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# OXIDANTS AND THE PATHOGENESIS OF LUNG DISEASES

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## Abstract

The increasing number of population-based and epidemiological associations between oxidant pollutant exposures and cardiopulmonary disease exacerbation, decrements in pulmonary function, and mortality underscores the important detrimental effects of oxidants on public health. Because inhaled oxidants initiate a number of pathologic processes, including inflammation of the airways which may contribute to the pathogenesis and/or exacerbation of airways disease, it is critical to understand the mechanisms through which exogenous and endogenous oxidants interact with molecules in the cells, tissues, and epithelial lining fluid (ELF) of the lung. Furthermore, it is clear that inter-individual variation in response to a given exposure also exists across an individual lifetime. Because of the potential impact that oxidant exposures may have on reproductive outcomes and infant, child, and adult health, identification of the intrinsic and extrinsic factors that may influence susceptibility to oxidants remains an important issue. In this review, we discuss mechanisms of oxidant stress in the lung, the role of oxidants in lung disease pathogenesis and exacerbation (e.g. asthma, COPD, and ARDS), and the potential risk factors (e.g. age, genetics) for enhanced susceptibility to oxidant-induced disease.

### Keywords

oxidative stress; antioxidant; genetics; susceptibility; infant; reproductive outcome; premature; children; elderly; asthma; chronic obstructive pulmonary disease; ozone; pollutants; particulates; PM; acute respiratory distress syndrome; hyperoxia; SNP; single nucleotide polymorphism

## INTRODUCTION

As epidemiologic studies emerge from developed and developing industrialized countries, it has become clear that air pollution is associated with dramatic increases in the risk of acute and chronic diseases and death in children and adults. Many air pollutants exert their major effect by causing oxidative stress in cells and tissues that they contact. Gaseous pollutants [including ozone ($O_3$), sulphur dioxide ($SO_2$), and nitrogen dioxide ($NO_2$)] and particulate matter (PM) [including ultrafine, $PM_{2.5}$, $PM_{10}$, and diesel exhaust particles (DEP)] are known to form reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, and hydroxyl radicals. ROS may damage proteins, lipids, and DNA directly, and form distinct products that can be used as biomarkers and help in measurement of ROS activity. ROS react with proteins to form nitrotyrosine[1] and bromotyrosine[2] whereas reaction with lipids leads primarily to the formation of isoprostanes[3,4] and ethane[5]. In scenarios where DNA damage occurs, single-strand breaks and 8-hydroxyguanosine are generated.[6]

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Health effects of air pollution can be classified into short-term and long-term effects, and a number of excellent reviews discuss these effects.[7] Pollutant effects include reversible decrements in pulmonary function, airway inflammation, airways hyperreactivity, compromised immune function, enhanced responsivity to respiratory infection, increased incidence and exacerbation of lung disease (e.g. asthma), and mortality. For example, high-dose exposure of DEP can aggravate bacterial infection and induce a strong T cell mediated response.[7,8] whereas $O_3$ exposure combined with exercise is known to decrease respiratory frequency, $FEV_1$, and FVC concurrent with an increase in airways resistance.[7,9] Ozone also exacerbates allergic asthma which is characterized by increased eosinophils in induced sputum.[10] Further, studies have shown that air pollutant exposures can increase susceptibility and response to bacterial and viral respiratory infections.[11–13] In particular, individuals with one or more risk factors for adverse effects of oxidant exposures are of public health concern. Some of these risk factors include, but are not limited to age, gender, genetic background, nutrition, or pre-existing pulmonary disease.

Despite current regulations and an increasing awareness of the quality of the air we breathe, levels of common air pollutants remain an issue in many areas worldwide. Furthermore, the mechanisms of oxidant toxicity in the lung and other organ systems remain incompletely understood. In this review, we 1) provide a brief overview of the mechanisms through which exogenous (airborne pollutants) and endogenous oxidants may interact with bioreactive molecules (i.e., proteins, lipids, and DNA) (cigarette smoke exposures are not discussed as many reviews on this topic currently exist); 2) describe the role of oxidants in the pathogenesis of asthma, chronic obstructive airways disease (COPD), acute respiratory distress syndrome (ARDS), and cystic fibrosis; 3) discuss a number of intrinsic and extrinsic factors that may enhance susceptibility to oxidants. The review is not meant to be exhaustive, but was intended to highlight representative areas of investigation in this important field, and to identify questions that remain to be answered.

## MECHANISMS OF OXIDANT-INDUCED TOXICITY

The mechanisms whereby oxidants exert their pathological effects on the lungs have been the focus of numerous studies and are still the subject of debate. Despite the diversity of these agents and the multitude of complex mechanisms that exist, several common themes have been identified which can serve as a platform for future research. Many ambient air pollutants may induce oxidative stress in the lung that arises when ROS overwhelm antioxidant defenses (Fig 1). After this imbalance is reached, ROS readily react with proteins, lipids, and DNA resulting in a number of pathological consequences.

### Oxidant interaction with molecules

A primary consequence of oxidative stress is lipid peroxidation, or the oxidative degeneration of lipids. Lipid peroxidation is caused by a free radical chain reaction mainly involving membrane polyunsaturated fatty acids. If not quenched, this reaction can permanently damage cell membranes, ultimately leading to cell death. Exposures to oxidant air pollutants cause lipid peroxidation in humans and rodents.[15–19] Furthermore, the end products of lipid peroxidation can lead to subsequent pathological consequences. One of these end products, 4-hydroxy-2-nonenal (HNE), has numerous downstream effects. *In vitro* treatment of cells with HNE can cause lipid peroxidation[20] and may potentiate oxidative stress through a depletion of intracellular glutathione and induction of peroxide production.[21] HNE may also play a role in airway remodeling through activation of the epidermal growth factor receptor[22] and induction of fibronectin production.[23] Additionally, HNE-protein adducts have been found in the lungs of mice and humans after $O_3$ exposure.[24,25] Finally, HNE can induce cell death of alveolar macrophages in mice.[26] These studies provide evidence for the hypothesis that secondary

mediators generated by oxidant reactions with lipids, proteins, and other biomolecules contribute to toxic effects of pollutants.

