diseases, like COPD, result from a diversity of genetic, developmental, and environmental factors. The vast majority of COPD patients (80–90%) share chronic cigarette smoke exposure as a common (and primary) environmental etiological factor. The individual genetic profiles of these patients are likely to be very different, comprised of multiple allelic variations across a number of different genes. However, we propose that their genetic profiles also share at least one common phenotypic outcome—a deficit in their overall aerobic capacity.

Two billion years of evolution in an oxygen-rich environment determined that oxygen metabolism is a central feature of multicellular organisms (Des Marais, 2000). It appears that evolution followed the increased free energy transfer afforded by the widened redox potential when oxygen is the final electron acceptor in oxidation reactions (Baldwin & Krebs, 1981). Obligatory for using oxygen in energy transfer pathways was the simultaneous co-evolution of enzymes that detoxify the reactive species formed as by-products. Thus, pathways that mediate both oxidation reactions and oxygen detoxification reactions constitute a large part of our biology (Myers et al., 2002; Young & Woodside, 2001).

These ideas about evolution are also consistent with the free radical theory of aging, which proposes that aging is a function of metabolic rate (Harman, 1956). Age-related pathological changes and declines in physiological functions are believed to result from a lifetime of free radical and oxidative damage to essential cellular components and macromolecules like nucleic acids, lipids, and proteins. These radicals and oxygen

metabolites are derived from both exogenous (environmental pollutants, radiation, etc.) or endogenous sources (cell metabolism/mitochondrial function). An individual's metabolic capacity is determined clinically by measuring whole-body aerobic capacity. Therefore, in combination, these ideas imply that those individuals with higher current aerobic function will have greater antioxidant and metabolic capacities to deal with the stresses associated with life, including environmental stresses such as chronic cigarette smoke exposure.

Our hypothesis proposes that smokers susceptible to developing COPD have a genetic profile that results in an attenuated aerobic capacity and therefore deficiencies in their antioxidant defenses and mitochondrial functions. With a diminished ability to metabolize and detoxify oxygen, they are left more susceptible to the oxidant burden associated with cigarette smoking that progressively worsens with age. We will provide information in support of this hypothesis by reviewing (a) how oxidant stress can drive COPD progression, (b) the importance of mitochondria and how smoke affects this organelle's function, and (c) evidence that individual aerobic capacity may be the major determinant in the continuum between health and disease.

3. Smoking and oxidant stress

Smoking is the main etiological factor in the development of COPD (U.S. Department of Health and Human Services, 2004). Cigarette smoke contains the equivalent of 10^{17} radicals per gram in the tar phase of smoke (the approximate daily intake of a pack-a-day smoker) and 10^{15} radicals per puff in the gas phase (Church & Pryor, 1985). Reactive oxygen species (ROS), reactive nitrogen species (RNS), and carbon-centered radicals are constituents in both the tar and gas phases of smoke and more can be readily produced by the reactive compounds present in smoke (olefins and dienes). Many of the radicals from the tar fraction of cigarette smoke are stable, water soluble, and can form ROS by reducing oxygen to form superoxide anion (O_2^-), which can dismutate to H_2O_2. Furthermore, tar can also chelate divalent iron and copper ions which can in turn produce damaging hydroxyl radicals (OH·) via the Fenton reaction (Church & Pryor, 1985) (Fig. 1). In contrast, the organic radicals present in the gas phase of smoke have short half-lives (<1 sec). However, radical concentrations in the gas phase are maintained at high levels for more than 10 min (Church & Pryor, 1985). To explain this anomaly, it has been suggested that these radicals exist in a steady state in which they are continuously formed and destroyed (see Pryor et al., 1983).

In addition, there are also endogenous sources of these ROS (O_2^-, H_2O_2, and OH·) such as those produced by activated leukocytes recruited to the lungs in response to smoke (generated by the NADPH oxidase system). Myeloperoxidase in neutrophils and macrophages can catalyze a reaction between H_2O_2 and chloride to generate highly reactive and destructive hyperchlorous acid. In addition, cells can produce ROS during arachidonic acid metabolism and as by-products of the mitochondrial respiratory chain (MRC), the largest source of intracellular ROS.

The radicals, ROS, and RNS derived from cigarette smoke and endogenous sources can alter and damage lipids, proteins, and nucleic acids, which in some cases leads to the formation of more stable reactive molecules, such as 4-hydroxy-nonenal with half-lives that last for minutes rather than seconds. Thus, their presence prolongs the oxidant-mediated damage initiated by smoke inhalation, forms additional ROS and a chain reaction of oxidant-mediated damage results (Frei et al., 1991; Halliwell & Chirico, 1993).

4. Oxidant stress and chronic obstructive pulmonary disease

The proposal of the oxidant/antioxidant imbalance hypothesis for COPD is based on evidence for an increased level of

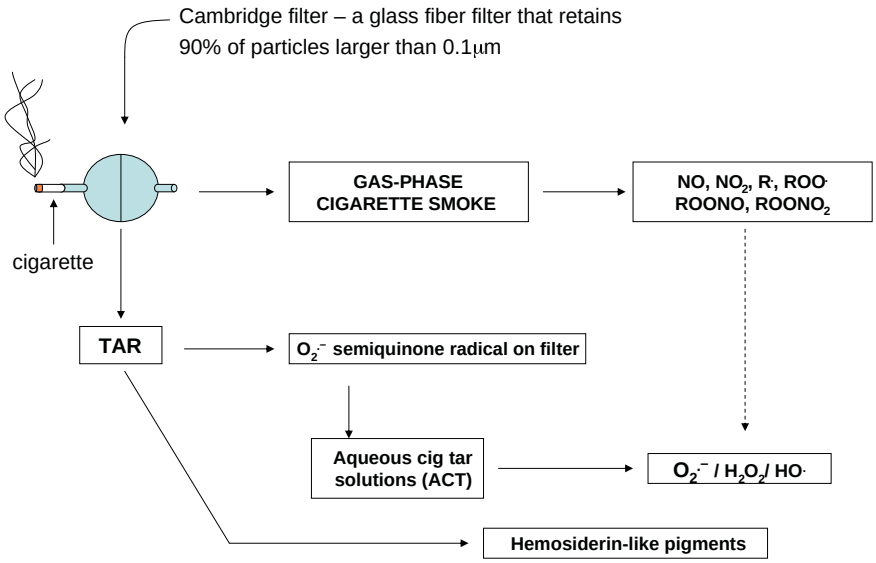

Fig. 1. Illustrates the oxidant chemistry in relation to cigarette smoke constituents (from Pryor & Stone, 1993, with permission).

oxidant damage, both locally and systemically, in COPD patients compared to healthy smokers (Taylor et al., 1986; Rahman & MacNee, 1996; Repine et al., 1997). Montuschi et al. (2000) measured 8-isoprostane, a marker of free radical-induced lipid peroxidation of arachidonic acid, in the exhaled breath of the following: (1) healthy subjects, (2) healthy smokers, (3) COPD patients who are ex-smokers, and (4) COPD patients who are current smokers. This evaluation showed that while smoking alone will induce oxidant damage, there are increased levels of oxidative stress in COPD patients (both smokers and ex-smokers) compared to both sets of healthy controls (Fig. 2) (Montuschi et al., 2000). Other groups have demonstrated increased oxidant damage in the lungs of both smokers and COPD patients by measuring exhaled ethane and H_2O_2 levels or systemically by measuring plasma and urinary isoprostane-2F-α levels (Morrow et al., 1995; Pratico et al., 1998; Paredi et al., 2000a, 2000b). More recently, Montes de Oca et al. (2004) reported a correlation between markers of oxidative stress and the BODE index, a multi-dimensional staging index that includes measures of body mass index, airflow obstruction, dyspnea, and exercise performance. Moreover, the molecular actions of oxidants have been linked to most of the local and systemic pathological features associated with COPD such as inflammation, emphysema, airway remodeling, mucus hypersecretion, and skeletal muscle wasting.