Another secondary mediator can be generated by a reaction of $O_3$ with unsaturated fatty lipids. Ozone can react directly with unsaturated fatty lipids in the epithelial lining fluid and cell membranes to produce lipid ozonation products (LOPs), which also have pathological downstream effects.[27–29] These products are small, diffusible, and relatively stable, making them ideal mediators of $O_3$ toxicity. *In vitro* exposure of human airway epithelial cells to different LOPs has shown that these products can activate eicosanoid metabolism similar to $O_3$ exposure.[30] Furthermore, products involved in eicosanoid metabolism are themselves highly reactive peroxides, which can contribute to the oxidative stress-induced damage. Other studies have shown that exposure of bronchial epithelial cells to LOPs caused activation of phospholipases A2, C, and D as well as the induction of inflammatory mediators such as platelet-activating factor, prostaglandin E2, interleukin (IL)-6, and IL-8.[28,29] Treatment with oxidized phospholipids from $O_3$-exposed lung surfactant reduced the viability of macrophages and epithelial cells by necrosis and apoptosis, respectively.[31] This treatment also stimulated the release of IL-8 from epithelial cells. Taken together, these studies provide evidence of a direct link between LOPs produced by $O_3$ exposure and $O_3$-induced inflammation and cell damage.

A primary function of ELF is to protect underlying tissue from inhaled pathogens and toxins. However, current evidence suggests that antioxidants and lipids found in the ELF mediate oxidant-induced membrane oxidation. Thus, some defenses within this barrier may also contribute to the toxicity of certain agents. The capacity of $O_3$ to oxidize cell membrane proteins and lipids *in vitro* was shown to be dependent on the presence of either of the antioxidants ascorbate or glutathione in the lining fluid.[32] These results were corroborated by a study demonstrating that addition of ascorbate to the lining fluid increased cell injury in response to $O_3$.[33] Other studies have shown similar mechanisms for $NO_2$. Glutathione and/or ascorbate are necessary components of the lining fluid for $NO_2$-mediated membrane oxidation *in vitro*.[34]

Another mechanism whereby oxidant pollutants may exert their pathological effects is through the modification of proteins. ROS can act directly or indirectly on proteins to cause oxidation of the polypeptide backbone, peptide bond cleavage, protein-protein cross linking, or amino acid side chain modifications.[35] Amino acid composition, particularly cysteine and methionine residues, can render proteins more susceptible to oxidation.[35] For example, oxidation of methionine residues in α-1-antitrypsin by ROS *in vitro* results in loss of anti-neutrophil elastase activity.[36] Without protection from α-1-antitrypsin, the alveolar matrix is susceptible to destruction by neutrophil elastase, which can eventually contribute to emphysema. Oxidation of multiple methionine residues by ROS impair rapid sodium channel inactivation.[37] ROS also oxidize methionine residues in surfactant protein (SP)-B leading to inactivation.[38] Inactivation of SP-B reduced the ability of the surfactant film to reduce lung surface tension during breathing, which can contribute to respiratory distress syndrome. Similarly, acute exposure of guinea pigs to $O_3$ altered SP-A function contributing to the inflammatory response.[39] Another study found that *in vitro* and *in vivo* $O_3$ exposure caused oxidative modifications in SP-A that reduced ability to enhance phagocytosis of bacteria.[40] Oxidative modification of surfactant proteins may also render the lung more susceptible to lipid peroxidation, inflammation, and oxidative damage since these proteins have been reported to inhibit these processes.[41–43]

Epidemiological and experimental studies have shown that exposure to air pollutants increases the risk of lung cancer.[44] A potential mechanism for the increased cancer incidence in exposed individuals is DNA damage. Prahalad et al.[45] demonstrated that PM can cause DNA damage

and that this effect was inhibited by an OH scavenger and metal ion chelators, suggesting a role for PM-generated free radicals and metals adsorbed onto the particles. Further evidence also showed that PM caused increased DNA oxidative damage to human airway epithelial cells and was associated with the amount of water-soluble metals contained on these particles.[46] Another group demonstrated that DEP induced DNA damage in mice and that this effect was dependent on the particle and not the organic chemicals adsorbed onto the particle surface.[47] The authors proposed that alveolar macrophage generation of hydroxyl radicals during particle phagocytosis may contribute to DNA damage. These and other studies suggest that PM-induced DNA damage results from free radical formation.[48] DNA damage has also been shown in lung epithelial cells exposed to $O_3$ and this effect was reduced by pretreatment with vitamins C and E.[49] It had also been reported that DNA backbone cleavages caused by $O_3$ were dependent on hydroxyl radicals, while DNA base modifications were mainly caused by a direct effect of $O_3$.[50] Furthermore, DNA-protein cross-linking has been shown in the lungs of mice exposed to $SO_2$.[51] In addition to potential cancer etiology, DNA damage may alter gene and protein expression as well as cell death.

## Oxidant-induced Cell Signaling

Activation of signaling pathways is another way in which oxidant pollutants may cause pathological responses in the lung. Air pollutants and ROS can activate MAPK signaling, which may ultimately promote inflammation. For example, inhibition of c-Jun $NH_2$ terminal kinase (JNK) in mice attenuated $O_3$-induced inflammation and hyper-responsiveness.[52] Additionally, end products of lipid peroxidation activate extracellular signal-regulated kinase p44/42 (Erk1/2), JNK, and p38MAPK and activation can be blocked by NAC.[21,23] Activation of these kinases was also accompanied by increased DNA binding activity of the transcription factor activator protein-1 (AP-1), which can lead to the transcription of stress response genes including phase II enzymes (Fig 1). Another study demonstrated that HNE could induce DNA binding of the transcription factors nuclear factor erythroid-2 related factor (NRF) 1, NRF2, JunB, c-Jun, FosB, c-Fos, Fra1, and Fra2.[53] Oxidants also increase NF-κB DNA binding along with the release of the pro-inflammatory cytokine IL-8 in lung epithelial cells and this effect can be abrogated by antioxidant pretreatment.[54] Other studies have also demonstrated the ability of air pollutants to activate NF-κB.[55,56] Activation of stress response pathways due to oxidative stress is likely a cause of pollutant-induced NF-κB activation. PM-induced activation of NF-κB has been shown to be dependent on EGFR activation and the MAPK signaling pathway, which is involved in the stress response.[57] Conversely, there is also evidence that oxidants can downregulate inflammatory pathways through an inhibitory effect on NF-κB.[58]