4.1. Inflammation

In vitro studies have demonstrated that oxidants increase the activation of transcription factors NF-κB and AP-1 (Schreck et al., 1992; Meyer et al., 1993; Lo & Cruz, 1995; Puri et al., 1995). Cigarette smoke has also been shown to impair the function of histone deacetylaces, resulting in a prolonged duration of transcription (Marwick et al., 2004; Barnes et al., 2005). In combination, the activation of transcription factors and inactivation of histone deacetylase can result in the increased expression of inflammatory genes like tumor necrosis factor-α, interleukin-1, and interleukin-8 from macrophages and alveolar/bronchial epithelial cells (reviewed in Rahman, 2003, and Rahman et al., 2002). There is also evidence that smoke can increase neutrophil sequestration/adherence and

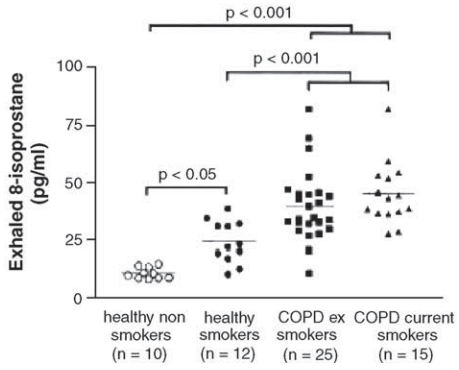

Fig. 2. Exhaled breath condensate levels of 8-isoprostane from healthy non-smokers, smokers, and COPD patients (current and ex-smokers) (from Montuschi et al., 2000, with permission).

macrophage adherence in the lung through oxidant-mediated mechanisms. This enhances the duration over which they are able to release additional mediators in the lung and blood stream and may also affect their ability to function properly (i.e., phagocytosis) (Selby et al., 1991; MacNee & Selby, 1993; Kirkham et al., 2003). As such, these may be critical molecular mechanisms that help orchestrate the "abnormal" inflammatory response associated with COPD.

4.2. Emphysema

Oxidant stress is also believed to be at the center of the pathogenesis of emphysema by potentiating proteolytic damage, inducing cell death and inhibiting lung repair mechanisms. Proteases produced by infiltrating leukocytes and resident cells can cleave the matrix, specifically the elastin fibers that are vital for lung mechanics. It is thought that these enzymes become toxic when their endogenous inhibitors are inactivated by oxidants, allowing proteases to destroy the structural integrity of the lung (Abboud et al., 1983; Janoff et al., 1984). In addition, cigarette smoke and related oxidants have been shown to induce endothelial and epithelial cell death through activation of the mitochondrial pathway of apoptosis (Tuder et al., 2000; Wang et al., 2001; Carnevali et al., 2003; Voelkel & Cool, 2003). It has also been demonstrated that oxidants can interfere with fibroblast repair processes essential for restoring the correct architecture in the lung after injury (Kim et al., 2002). The combined effect of enhanced proteolytic damage, increased cell death, and decreased lung repair leads to the destroyed the nature of the emphysematous lung.

4.3. Small airways disease

Airway fibrosis and smooth muscle hypertrophy are typical pathological consequences of oxidant exposure (Chitano et al., 1995). ROS are critical signaling molecules in pathways regulating cell proliferation and smooth muscle hypertrophy and hyperplasia (Rhee, 1999). Superoxide and H_2O_2, in particular, are involved in growth factor-induced proliferative signaling pathways by inhibiting tyrosine phosphatase activity and prolonging the kinase activation signal (Callsen et al., 1999). They are also involved in angiotensin II and serotonin-mediated smooth muscle hypertrophy and hyperplasia signaling pathways by activating mitogen activated protein kinases, p38 and ERK (Puri et al., 1995; Ushio-Fukai et al., 1998; Lee et al., 1999). Tumor growth factor-β1 is a cytokine believed to contribute to airway remodeling in a number of ways including stimulating the production of connective tissue matrix proteins (Hogg, 2004). It, too, uses ROS as signaling molecules in the pathways which mediate these effects (Thannickal & Fanburg, 2000; Chapman, 2004). In addition, a number of oxidant-driven in vivo models have demonstrated that fibrosis is a primary pathological consequence of their actions (Chitano et al., 1995; Manoury et al., 2005). Therefore, these cellular activities may contribute to the remodeling and thickening of the airways observed in COPD patients.

4.4. Chronic bronchitis—mucus hypersecretion

Mucin is a glycoprotein with a structure rich in sulfhydryl and disulfide moieties. Thus, it can act as an effective scavenging antioxidant and is secreted by goblet cells in the epithelium as a protective response to a variety of insults including cigarette smoke. However, the mucus secretory and mucociliary clearance apparatuses in the lung are also affected by oxidants. Cigarette smoke and related oxidants have been shown to induce mucus hypersecretion in a variety of in vitro and in vivo modeling systems (Jeffery et al., 1982; Kreindler et al., 2005; Stevenson et al., 2005). It has been suggested that mucus production may be governed in part by epidermal growth factor, which uses ROS as signaling molecules in its activation pathways (Sundaresan et al., 1995; Halvey et al., 2005). In addition, smoke can also impair mucociliary clearance through oxidant-mediated mechanisms leading to damaged ciliary motility and attenuated fluid secretion (Welsh, 1983; Feldman et al., 1994; Verra et al., 1995; Kreindler et al., 2005). The result is excessive mucus production in the airway that cannot be moved due to a combination of malfunctioning cilia and mucus that may not be properly hydrated, thus forming plaques. This mucus hypersecretory phenotype (similar in many ways to cystic fibrosis) leaves portions of the lung obstructed with mucus plugs (possibly affecting airflow) and susceptible to bacterial colonization.

4.5. Chronic obstructive pulmonary disease and skeletal muscle dysfunction

Skeletal muscle wasting is a prominent systemic feature in COPD patients that can influence symptoms and prognosis. Here too, oxidant stress is believed to play a key role by stimulating muscle proteolysis and causing myocyte cell apoptosis (Stangel et al., 1996; Gosker et al., 2000; Panda et al., 2001). Oxidant-mediated carbonyl modifications to muscle proteins can make them more susceptible to proteolytic degradation (Nagasawa et al., 1997; Panda et al., 2001). Oxidant activation of Nf-κB may also serve to inhibit myogenic differentiation preventing the regeneration of damaged tissue (Guttridge et al., 2000). In addition, oxidant stress can disrupt the mitochondrial respiratory chain (MRC), disturbing the bioenergetic requirements of the muscle, and causing a greater oxidant burden (Haycock et al., 1996).

5. Antioxidant capacity and chronic obstructive pulmonary disease

It is clear that the molecular effects of ROS and RNS can lead to a number of the pathological and physiological changes that comprise COPD (Fig. 3). In addition, clinical studies have demonstrated that oxidant damage is greater in COPD patients compared to healthy smokers. However, questions remain with respect to the role that antioxidant capacity plays in the disease process. First, is antioxidant capacity important for protection from these cigarette smoke-induced pathologies? And second, is there evidence for decreased antioxidant capacity in COPD patients? In vivo data suggest that antioxidant capacity is important for protection from cigarette smoke. The transcription factor Nrf2 controls the transcription of a number of antioxidant enzymes. Nrf2 knockout mice have a deficient antioxidant response and compared to wild type develop a greater degree of inflammation and emphysema after exposure to cigarette smoke or elastase (Ishii et al., 2004; Rangasamy et al., 2004). In addition, the catalytic antioxidant, AEOL 10150, can reduce the level of inflammation by cigarette smoke in rats (Smith et al., 2002). Both studies suggest increasing antioxidant capacity will lead to greater protection from damage elicited by cigarette smoke.

So do smokers who develop COPD have lower antioxidant capacity? In humans, association studies for “susceptibility genes ” in COPD patients show that there are associated allelic variants in antioxidant and xenobiotic metabolizing enzymes. Polymorphisms in microsomal epoxide hydrolase, heme oxygenase-1, alleles of glutathione-*S*-transferase, and cytochrome P450A1 have been identified in certain COPD

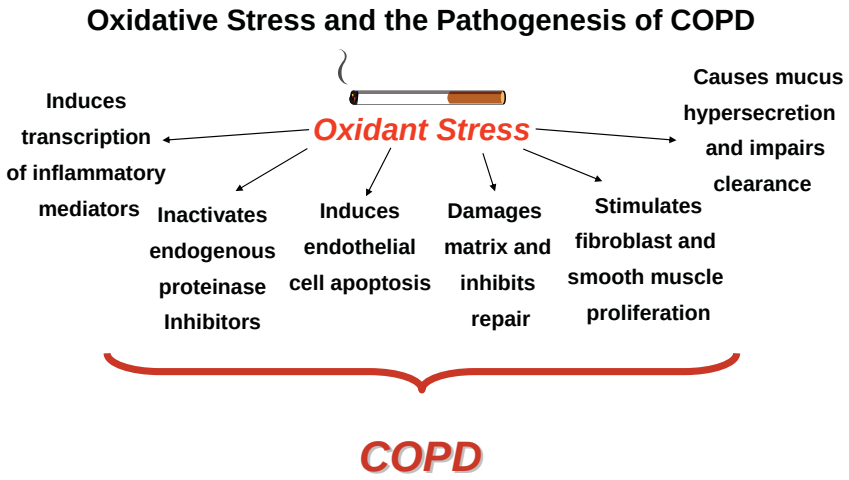

Fig. 3. ROS and RNS play important intracellular signaling roles in mediating a number of cellular processes. When their concentration exceeds a certain threshold, disrupting the redox balance, their effects can have detrimental cellular effects leading to a number of pathologies associated with COPD.

populations, although it is difficult to interpret these results. In some cases, the observations could not be repeated in different populations and the patients studied often had multiple co-morbidities (reviewed in Langen et al., 2003, and Molfino, 2004). Clinically, it has been demonstrated that COPD patients have lower anti-oxidant capacities in their plasma and skeletal muscle at rest (Sastre et al., 1992; Engelen et al., 2000). In addition, Rabinovich et al. (2001) found that COPD patients have reduced capacity to adapt their redox potential in response to exercise training to accommodate the added cellular demand for O_2 consumption and transport during exercise.

6. The effects of smoking on mitochondrial function

The observation that COPD patients have less of a change in redox potential in response to exercise training has interesting implications for mitochondrial function, which is related to both O_2 consumption, and ROS generation during exercise (Rabinovich et al., 2001). Inefficient mitochondrial function is known to lead to added oxidant stress levels; therefore, mitochondrial dysfunction may play a direct role in the development of COPD.

6.1. Mitochondrial functions

The mitochondria are often referred to as the powerhouse of the cell because it is where 2 key enzymatic processes occur – the citric acid cycle and the mitochondrial respiratory chain (MRC) – resulting in the production of adenosine triphosphate (ATP). To review these processes in detail is beyond the scope of this review, but will be summarized very briefly (for more extensive reviews, see Duchen, 1999, 2004). In the citric acid cycle, acetyl CoA (derived from the breakdown of pyruvate, fatty acids, or amino acids) is broken down to CO_2 and the reducing equivalents NADH and $FADH_2$. In the MRC, electrons from these reducing equivalents are then transferred onto O_2 through a series of oxidation–reduction reactions to produce a molecule of H_2O and ATP. This has been the principle function attributed to mitochondria; however, the importance of this organelle to other important cellular functions is now just beginning to be understood. In addition to meeting the cell's energy requirements via oxidative phosphorylation, the mitochondria are centrally involved in calcium signaling, ion transport, apoptosis, and antioxidant protection (for more in depth descriptions, see Duchen, 2004). All of these processes appear to be closely linked together, making the mitochondria vital cellular sensors whose activities are fundamental for processing internal and external cell signals. Because they are involved in all these processes, it is clear that mitochondrial dysfunction can lead to enhanced oxidant damage, cellular dysfunction and, in some cases, eventually to disease (Duchen, 2004).

6.2. Smoking damages mitochondrial function

One common defect in mitochondrial function is electron leak – displacement of electrons from the MRC – leading to increased intracellular oxidant levels. This flaw is not a problem short-term, but over the years it is thought that these insults accumulate and link to the destructive pathological changes associated with the aging process, such as sarcopenia and emphysema—also 2 key features of COPD patients (Ladislas, 2000; Lane, 2003). To limit the amount of damage that can be done by aberrant function and subsequent ROS production, mitochondria have evolved with the ability to produce reducing molecules and house their own enzymatic antioxidant system (superoxide dismutase-2 and catalase). In addition to intracellular ROS, it has also been observed that extracellular ROS appear to specifically attack the mitochondria and that mitochondria are vital for their correct detoxification (Thorpe et al., 2004). Several groups have shown that cigarette smoking can damage mitochondria and the MRC. In studies using isolated platelets or isolated peripheral blood lymphocytes from healthy smokers and non-smokers, it was demonstrated that certain components of the MRC did not function properly in the cells of smokers resulting in an increased oxidant burden to those cells (Gairola & Aleem, 1974; Smith et al., 1993; Miro et al., 1999; Cardellach et al., 2003).

Smoke also causes mutations in mitochondrial DNA (mtDNA) (Ballinger et al., 1996; Fahn et al., 1998). The genes in mtDNA encode 13 of the over 100 known polypeptides involved in the MRC and some of the machinery needed to translate these proteins. mtDNA is less protected from oxidant damage and repaired less effectively compared to nuclear DNA. Mutations can damage mitochondrial functions, again a process implicated in the aging process and common to those suffering from primary mitochondrial disorders (Wei & Lee, 2002). Fahn et al. (1998) have demonstrated that chronic smoking can cause large-scale deletions of mtDNA (4389 bp deletion). They reported that this deletion correlated to years of smoking, the lipid peroxide content of lung tissue, and FEV_1/FVC ratio, suggesting a potential link between mitochondrial dysfunction and COPD (Fahn et al., 1998).