Another important transcription factor involved in the response to oxidative stress is Nrf2. Nrf2 contributes to the oxidative stress response through its binding of antioxidant response elements (AREs) leading to the induction of various genes involved in mitigating oxidative damage.[63] Oxidant-induced activation of Nrf2 leads to the transcription of genes for antioxidants, DNA damage recognition, glutathione homeostasis, free radical metabolism, as well as a number of others involved in the oxidative stress response.[59] Mutations in Nrf2 or a disruption of the signaling pathway would likely render individuals more susceptible to the adverse effects of pollutant exposure. Further investigation of pollutant-induced activation of Nrf2 and the consequences of Nrf2 mutation in the response to pollutant exposure is needed to fully elucidate the role of Nrf2 in mitigating the effects of oxidant pollutant exposure.

## Endogenous Sources of Oxidants

Endogenous sources of ROS may have an indirect role in the toxicity induced by exposure to air pollutants. The main cellular sources of ROS in the lung include neutrophils, eosinophils, alveolar macrophages, epithelial cells, and endothelial cells. Air pollutant-induced lung inflammation involves the recruitment of inflammatory cells that release ROS, which can

enhance inflammation, tissue damage, and other pathological effects. Additionally, phagocytes can be activated by PM deposition in the lung and cause ROS release contributing to the oxidative damage.[47,60] Similarly, $NO_2$ has also been shown to induce the release of ROS from macrophages.[61] The predominant ROS produced by inflammatory cells and macrophages are superoxide and hydrogen peroxide, respectively. Both oxidants can react with a number of substrates and biomolecules to cause damage and generation of harmful radicals. ROS are also produced in the body during normal metabolic reactions such as aerobic respiration involving the electron transport chain within the mitochondria, and enzyme reactions involving cycloxygenases, lipoxygenase, peroxidases, and cytochrome-P450. Any alteration in these processes or decrement in the antioxidants that offset the production of ROS from them may also lead to tissue damage and other pathological consequences. Additionally, endogenously produced nitric oxide can react with oxygen to form damaging nitrogen oxides or it can react with superoxide to form peroxynitrate.[62] Peroxynitrate has been shown to induce lipid peroxidation, DNA damage, and protein oxidation.[63–66] Furthermore, peroxynitrate can also react with $CO_2$ to form $NO_2$, which can lead to further oxidant-induced damage.[67] Taken together these studies demonstrate how endogenous sources of ROS can contribute to air pollutant-induced toxicity through an enhancement of the oxidative burden within the lung.

## OXIDANTS AND LUNG DISEASE

Because the lung interfaces with the external environmental, it is frequently exposed to airborne oxidant gases and particulates, and thus prone to oxidant-mediated cellular damage. Enhanced levels of oxidant production and cellular injury have been implicated in many pulmonary diseases including asthma and other allergic diseases, COPD, ARDS, and cystic fibrosis. In the following section, we briefly describe investigations on exacerbation of these important pulmonary diseases due to ROS.

### Asthma

It is widely agreed that a link exists between oxidants and their effect on various allergic diseases, particularly asthma pathogenesis. Oxidants can cause airway inflammation and airway hyperresponsiveness (AHR) which are major characteristics of asthma.[68] Asthmatics have increased ROS production by macrophages, eosinophils and neutrophils which leads to increased hydrogen peroxide[69], 8-isoprostance and CO in their breath condensates, increased pulmonary glutathione peroxidase and superoxide dismutase in lung cells,[70] as well as increased pulmonary, serum and urinary peroxidation products. Because eosinophils and neutrophils are the major cells in the inflammatory infiltrate in asthma, increased levels of eosinophil peroxidase and myeloperoxidase in the peripheral blood, induced sputum and BAL fluid from patients have been documented.[71,72] Other markers of oxidant activity such as malondialdehyde (MDA) and thiobarbituric acid reactive products (TBARs) have also been detected in urine, plasma, sputum and BAL fluid which relate to the severity of asthma in these patients.[73–76] Elevated levels of nitrotyrosine[1] and chlorotyrosine[2,77] in BAL fluid from asthma patients also suggests oxidative protein damage.

External oxidant stimuli also worsen existing allergic disease. For example, $O_3$ may evoke asthma exacerbations. Ozone inhalation was shown to increase AHR, induce higher IL-5, GM-CSF and G-CSF levels and indirectly enhancing the longevity of eosinophils via suppressing apoptosis, in a mouse model of allergic asthma.[78]

DEP and its components have been demonstrated to enhance AHR in a murine model of asthma.[79] Human studies have also revealed that antioxidant enzymes, GSTM1 and GSTP1, can alter adjuvant function of DEP in allergic inflammation, block DEP-induced IgE and IL-4 cytokine production.[80] DEP exposure leads to generation of ROS *in vitro*[81] and *in vivo*.[82] DEP has also been advocated as an adjuvant in allergic sensitization mediating its effect via dendritic

cells (DCs). Chan et al[83] showed that DEP downregulated LPS-induced CD86 and CD54 and MHC class II maturation makers and IL-12 production by DCs thereby, interfering with DC function. The interference in DC function was attributed to the Nrf2 signaling pathway.[83]

Recent studies have also suggested that $O_3$ and DEP have an additive effect on AHR and pulmonary inflammation in asthma. For example, higher PenH, increased IL-4 and reduced IFN-γ levels in BAL fluid from OVA-sensitized-challenged $O_3$- and DEP-exposed groups were observed compared to OVA-sensitized-challenged $O_3$-exposed groups and OVA-sensitized-challenged DEP-exposed groups.[84]