6.3. Consequences of cigarette smoke-mediated mitochondrial damage

Smoke-induced alterations in mitochondrial function could have a substantial impact on cellular stability and function—that is, lead to the impaired function of immune cells, decreased muscle strength, etc. It has been demonstrated that smoke can disrupt mitochondrial membrane depolarization in monocytes and macrophages in vitro and in vivo and this affects mitochondrial control of apoptosis after DNA damage (Banzet et al., 1999; Aoshiba et al., 2001). Miro et al. (1999) showed that the activity of complex IV of the MRC is decreased in the lymphocytes of smokers leading to an unbalanced respiratory chain and greater ROS generation. These changes correlated with increases in lipid peroxidation of the plasma membranes of the cells from smokers, disrupting the stability of these barriers (Miro et al., 1999). Other groups have shown that cigarette smoke can alter mitochondrial function in endothelial cells by disrupting mitochondrial

membrane potential, leading to endothelial cell apoptosis and necrosis (Vayssier-Taussat et al., 2001; Yang & Liu, 2004). In addition, it has been established that the mitochondria in the peripheral skeletal muscle of COPD patients do not function as efficiently as mitochondria from healthy patients (Maltais et al., 1996). The resulting energetic deficiency leaves their muscles weaker and the patients are less mobile, which also associates with a poor prognosis for survival. Whether these changes in the muscle are a direct result of smoke exposure, a combination of inactivity and systemic hypoxia or due to an innate deficiency is difficult to determine. Furthermore, it appears that peripheral defects in oxygen utilization may also contribute (Antonucci et al., 2003). At the least, these observations suggest that mitochondrial function is indeed being altered and may contribute to the disease process of COPD.

6.4. Mitochondrial function, smoking, and the aging process

The adverse pathological consequences of the mitochondrial dysfunction due to chronic smoking could take a substantial length of time to develop. Each cell contains several hundred to more than a thousand mitochondria depending on cell type and function. Each mitochondrion contains 2–10 copies of mtDNA with both wild-type and mutant mtDNA co-existing (heteroplasmy). The degree to which these mutations occur varies in different cells in the same individual. As one ages, mtDNA mutations accumulate causing mitochondrial dysfunction and a decline in antioxidant capacity (Wei & Lee, 2002). These changes also correlate with a decline in aerobic capacity (Short et al., 2005). The added insult of chronic heavy cigarette smoking may accelerate these mutational processes and advance the decline of normal physiological functions. This could be why smoking appears to accelerate the aging process (Fig. 4). This idea is supported by the findings of Lundback et al. (2003) who found that as many as 50% of elderly smokers develop COPD. They suggest that the 2 most important factors in the development of COPD are smoking history and age (Lundback et al., 2003). The idea of accelerated aging process within the development of COPD was also put forth by Fahn et al. These investigators demonstrated that smoking and large-

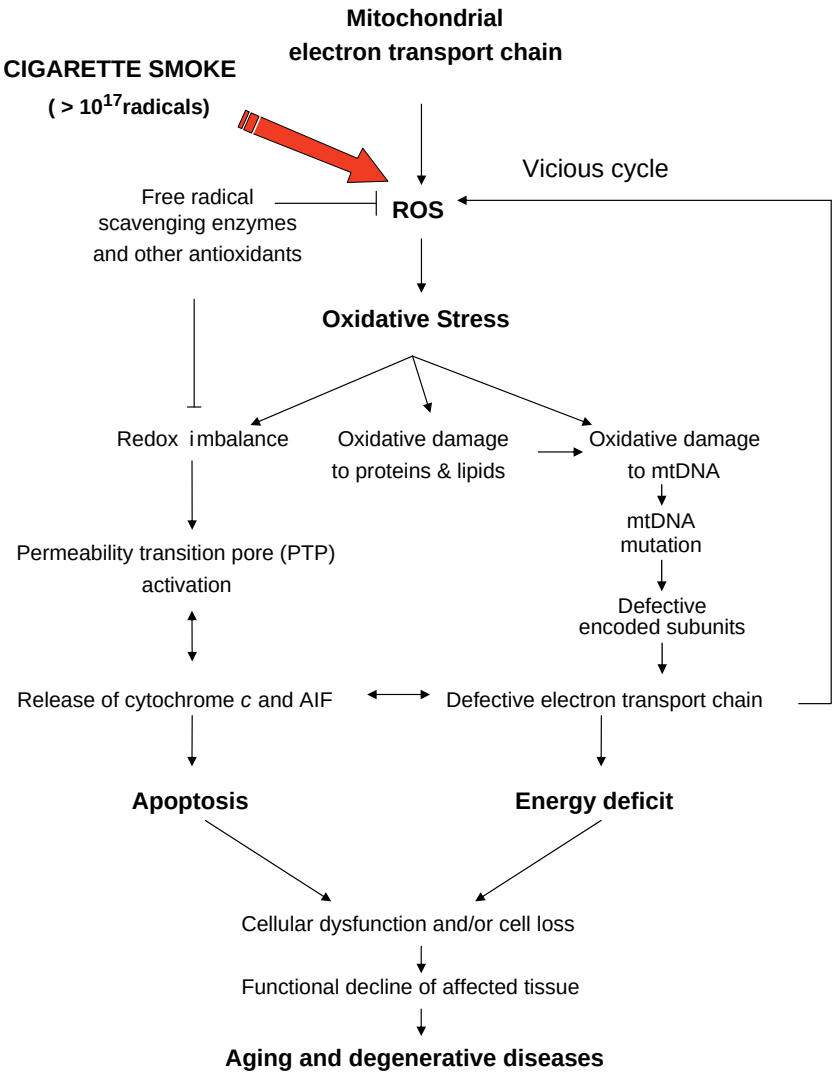

Fig. 4. Mitochondrial dysfunction and cigarette smoke (ROS generation) contribute to the aging process and chronic degenerative diseases associated with the aging process (adapted from Wei & Lee, 2002, with permission).

scale mtDNA deletions associate with a decline in lung function and age (Fahn et al., 1998). But if cause were simply smoke damaging mitochondrial function, all heavy smokers should get COPD.

7. The etiology of chronic obstructive pulmonary disease

The critical question remains: Is mitochondrial dysfunction the underlying causative feature in COPD? Once believed to be uncommon, deficiencies in mitochondrial function are associated with a number of multi-system disorders including degenerative diseases like Alzheimer's disease, neuromuscular diseases, type II diabetes, cardiovascular disease, and some cancers (Houten & Auwerx, 2004). These disorders are adult-onset, their prevalence increases with age, and smoking has been implicated as an etiological risk factor in most.

7.1. Low aerobic capacity and disease

In recent work, Britton et al. (Koch & Britton, 2001; Wisloff et al., 2005) proposed that the strong statistical association of high aerobic capacity and health suggests a link between impaired oxygen metabolism and disease. They hypothesized that artificial selection of rats based on low and high intrinsic aerobic exercise capacity would yield models that also contrast for disease risk. After 11 generations of selective breeding, rats bred for low aerobic capacity scored high on cardiovascular risk factors that constitute the metabolic syndrome (including insulin resistance, low heart function, and increased blood glucose, blood lipids, and visceral adiposity). The decrease in aerobic capacity was also associated with low levels of transcription factors required for mitochondrial biogenesis and oxidative enzymes in skeletal muscle. These results are consistent with the idea that impairment of mitochondrial function may link reduced aerobic capacity to cardiovascular and metabolic disease.

This hypothesis is also consistent with 2 informative clinical studies. First, Myers et al. (2002) found that age-predicted exercise capacity was the most powerful predictor of mortality for men suffering from cardiovascular disease (Fig. 5). They demonstrated that lower exercise/aerobic capacity correlated with increased risk of death in several chronic diseases including type II diabetes and COPD. The risk of death for those with an exercise capacity of <5 metabolic equivalent (MET; 1 MET is oxygen consumption at rest) was about double that compared to those whose exercise capacity was >8 MET. In general, it appears that each 1 MET improvement in aerobic capacity is associated with a 12% improvement in survival (Myers et al., 2002). Second, Mootha et al. (2003) using gene set enrichment analysis (GSEA), identified that the expression of genes involved in oxidative phosphorylation was coordinately decreased in muscle from humans with type II diabetes and correlated with total-body aerobic capacity.

7.2. Aerobic capacity, antioxidant capacity, and chronic obstructive pulmonary disease

The study by Wisloff et al. that provides evidence concerning the centrality of oxygen metabolism in disease has implications for the evolution of both metabolic and antioxidant pathways. Obligatory for using oxygen in energy transfer pathways was the simultaneous evolution of enzymes that detoxify the reactive species formed as by-products.

From this, one can propose that a greater aerobic capacity is not only associated with enhanced mitochondrial oxidative function, but simultaneously with a greater level of antioxidant capacity. Here we extend these ideas to propose that those high aerobic capacity individuals with greater antiox-

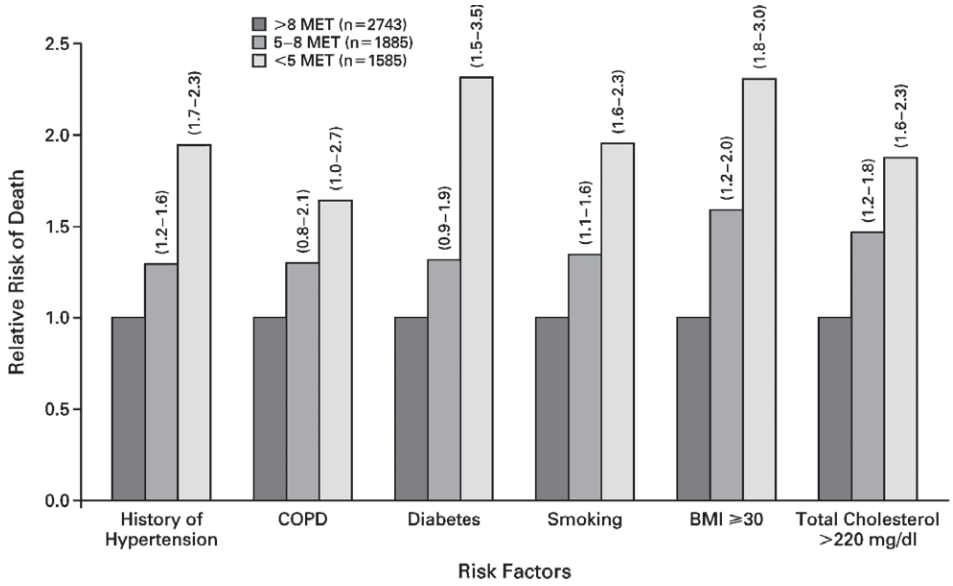

Fig. 5. Illustrates the correlation between exercise tolerance (measured in MET) and risk of death across several chronic diseases (from Myers et al., 2002, with permission).

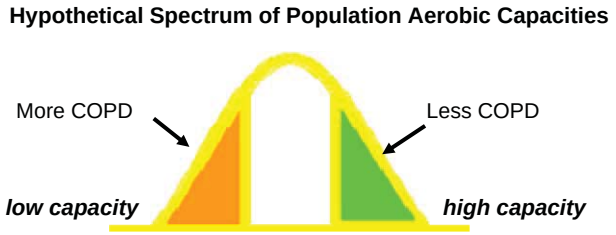

Fig. 6. Hypothetical plot of aerobic capacity/redox potentials across a population. Illustration of the idea that those with low aerobic capacity have more risk for developing diseases such as COPD while those with high aerobic capacity are at less risk for developing a chronic disease.

idant capacity will be protected from the oxidant damage elicited by cigarette smoke. In addition, their enhanced mitochondrial function suggests they will be less susceptible to the consequences of smoke-mediated damage to the mitochondria—that is, apoptosis, signaling abnormalities, and the resulting cellular energetic deficits. Thus, the heterogeneity that exists for aerobic capacity in humans may explain why some, but not all smokers are resistant to the effects of cigarette smoking and do not develop COPD. If this hypothesis is true, then those smokers with low aerobic capacity will develop the disease while those with higher aerobic capacity will be resistant (Fig. 6).

Aerobic functioning is a polygenetic trait that obviously involves much more than mitochondrial function and antioxidant capacity. Several other components also contribute to overall aerobic capacity, such as O_2 conductance and extraction, which also appear to be affected in the disease process (Sala et al., 1999; Antonucci et al., 2003). However, we think that oxygen metabolism and detoxification at the cellular level are at the core of determining an individual's aerobic capacity, and it is why we specifically focused on these processes for this review. Because total aerobic function is a complex trait, it is best studied in vivo and indeed, the creation of low and high aerobic capacity rat models (Wisloff et al., 2005) may be useful in elucidating mechanisms for resistance and susceptibility for COPD. We put forward the hypothesis that the low aerobic capacity rats will be more susceptible to the deleterious effects of exposure to cigarette smoke relative to the high aerobic capacity rats.

8. Summary

The concept that aerobic capacity is a major determinant in the continuum between health and disease could explain the heterogeneity observed in many multifactorial diseases, like COPD. Cigarette smoke creates an environmental oxidant hazard for chronic smokers both in the lung and systemically. This can lead to a number of destructive changes, but only a percentage of smokers develop severe pathologies resulting in COPD. The concept that COPD patients have a reduced ability to adapt their antioxidant defenses to meet the oxidant burden associated with chronic smoking has been around for some time. However, the mechanisms that underlie this proposed deficiency remain unclear. Variation in the alleles that determine intrinsic mitochondrial function and antioxidant capacity could explain the susceptibility of certain individuals to develop chronic diseases like COPD. Mitochondria are not only the most significant sources of endogenous ROS, but also appear to be specifically targeted by cigarette smoke and related oxidants (Thorpe et al., 2004). The role of mitochondria in the molecular pathogenesis of COPD is beginning to be studied more closely as their contribution to other complex disease conditions becomes better understood. This organelle's integral role in ROS generation, energy production, and cell death pathways make it predictable that mitochondrial dysfunction would lead to disease.