## Chronic Obstructive Pulmonary Disease (COPD)

COPD is a slow progressive and irreversible disease state characterized by limited airflow associated with gradual decline in lung function[85] with clinical manifestations such as emphysema and chronic bronchitis.[86,87] Clinical and experimental investigations suggest that oxidants play a role in pathogenesis of COPD. The major contributing factors in COPD etiology are direct exogenous sources of oxidants such a cigarette smoke rich in ROS. Increased amounts of ROS are also generated endogenously by various inflammatory and epithelial cells of the airways. Further, accumulating evidence has implicated indirect local and systemic effects of oxidants in COPD pathogenesis. Locally, higher levels of oxidants have been found in exhaled breath condensates, sputum and lavage fluid of patients with COPD. Large numbers of neutrophils and macrophages migrating into the lungs of COPD patients generate ROS in excess such that these patients have higher levels of superoxide anion and hydrogen peroxide release.[88–91] Recently, hydrogen peroxide in exhaled air and, IL-8 and soluble ICAM-1 in serum were found to be suitable markers in monitoring patients with exacerbated COPD.[92] Impairment in gene expression of protective mechanisms (GSTP1, GSTM1, microsomal epoxide hydrolase and TIMP2) against oxidants in lung samples of COPD patients was observed along with upregulation of chemokines involved in the inflammatory process.[93] Locally generated 4-HNE has been shown to modify protein levels in airway and alveolar epithelial cells, and endothelial cells in human subjects with airway obstruction.[94] It also interacts with glutathione thereby reducing cells antioxidant ability.[95]

Systemically, oxidants cause elevation of plasma lipid peroxidation products such as malondialdehyde.[96,97] Higher levels of 8-isoprostanes, products of ROS-mediated peroxidation of arachidonic acid, are also found in breath condensates as well as in the urine.[98,99] Erythrocyte superoxide dismutase (SOD) which scavenges superoxide radical was significantly higher in plasma of COPD patients than in healthy non-smokers.[100]

## Acute Respiratory Distress Syndrome (ARDS)

ARDS is a severe form of acute lung injury (ALI) and a syndrome of acute pulmonary inflammation characterized by sudden reduction in gas exchange and static compliance as well as nonhydrostatic pulmonary edema.[101] The mechanisms of ARDS are an ongoing field of investigation. However, ROS have been suggested to play an important role in pulmonary vascular endothelial damage[102] which is hypothesized to be responsible for clinical manifestation of ARDS. Recently, studies have suggested that pathogenesis of ARDS involves enhanced production of ROS and diminished antioxidant levels.[103,104] Patients with ARDS have high levels of hydrogen peroxide in exhaled air and urine[105], and high circulating levels of 4-HNE.[106] Levels of antioxidant defense system including enzymes like superoxide dismutase and catalase as well as other scavengers like glutathione, vitamin E and C have been shown to drop with increasing levels of ROS.[107]

Iron is also known to be a mediator of oxidative stress as it can catalyze prooxidant reactions. Decreased plasma iron-binding activity leading to decreased ability to prevent iron dependent

ROS formation has been detected in patients with ARDS. Transferrin receptor protein levels were found to be significantly increased in lung biopsies of patients with ARDS implicating iron a mediator of oxidative stress.[108]

Inflammatory mediators such as cytokines, chemokines and adhesion molecules expressed during ARDS can also indirectly mediate production of ROS[109] and thereby lead to further damage. High concentrations of TNF-α and IL-1β have been detected in high concentrations of BALF of patients with ARDS. IL-6 levels in the circulation are known to be a detector of ARDS of different etiologies such a sepsis and acute pancreatitis.[109]

### Cystic fibrosis (CF)

CF is a disease caused by an autosomal recessive mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, resulting in dysfunction of a protein involved in the transport of chloride ions across cell membranes. The deficiency in chloride transport leads to production of thickened mucus secretions in the respiratory system of CF patients who are commonly afflicted with recurrent episodes of bronchitis and pneumonia. Exhaled breath condensates (EBC) from children with cystic fibrosis expressed high levels of oxygen and carbon centered radicals in CF groups versus the healthy controls. Catalase abolished oxygen radicals in EBC whereas addition of hydrogen peroxide led to a dramatic increase.[110] In a similar study, myeloperoxidase and 3-cholorotryosine levels were 10 and 5-fold elevated respectively, in the BALF of young children with CF as compared to the controls.[111] Nitrotyrosine was found elevated in sputum of CF patients.[112]

Recent investigations have helped in understanding the important role of CFTR in CF pathogenesis. CFTR is known to regulate cellular glutathione (GSH) transport. CFTR gene expression is suppressed by oxidative stress caused by tert-butylhydroquinone, BHQ as it enhanced cellular glutathione in CFTR-expressing T84 and Calu-3 epithelial cells.[113] In another recent study, mutated CFTR caused increased ROS levels and mitochondrial oxidative stress as a consequence of lower GSH levels.[114] The submucosal gland serous cell is the principal site of expression of CFTR chloride ion channel which is known to dysfunction in CF.[115] Cowley and Lindsell[115] proposed that ROS-stimulated anion secretion from serous cells is CFTR dependent. Absence of this compensatory protective mechanism might expose lung to ROS for extended periods, which could be important in the pathogenesis of CF lung disease.

Neutrophil rich inflammation is a determinant of CF severity, and findings from a recent genetic study show that the level of myeloperoxidase gene expression which governs the microbicidal and proinflammatory activities of neutrophils may influence CF pathogenesis.[116] Specifically the -463GA MPO promoter polymorphism has been shown to control the severity of CF-related pulmonary inflammation.

## SUSCEPTIBLE POPULATIONS

Inter-individual differences in responses to air pollutant exposures have been well-documented.[117–120] That is, in populations and clinical studies exposed similarly to air pollutants, pulmonary inflammatory and function responses are more severe in some individuals than in others. Importantly, investigators have also demonstrated high within-individual reproducibility of the responses to air pollutant exposures.[120] The wide spectrum of adverse responses to the pollutants has been attributed to multiple intrinsic (e.g. age, gender, genetic) and extrinsic (e.g. nutrition, pre- or concurrent exposure, pre-existing disease) factors. In the following section, we identify and briefly discuss intrinsic (host) and extrinsic factors that may influence susceptibility to oxidant air pollutants, and the potential implications for

increased risk of allergic disease. Because of space limitations, most of the discussion is focused on age and genetic background.

## Age

**Pre-neonatal effects—**Maternal exposure to air pollutants during pregnancy may have adverse effects on the developing fetus (for comprehensive review, see [121–123]). Pre-term neonates are particularly susceptible to potential injurious effects of air pollutants because exposure may disrupt normal fetal developmental processes. Adverse birth outcomes, including preterm birth, low (<2,500 g) and very low (<1,500 g) birth weight, intrauterine growth restriction, birth defects, and intrauterine and infant mortality have been associated with maternal exposure to $O_3$, $NO_2$, $SO_2$, and PM (Table 1). Because low/very low birth weight and prematurity are important predictors of children's health (e.g. mortality, cardiovascular, pulmonary, immunologic, renal, central nervous system, and neurocognitive deficits[145–148]), understanding of the effects of oxidants have important economic and public health implications. Interestingly, while some investigations found positive associations between maternal exposures to oxidant pollutants and adverse birth outcomes, others suggested that exposures have little or no effects.[132] However, the majority of studies suggest that maternal exposures to oxidant air pollutants and the resultant effects on birth weight and other adverse birth outcomes represent an important determinant of susceptibility to lung development and diseases including allergic diseases such as asthma (Fig 2).

**Infants and children—**Development of the lung is a complex, highly orchestrated process that begins in the embryo where the lung begins as an avascular epithelial bud and reaches maturation at approximately 6–8 years of age.[149,150] Transcription factors and other molecular signals control development of respiratory bronchioles, epithelium, capillaries, and immune cell populations and processes over a number of well-defined stages.[150] The lung is particularly vulnerable to adverse effects of oxidant pollutants and other toxicants during post-natal growth and development processes.[121,123] However, little is known about the mechanisms through which inhaled oxidant toxicants affect the human developing lung, though animal models have provided important insight.[150] The airway epithelium, in particular, is thought to be highly vulnerable, and the immune system, such as polarization of T helper cells, could also be affected by oxidants and inflammatory stimuli.[151]

Large cohort investigations have associated exposure to air pollutants with changes in children's lung function.[123,152–155] Children are particularly susceptible not only because the lung is developing (above), but children are also often very active outdoors and have very different ventilatory parameters compared to adults[150] that facilitates deeper and greater lung deposition of particles and gas/cell membrane interactions. Gauderman et al[154] reported that among over 3,600 children in southern California communities, those who lived within 500 m of a freeway had significant deficits in pulmonary function ($FEV_1$, MMEF) compared with children who lived at least 1,500 m away from the freeway. Delfino et al[155] evaluated the relationship between daily changes in $FEV_1$ and ambient and personal air pollutant exposures in asthmatic subjects between the ages of 9 and 18 yr. These investigators found that decrements in $FEV_1$ were significantly associated with increasing hourly peak and daily average personal $PM_{2.5}$ and $NO_2$. Interestingly, $FEV_1$ decrements were not found with ambient $PM_{2.5}$ and only weakly with ambient $NO_2$. They concluded that pollutant associations with lung function deficits might be missed using ambient data alone, and stress the importance of using personal exposure to identify independent effects of specific pollutants.

An increasing body of population-based and epidemiological literature also indicates that asthmatic children are at great risk for asthma exacerbation with exposure to traffic-related air pollution (for review[156]). Asthma exacerbations have been demonstrated in many urban

environments worldwide including Hong Kong[157], Mexico City[1158], and Los Angeles[159]. Air pollutants have also been associated with the development of asthma in children. For example, McConnell et al[184] found that the relative risk of developing asthma was 3.3 times greater in children who played 3 or more outdoor sports in southern California communities with high $O_3$ concentrations compared with children playing no outdoor sports. The investigators found that the number of sports had no influence on asthma incidence in low $O_3$ communities. Further support for the hypothesis that air pollutants contribute to atopic diseases in children was provided by a prospective birth cohort study that found strong positive associations between the distance to nearest main road and asthmatic bronchitis, hay fever, eczema, and sensitization during the first 6 years of life.[186]

The relatively greater increases in urban asthma have been attributed to many factors, including socioeconomic status. Socioeconomic status and ethnic disparities in asthma prevalence and morbidity have been well documented[162], and environmental factors may account for asthma disparities including greater traffic air pollution, disparities in treatment and access to care, housing conditions, and indoor exposure to allergens.

**Elderly—**Evidence has accumulated that suggests the elderly (>65 yr) may be susceptible to adverse effects of air pollutant exposures (for reviews[163,164]). The mechanisms for increased susceptibility in this subpopulation are not well understood, but age-related decline in lung function[165,166], underlying cardiovascular and/or pulmonary disease[167,168], and potential decline in antioxidant defense capacity of the respiratory tract lining fluid[164] have been proposed. It is generally agreed that lung function declines with age, and oxidant stress related to smoking, chronic lung inflammation, and related diseases may increase the rate of decline.[164] Furthermore, decline in antioxidant capacity has been correlated with increased risk of mortality due to multiple causes.[169] Supplementation with antioxidants (for review[164]) or treatments with other oxidant-reducing drugs (e.g. statins[170]) may reduce the rate of decline in lung function. Interestingly, a recent investigation found in relatively small groups of young subjects, older current smokers, and older non-smokers that Nrf2 expression decreased in the alveolar macrophages of older current smokers and COPD patients relative to the other groups.[171] However, the use of antioxidant therapies to treat diseases such as COPD that are associated with decreased lung function have been equivocal.[164] The explanation for inconsistent protective effects of antioxidant therapies for these diseases is not clear, though the presence of large inter-individual variation in antioxidant levels in smokers suggest that protective response mechanisms may exist in which oxidant stress stimulates upregulation of antioxidant mechanisms.[164] Understanding whether differential inducibility of antioxidant defenses due to loss-of-function polymorphisms in antioxidant enzyme genes or other mechanisms (e.g. post-translational modification of proteins) in aging normal and diseased individuals could provide important insight to development of effective strategies to prevent or reduce loss of lung function. For example, Alexeeff et al[172] found that acute effects of $O_3$ exposure were exacerbated in elderly men with polymorphisms in the antioxidant genes *GSTP1* (glutathione S-transferase pi) and *HMOX1* (heme oxygenease-1) relative to those with wild-type genotypes. Interestingly, antioxidant supplementation to reduce change in lung function due to $O_3$ in asthmatic children was effective only in those with genetic deficiency in GSTM1, suggesting nutrition by gene interaction may be important in this setting[173]. Further investigation of interactions between antioxidant supplementation and genetic background may have implications for other subpopulations, including the elderly.