Greater mitochondrial function and antioxidant protection could provide some smokers with the added protection needed to escape the toxic effects of cigarette smoke. Others may not be as fortunate, especially with increased age and the accompanying decrease in aerobic capacity. The association between intrinsic aerobic capacity and susceptibility to smoke can now be tested more rigorously with the use of the new aerobic rat models (Wisloff et al., 2005) combined with novel approaches to analysis of array data (Mootha et al., 2003). Future studies will hopefully provide a greater mechanistic insight into the common pathways that confer susceptibility to chronic, complex diseases such as COPD.

Acknowledgments

We are grateful to Dr. Yong Han, Dr. Henry Danahay, Dr. Alan Jackson, Dr. Alex Trifilieff, and Dr. John Fozard for their insights, suggestions, and careful review of this manuscript. In addition, we would like to thank Prof. Peter Barnes, Dr. William Pryor, Dr. Jonathan Myers, Prof. Yau-Wuei Wei, the New England Journal of Medicine, the American Journal of Respiratory and Critical Care Medicine, the New York Academy of Sciences, and the Society for Experimental Biology and Medicine for their permission to use their figures in this manuscript. The research of LGK and SLB was supported by NIH grants HL 64270 and RR 17718.

References

Abboud, R. T., Johnson, A. J., Richter, A. M., & Elwood, R. K. (1983). Comparison of in vitro neutrophil elastase release in nonsmokers and smokers. *Am Rev Respir Dis 128*, 507–510.

American Lung Association. (2005). COPD Fact Sheet. www.lungusa.com, Home > Chronic Obstructive Pulmonary Disease (COPD) center > COPD Fact Sheet (http://www.lungusa.org/site/pp.asp?c=dvLUK9O0E&b=35020).

Antonucci, R., Berton, E., Huertas, A., Laveneziana, P., & Palange, P. (2003). Exercise physiology in COPD. *Monaldi Arch Chest Dis 59*, 134–139.

Aoshiba, K., Tamaoki, J., & Nagai, A. (2001). Acute cigarette smoke exposure induces apoptosis of alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol 281*, L1392–L1401.

Baldwin, J. E., & Krebs, H. (1981). The evolution of metabolic cycles. *Nature 291*, 381–382.

Ballinger, S. W., Bouder, T. G., Davis, G. S., Judice, S. A., Nicklas, J. A., & Albertini, R. J. (1996). Mitochondrial genome damage associated with cigarette smoking. *Cancer Res 56*, 5692–5697.

Banzet, N., Francois, D., & Polla, B. S. (1999). Tobacco smoke induces mitochondrial depolarization along with cell death: effects of antioxidants. *Redox Rep 4*, 229–236.

Barnes, P. J., Adcock, I. M., & Ito, K. (2005). Histone acetylation and deacetylation: importance in inflammatory lung diseases. *Eur Respir J 25*, 552–563.

Barreiro, E., de la Puente, B., Minguella, J., Corominas, J. M., Serrano, S., Hussain, S. N., et al. (2005). Oxidative stress and respiratory muscle dysfunction in severe chronic obstructive pulmonary disease. *Am J Respir Crit Care Med 171*, 1116–1124.

Callsen, D., Sandau, K. B., & Brune, B. (1999). Nitric oxide and superoxide inhibit platelet-derived growth factor receptor phosphotyrosine phosphatases. *Free Radic Biol Med 26*, 1544–1553.

Cardellach, F., Alonso, J. R., Lopez, S., Casademont, J., & Miro, O. (2003). Effect of smoking cessation on mitochondrial respiratory chain function. *J Toxicol Clin Toxicol 41*, 223–228.

Carnevali, S., Petruzzelli, S., Longoni, B., Vanacore, R., Barale, R., Cipollini, M., et al. (2003). Cigarette smoke extract induces oxidative stress and apoptosis in human lung fibroblasts. *Am J Physiol Lung Cell Mol Physiol 284*, L955–L963.

Chapman, H. A. (2004). Disorders of lung matrix remodeling. *J Clin Invest 113*, 148–157.

Chitano, P., Hosselet, J. J., Mapp, C. E., & Fabbri, L. M. (1995). Effect of oxidant air pollutants on the respiratory system: insights from experimental animal research. *Eur Respir J 8*, 1357–1371.

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

Des Marais, D. J. (2000). Evolution: when did photosynthesis emerge on earth? *Science 289*, 1703–1705.

Duchen, M. R. (1999). Contributions of mitochondria to animal physiology: from homeostatic sensor to calcium signalling and cell death. *J Physiol 516*, 1–17.

Duchen, M. R. (2004). Mitochondria in health and disease: perspectives on a new mitochondrial biology. *Mol Aspects Med 25*, 365–451.

Engelen, M. P., Schols, A. M., Does, J. D., Deutz, N. E., & Wouters, E. F. (2000). Altered glutamate metabolism is associated with reduced muscle glutathione levels in patients with emphysema. *Am J Respir Crit Care Med 161*, 98–103.

Fahn, H. J., Wang, L. S., Kao, S. H., Chang, S. C., Huang, M. H., & Wei, Y. H. (1998). Smoking-associated mitochondrial DNA mutations and lipid peroxidation in human lung tissues. *Am J Respir Cell Mol Biol 19*, 901–909.

Feldman, C., Anderson, R., Kanthakumar, K., Vargas, A., Cole, P. J., & Wilson, R. (1994). Oxidant-mediated ciliary dysfunction in human respiratory epithelium. *Free Radic Biol Med 17*, 1–10.

Frei, B., Forte, T. M., Ames, B. N., & Cross, C. E. (1991). Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma. Protective effects of ascorbic acid. *Biochem J 277*, 133–138.

Gairola, C., & Aleem, H. M. (1974). Cigarette smoke: in vitro effects of condensate fractions on mitochondrial respiration. *Life Sci 14*, 2199–2207.

Gosker, H. R., Wouters, E. F., van der Vusse, G. J., & Schols, A. M. (2000). Skeletal muscle dysfunction in chronic obstructive pulmonary disease and chronic heart failure: underlying mechanisms and therapy perspectives. *Am J Clin Nutr 71*, 1033–1047.

Guttridge, D. C., Mayo, M. W., Madrid, L. V., Wang, C. Y., & Baldwin Jr. A. S. (2000). NF-kappaB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. *Science 289*, 2363–2366.

Halliwell, B., & Chirico, S. (1993). Lipid peroxidation: its mechanism, measurement, and significance. *Am J Clin Nutr 57*, 715S–725S.

Halvey, P. J., Watson, W. H., Hansen, J. M., Go, Y. M., Samal, iA., & Jones, D. P. (2005). Compartmental oxidation of thiol-disulphide redox couples during epidermal growth factor signalling. *Biochem J 386*, 215–219.

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. *J Gerontol 11*, 298–300.

Haycock, J. W., Jones, P., Harris, J. B., & Mantle, D. (1996). Differential susceptibility of human skeletal muscle proteins to free radical induced oxidative damage: a histochemical, immunocytochemical and electron microscopical study in vitro. *Acta Neuropathol (Berl) 92*, 331–340.

Hogg, J. C. (2004). Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet 364*, 709–721.

Houten, S. M., & Auwerx, J. (2004). PGC-1alpha: turbocharging mitochondria. *Cell 119*, 5–7.

Ishii, Y., Mochizuki, M., Morishima, Y., Kimura, T., Saito, W., Iizuka, H., et al. (2004). Nrf2 is important for protection against elastase-induced pulmonary inflammation and subsequent emphysema. *Am J Respir Crit Care Med 169*, A831.

Janoff, A., Chan, S. K., Abboud, R. T., Richter, A., Fera, T., & Johal, S. (1984). Is alpha 1-protease inhibitor inactivated by smoking? *Science 224*, 755–756.

Jeffery, P. K., Ayers, M., & Rogers, D. (1982). The mechanisms and control of bronchial mucous cell hyperplasia. *Adv Exp Med Biol 144*, 399–409.

Kim, H. J., Liu, X., Wang, H., Kohyama, T., Kobayashi, T., Wen, F. Q., et al. (2002). Glutathione prevents inhibition of fibroblast-mediated collagen gel contraction by cigarette smoke. *Am J Physiol Lung Cell Mol Physiol 283*, L409–L417.

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 Radic Biol Med 35*, 697–710.

Koch, L. G., & Britton, S. L. (2001). Artificial selection for intrinsic aerobic endurance running capacity in rats. *Physiol Genomics 5*, 45–52.

Kreindler, J. L., Jackson, A. D., Kemp, P. A., Bridges, R. J., & Danahay, H. (2005). Inhibition of chloride secretion in human bronchial epithelial cells by cigarette smoke extract. *Am J Physiol Lung Cell Mol Physiol 288*, L894–L902.

Ladislas, R. (2000). Cellular and molecular mechanisms of aging and age related diseases. *Pathol Oncol Res 6*, 3–9.

Lane, N. (2003). A unifying view of ageing and disease: the double-agent theory. *J Theor Biol 225*, 531–540.

Langen, R. C., Korn, S. H., & Wouters, E. F. (2003). ROS in the local and systemic pathogenesis of COPD. *Free Radic Biol Med 35*, 226–235.

Lee, S. L., Wang, W. W., Finlay, G. A., & Fanburg, B. L. (1999). Serotonin stimulates mitogen-activated protein kinase activity through the formation of superoxide anion. *Am J Physiol 277*, L282–L291.

Lo, Y. Y., & Cruz, T. F. (1995). Involvement of reactive oxygen species in cytokine and growth factor induction of c-*fos* expression in chondrocytes. *J Biol Chem 270*, 11727–11730.

Lundback, B., Lindberg, A., Lindstrom, M., Ronmark, E., Jonsson, A. C., Jonsson, E., et al. (2003). Obstructive lung disease in Northern Sweden studies. Not 15 but 50% of smokers develop COPD?—Report from the Obstructive Lung Disease in Northern Sweden Studies. *Respir Med 97*, 115–122.

MacNee, W., & Selby, C. (1993). New perspectives on basic mechanisms in lung disease: 2. Neutrophil traffic in the lungs: role of haemodynamics, cell adhesion, and deformability. *Thorax 48*, 79–88.

Maltais, F., Simard, A. A., Simard, C., Jobin, J., Desgagnes, P., & LeBlanc, P. (1996). Oxidative capacity of the skeletal muscle and lactic acid kinetics during exercise in normal subjects and in patients with COPD. *Am J Respir Crit Care Med 153*, 288–293.

Manoury, B., Nenan, S., Leclerc, O., Guenon, I., Boichot, E., Planquois, J. M., et al. (2005). The absence of reactive oxygen species production protects mice against bleomycin-induced pulmonary fibrosis. *Respir Res 21*, 11.

Marwick, J. A., Kirkham, P. A., Stevenson, C. S., Danahay, H., Giddings, J., Butler, K., et al. (2004). Cigarette smoke alters chromatin remodeling and induces proinflammatory genes in rat lungs. *Am J Respir Cell Mol Biol 31*, 633–642.

Meyer, M., Schreck, R., & Baeuerle, P. A. (1993). H2O2 and antioxidants have opposite effects on activation of NF-kappa B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J 12*, 2005–2015.

Miro, O., Alonso, J. R., Jarreta, D., Casademont, J., Urbano-Marquez, A., & Cardellach, F. (1999). Smoking disturbs mitochondrial respiratory chain function and enhances lipid peroxidation on human circulating lymphocytes. *Carcinogenesis 20*, 1331–1336.

Molfino, N. A. (2004). Genetics of COPD. *Chest 125*, 1929–1940.

Montes de Oca, M., Torres, S. H., De Sanctis, J. B., Talamo, C., & Celli, B. R. (2004). Systemic inflammation: relation between oxidative stress and the multiple component staging system BODE in COPD patients. *Am J Respir Crit Care Med 169*, A616.

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. *Am J Respir Crit Care Med 162*, 1175–1177.

Mootha, V. K., Lindgren, C. M., Eriksson, K. F., Subramanian, A., Sihag, S., Lehar, J., et al. (2003). PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet 34*, 267–273.

Morrow, J. D., Frei, B., Longmire, A. W., Gaziano, J. M., Lynch, S. M., Shyr, Y., et al. (1995). Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. *N Engl J Med 332*, 1198–1203.

Myers, J., Prakash, M., Froelicher, V., Do, D., Partington, S., & Atwood, J. E. (2002). Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med 346*, 793–801.

Nagasawa, T., Hatayama, T., Watanabe, Y., Tanaka, M., Niisato, Y., & Kitts, D. D. (1997). Free radical-mediated effects on skeletal muscle protein in rats treated with Fe-nitrilotriacetate. *Biochem Biophys Res Commun 231*, 37–41.

Panda, K., Chattopadhyay, R., Chattopadhyay, D., & Chatterjee, I. B. (2001). Cigarette smoke-induced protein oxidation and proteolysis is exclusively caused by its tar phase: prevention by vitamin C. *Toxicol Lett 123*, 21–32.

Paredi, P., Kharitonov, S. A., & Barnes, P. J. (2000a). Elevation of exhaled ethane concentration in asthma. *Am J Respir Crit Care Med 162*, 1450–1454.

Paredi, P., Kharitonov, S. A., Leak, D., Ward, S., Cramer, D., & Barnes, P. J. (2000b). Exhaled ethane, a marker of lipid peroxidation, is elevated in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med 162*, 369–373.

Pauwels, R. A., Buist, A. S., Ma, P., Jenkins, C. R., & Hurd, S. S. (2001). GOLD Scientific Committee. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: National Heart, Lung, and Blood Institute and World Health Organization Global Initiative for Chronic Obstructive Lung Disease (GOLD): executive summary. *Respir Care 46*, 798–825.

Pratico, D., Basili, S., Vieri, M., Cordova, C., Violi, F., & FitzGerald, G. A. (1998). Chronic obstructive pulmonary disease is associated with an increase in urinary levels of isoprostane F2alpha-III, an index of oxidant stress. *Am J Respir Crit Care Med 158*, 1709–1714.

Pryor, W. A., Prier, D. G., & Church, D. F. (1983). Electron-spin resonance study of mainstream and sidestream cigarette smoke: nature of the free radicals in gas-phase smoke and in cigarette tar. *Environ Health Perspect 47*, 345–355.

Pryor, W. A., & Stone, K. (1993). Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. *Ann N Y Acad Sci 686*, 12–28.