## Genetic Background

A role for genetic background in pulmonary responses to the adverse effects of oxidant exposures was initially suggested based on reproducible inter-individual variation in pulmonary spirometric responses ($FEV_1$, sRaw) by normal healthy volunteers following

controlled $O_3$ exposures.[120] Similar observations were independently reported by other laboratories.[119] Furthermore, investigators found that $O_3$-induced inflammation as indicated by the number of polymorphonuclear leukocytes found in bronchoalveolar lavage fluid also varied widely between subjects.[117] Because the subjects in all of these studies were otherwise healthy, non-smoking, young adults, an intrinsic factor was suggested to be an important determinant of the variation.[174]

To more formally evaluate whether genetic background was an important determinant of susceptibility to $O_3$-induced lung inflammation and injury, multiple inbred strains of mice were exposed to $O_3$.[175] Significant inter-strain variation in $O_3$-induced inflammation was found, and linkage analyses identified quantitative trait loci (QTL) that harbor candidate susceptibility genes. Proof of concept investigations have implicated tumor necrosis factor (*Tnf*176) and toll-like receptor 4 (*Tlr4*[177]) as determinants of $O_3$-induced lung inflammation and hyperpermeability, respectively. However, these susceptibility QTLs (and candidate genes) account for only approximately 20–30% of the genetic variance in $O_3$ responsiveness which indicates that other QTLs likely interact to determine $O_3$ response phenotype.

Evidence exists that genetic loci for inflammatory and antioxidant processes are also important in human responses to air pollutants (for review[178]). For example, Bergamaschi et al[179] found that polymorphisms in genes for phase II xenobiotic metabolizing enzymes NAD(P)H:quinone oxidoreductase 1 (*NQO1*) and glutathione-S-transferase (GST) M1 (*GSTM1*) associated with pulmonary function and epithelial injury responses to $O_3$ in exercising subjects. Yang et al[180] found among 51 adult subjects exposed to $O_3$ during intermittent exercise, those with the *TNF* -308 G/G genotype (wild type) had a significantly greater fall in $FEV_1$ (−9% of baseline) compared to subjects with a loss of function −308 A allele (G/Aor A/A genotype). Interestingly, a similar association of *TNF* genotypes was found in adult asthmatic subjects exposed to 0.5 ppm SO $O_3$ during moderate exercise.[181]

The role of *TNF* was also investigated by Li et al[182] who found that children with −308 G/G *TNF* genotype had decreased risk of asthma and lifetime wheezing compared to children who had one or more of the mutant alleles. They also found that the protective effect of the G/G genotype on wheezing was greater in low $O_3$ communities compared with high $O_3$ communities. Furthermore, they found that reduction of the protective effect of the −308 G/G genotype with higher $O_3$ exposure was most evident in children who had antioxidant *GSTM1* null and *GSTP1* Ile/Ile genotypes. Results suggested that the −308 G/G genotype may be important in the pathogenesis of asthma and wheezing, which in turn is dependent on airway oxidative stress. Others[183,184] have also found that polymorphisms in *NQO1* and *GSTM1* confer differential risk to asthma in oxidant environments, thus strengthening the notion that interaction of environmental oxidants and these phase II enzyme genes (i.e. gene by environment interaction) are important in the pathogenesis of asthma.

Hyperoxic lung injury induces inflammation and noncardiogenic edema in the lung which are phenotypes of acute lung injury (ALI) and ARDS. In positional cloning studies, the nuclear transcription factor *Nrf2* was identified as a potential candidate gene for susceptibility to hyperoxic lung injury in inbred mice.[185] Studies in mice with targeted deletion of *Nrf2* confirmed a role for this gene in the hyperoxia model.[186] Interestingly, Nrf2 has also subsequently been found to be important in the pathogenesis of asthma phenotypes in a mouse model.[187] Furthermore, recent investigations have also suggested a role for Nrf2 in PM-induced exacerbation of asthma.[188,189]

We therefore hypothesized that polymorphisms in *NRF2* resulting in decreased function similarly predispose humans to ALI. Resequencing of *NRF2* in four different ethnic populations and identified three new *NRF2* promoter polymorphisms at positions −617 (C/A),

−651 (G/A) and −653 (A/G).[190] The −617 polymorphism alters the consensus recognition sequences for NRF2, and suggested that this polymorphism may affect *NRF2* transcription. *In vitro* transcription factor binding analyses confirmed a loss-of-function effect of the −617 C/A polymorphism. Furthermore, among major trauma patients, those with the −617 A SNP had a significantly higher risk for developing ALI [OR 6.44; 95% CI 1.34, 30.8; $p = 0.021$] relative to patients with the wild type (−617 CC).[223] These studies provided insight into the molecular mechanisms of susceptibility to ALI, and may help to identify patients who are predisposed to develop ALI under at risk conditions, such as trauma and sepsis. Furthermore, because animal models have implicated an important role for *Nrf2* in asthma/allergy phenotypes, evaluation of the *NRF2* promoter polymorphisms may have relevance in these and other diseases with oxidant stress etiologies.

## CONCLUDING REMARKS AND FUTURE DIRECTIONS

Oxidative stress can cause cellular damage by oxidizing nucleic acids, proteins, and membrane lipids. ROS have been implicated in the pathogenesis of many diseases and important biological processes including carcinogenesis, atherosclerosis, aging, and inflammatory disorders. Moreover, because of its interface with the environment, the lung is a major target organ for injury by exogenous oxidants such as environmental pollutants and endogenous ROS generated by inflammatory cells. Lipid peroxidation products such as isoprostanes, TBARS, and MDA can be detected in EBC, BALF, urine or plasma to give an indirect measure of oxidative stress. Levels of $H_2O_2$ in EBC can also be used to estimate oxidative stress within the lungs. Furthermore, levels of antioxidants such as GSH in EBC or BALF are another indirect measure of oxidative stress. These endpoints are indirect measures of oxidative stress, are not specific to air pollutant exposure, and considerable variability may arise from confounding factors such as collection methods and individual lifestyle habits. Markers of DNA oxidation such as 8-OHdG and and 8-oxoGuo can be detected in the urine. Protein carbonyl levels in the plasma can be used to assess protein oxidation. All of the biomarkers mentioned do not discriminate between different oxidative insults and are merely indicators of oxidative damage. Further investigation is needed to discover biomarkers that correlate well with severity of pollutant-induced injury as well as exposure to the pollutants. These markers are essential to provide a means to estimate exposure and facilitate identification of at risk individuals.

While considerable progress has been made to understand the mechanisms through which oxidants initiate and propagate cell and tissue toxicity, critical questions remain to be addressed. For example, specific signaling pathways and mechanisms of transcription factor activation by specific oxidant pollutants are not well understood. Characterization of precise cellular mechanisms may provide a means for intervention to prevent or protect against disease pathogenesis, particularly in populations that are at risk due to preexisting disease, age (very young and elderly), or other predisposing conditions such as poor nutrition.

Because of the impact that oxidants may have on lung function, a better understanding of factors that may influence individual susceptibility remains an important issue. *In utero* and neonatal exposures to oxidants can have profound effects on the developing lung and may impact considerably on childhood and adult health. Studies designed to investigate how early life exposures to specific oxidants affect airway morphology, immune function, as well as the epigenome are necessary if we are to understand the long-term differences in morbidity/mortality outcomes, including asthma and other allergic diseases.

Genetic background is also an important determinant of responsiveness to oxidant exposures in children and adults. Using association analyses in clinical and epidemiological investigations, functional polymorphisms in a number of candidate susceptibility genes (e.g. *NQO1*, *GSTM1*, *TNF*) have provided some insight to the importance of genetics in inter-

individual variation in oxidant responsiveness. However, responsivity to oxidant exposures and disease pathogenesis are complex, multigenic processes, and the contribution of each gene in a complex trait is relatively minor. Therefore, it is critical to identify each of the genes that ultimately determine complex traits such as environmental lung diseases. Discovery of genes that affect physiology and pathophysiology will occur only if multi-disciplinary investigations utilize model systems to exploit the accumulating data available for comparative genetics and genomics.[191] Furthermore, susceptibility genes almost certainly interact with multiple environmental exposures or stimuli that are important in the etiology of a disease, and these interactions may vary with age and from one population to another.[192] It is only through investigation of the basic mechanisms and translational application that we will understand the complex interplay of gene-environment interactions and oxidant-mediated lung disease.

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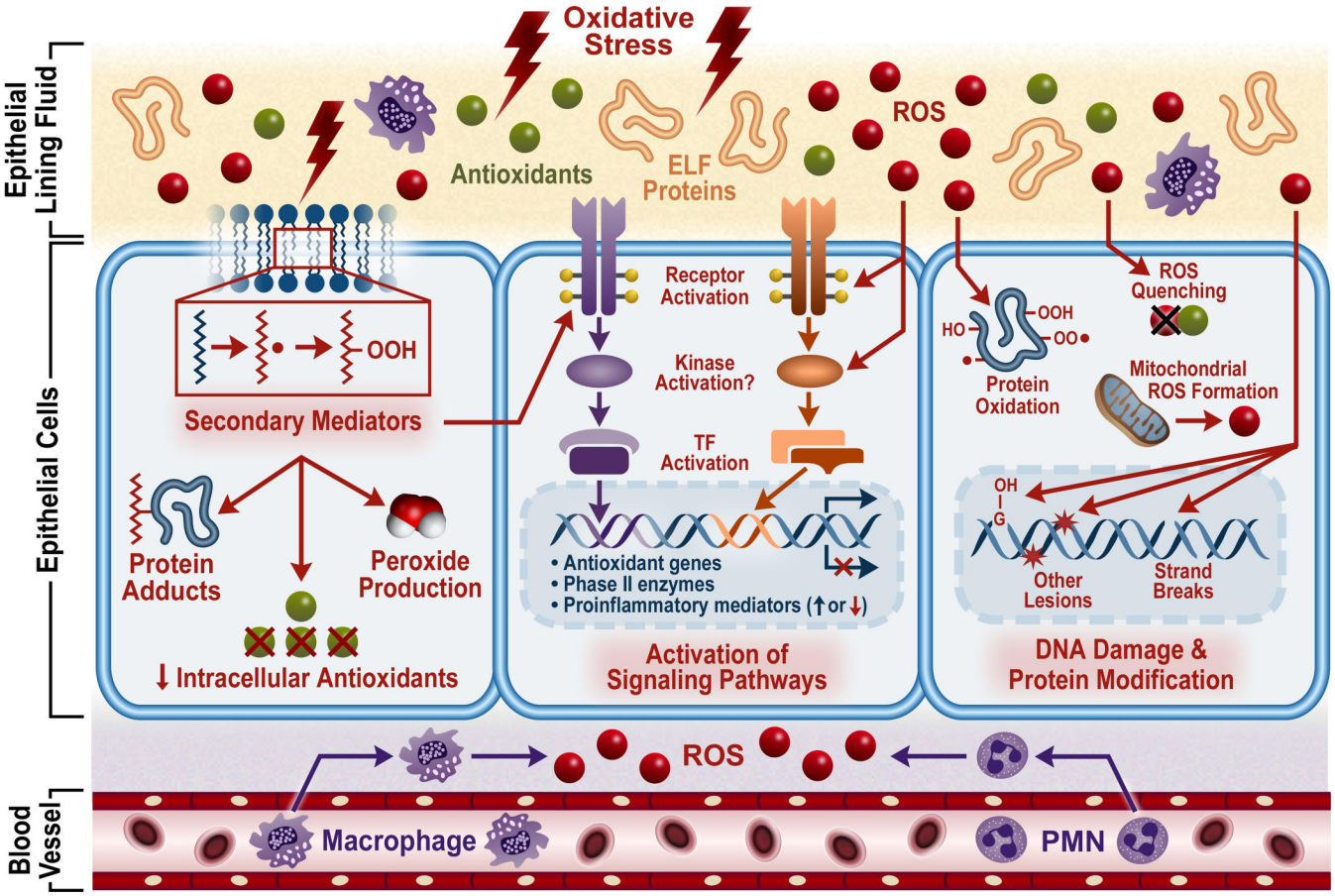