Puri, P. L., Avantaggiati, M. L., Burgio, V. L., Chirillo, P., Collepardo, D., Natoli, G., et al. (1995). Reactive oxygen intermediates mediate angiotensin II-induced c-Jun. c-Fos heterodimer DNA binding activity and proliferative hypertrophic responses in myogenic cells. *J Biol Chem 270*, 22129–22134.

Rabinovich, R. A., Ardite, E., Troosters, T., Carbo, N., Alonso, J., Gonzalez de Suso, J. M., et al. (2001). Reduced muscle redox capacity after endurance training in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med 164*, 1114–1118.

Rahman, I. (2003). Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. *J Biochem Mol Biol 36*, 95–109.

Rahman, I., & MacNee, W. (1996). Role of oxidants/antioxidants in smoking-induced lung diseases. *Free Radic Biol Med 21*, 669–681.

Rahman, I., Gilmour, P. S., Jimenez, L. A., & MacNee, W. (2002). Oxidative stress and TNF-alpha induce histone acetylation and NF-kappaB/AP-1 activation in alveolar epithelial cells: potential mechanism in gene transcription in lung inflammation. *Mol Cell Biochem 234–235*, 239–248.

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. *J Clin Invest 114*, 1248–1259.

Repine, J. E., Bast, A., & Lankhorst, I. (1997). Oxidative stress in chronic obstructive pulmonary disease. Oxidative Stress Study Group. *Am J Respir Crit Care Med 156*, 341–357.

Rhee, S. G. (1999). Redox signaling: hydrogen peroxide as intracellular messenger. *Exp Mol Med 31*, 53–59.

Sala, E., Roca, J., Marrades, R. M., Alonso, J., Gonzalez De Suso, J. M., Moreno, A., et al. (1999). Effects of endurance training on skeletal muscle bioenergetics in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med 159*, 1726–1734.

Sastre, J., Asensi, M., Gasco, E., Pallardo, F. V., Ferrero, J. A., Furukawa, T., et al. (1992). Exhaustive physical exercise causes oxidation of glutathione status in blood: prevention by antioxidant administration. *Am J Physiol 263*, R992–R995.

Schreck, R., Albermann, K., & Baeuerle, P. A. (1992). Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells. *Free Radic Res Commun 17*, 221–237.

Selby, C., Drost, E., Lannan, S., Wraith, P. K., & MacNee, W. (1991). Neutrophil retention in the lungs of patients with chronic obstructive pulmonary disease. *Am Rev Respir Dis 143*, 1359–1364.

Short, K. R., Bigelow, M. L., Kahl, J., Singh, R., Coenen-Schimke, J., Raghavakaimal, S., et al. (2005). Decline in skeletal muscle mitochondrial function with aging in humans. *Proc Natl Acad Sci U S A 12*, 5618–5623.

Smith, P. R., Cooper, J. M., Govan, G. G., Harding, A. E., & Schapira, A. H. (1993). Smoking and mitochondrial function: a model for environmental toxins. *Q J Med 86*, 657–660.

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 Radic Biol Med 33*, 1106–1114.

Stangel, M., Zettl, U. K., Mix, E., Zielasek, J., Toyka, K. V., Hartung, H. P., et al. (1996). H2O2 and nitric oxide-mediated oxidative stress induce apoptosis in rat skeletal muscle myoblasts. *J Neuropathol Exp Neurol 55*, 36–43.

Stevenson, C. S., Coote, K., Webster, R., Johnston, H., Atherton, H. C., Nicholls, A., et al. (2005). Characterization of cigarette smoke-induced inflammatory and mucus hypersecretory changes in rat lung and the role of CXCR2 ligands in mediating this effect. *Am J Physiol Lung Cell Mol Physiol 288*, L514–L522.

Sundaresan, M., Yu, Z. X., Ferrans, V. J., Irani, K., & Finkel, T. (1995). Requirement for generation of H2O2 for platelet-derived growth factor signal transduction. *Science 270*, 296–299.

Taylor, J. C., Madison, R., & Kosinska, D. (1986). Is antioxidant deficiency related to chronic obstructive pulmonary disease? *Am Rev Respir Dis 134*, 285–289.

Thannickal, V. J., & Fanburg, B. L. (2000). Reactive oxygen species in cell signaling. *Am J Physiol Lung Cell Mol Physiol 279*, L1005–L1028.

Thorpe, G. W., Fong, C. S., Alic, N., Higgins, V. J., & Dawes, I. W. (2004). Cells have distinct mechanisms to maintain protection against different reactive oxygen species: oxidative-stress-response genes. *Proc Natl Acad Sci U S A 101*, 6564–6569.

Tuder, R. M., Wood, K., Taraseviciene, L., Flores, S. C., & Voekel, N. F. (2000). Cigarette smoke extract decreases the expression of vascular endothelial growth factor by cultured cells and triggers apoptosis of pulmonary endothelial cells. *Chest 117*, 241S–242S.

U.S. Department of Health and Human Services. (2004). *The health consequences of smoking*. A Report of the Surgeon General.

Ushio-Fukai, M., Alexander, R. W., Akers, M., & Griendling, K. K. (1998). p38 Mitogen-activated protein kinase is a critical component of the redox-sensitive signaling pathways activated by angiotensin II. Role in vascular smooth muscle cell hypertrophy. *J Biol Chem 273*, 15022–15029.

Vayssier-Taussat, M., Camilli, T., Aron, Y., Meplan, C., Hainaut, P., Polla, B. S., et al. (2001). Effects of tobacco smoke and benzo[a]pyrene on human endothelial cell and monocyte stress responses. *Am J Physiol Heart Circ Physiol 280*, H1293–H1300.

Verra, F., Escudier, E., Lebargy, F., Bernaudin, J. F., De Cremoux, H., & Bignon, J. (1995). Ciliary abnormalities in bronchial epithelium of smokers, ex-smokers, and nonsmokers. *Am J Respir Crit Care Med 151*, 630–634.

Voelkel, N. F., & Cool, C. D. (2003). Pulmonary vascular involvement in chronic obstructive pulmonary disease. *Eur Respir J Suppl 46*, 28s–32s.

Wang, J., Wilcken, D. E., & Wang, X. L. (2001). Cigarette smoke activates caspase-3 to induce apoptosis of human umbilical venous endothelial cells. *Mol Genet Metab 72*, 82–88.

Wei, Y. H., & Lee, H. C. (2002). Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. *Exp Biol Med (Maywood) 227*, 671–682.

Welsh, M. J. (1983). Cigarette smoke inhibition of ion transport in canine tracheal epithelium. *J Clin Invest 71*, 1614–1623.

Wisloff, U., Najjar, S. M., Ellingsen, O., Haram, P. M., Swoap, S., Al-Share, Q., et al. (2005). Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. *Science 307*, 418–420.

Wouters, E. F., Creutzberg, E. C., & Schols, A. M. (2002). Systemic effects in COPD. *Chest 121*, 127S–130S.

Yang, Y. M., & Liu, G. T. (2004). Damaging effect of cigarette smoke extract on primary cultured human umbilical vein endothelial cells and its mechanism. *Biomed Environ Sci 17*, 121–134.

Young, I. S., & Woodside, J. V. (2001). Antioxidants in health and disease. *J Clin Pathol 54*, 176–186.