**Figure 1.**

Mechanisms involved in oxidant pollutant-induced adverse health effects. Oxidant pollutants may elicit their effects through 1) the production of secondary mediators generated by reaction of pollutants or pollutant-induced ROS or free radicals with lipids in the ELF or cell membrane as well as proteins and antioxidants; 2) activation of signaling pathways by reactive oxygen species (ROS) or secondary mediators; 3) oxidation of cellular proteins; 4) damage to DNA. In addition to pollutant-induced generation of ROS, endogenous sources of ROS such as inflammatory cells, phagocytes recruited to the site of injury, as well as other cellular processes, may contribute to the oxidative stress state caused by pollutant exposure. Antioxidant molecules and enzymes mitigate the effects of ROS in the body. TF, transcription factor.

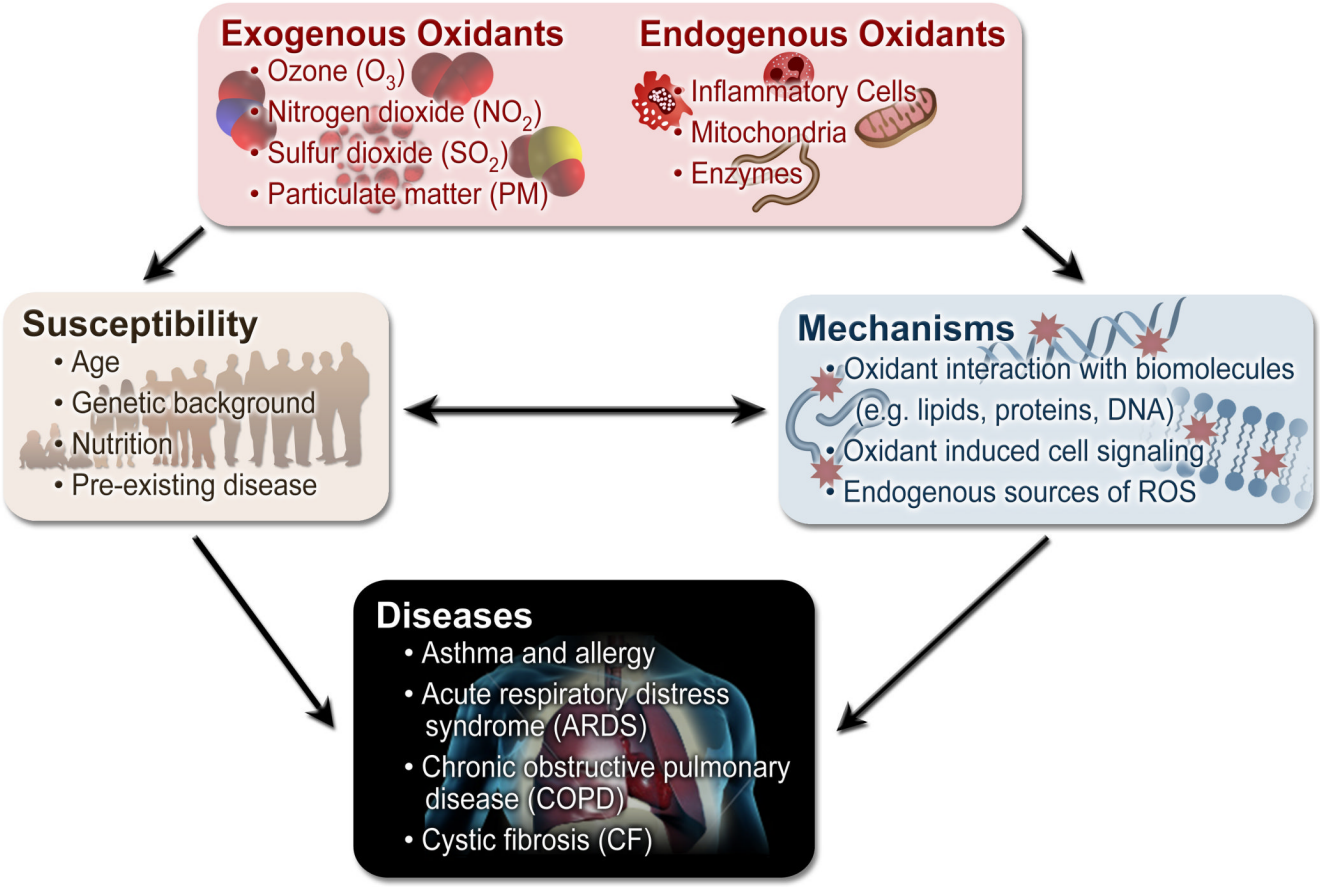


**Figure 2.**
Flowchart demonstrating the connection between oxidants and disease states, as well as the mechanisms and susceptibility factors involved in the development of oxidant-induced diseases.

**Table 1**

Summary of representative epidemiological investigations that have found effects of oxidant air pollutant exposures on pre- and post-neonatal outcomes in human populations.

| Age | Outcome | References |
|---|---|---|
| Pre-neonatal | Early fetal loss | Pereira[124]; Liu[125]; Sram[126] |
| | Pre-term delivery | Ritz[127]; Brauer[128]; Gessner[129] |
| | Low birth weight (<2,500 g) | Bobak[130]; Maisonet[131]; Bell[132]; Slama[133] |
| | Very low birth weight (<1,500 g) | Rogers[134]; Rogers[135] |
| | Birth defects | Ritz[136]; Brook[137]; Gilboa[138] |
| Post-neonatal | Mortality (respiratory disease) | Samet[139]; Ha[140]; Gliniana[141]; Woodruff[142] |
| | Mortality (SIDS) | Dales[143]; Tong[144]; Woodruff[142] |