Hindawi Publishing Corporation
Oxidative Medicine and Cellular Longevity
Volume 2012, Article ID 856918, 18 pages
doi:10.1155/2012/856918

Review Article

Lung Oxidative Damage by Hypoxia

O. F. Araneda[1] and M. Tuesta[2]

[1] *Laboratorio Integrativo de Biomecánica y Fisiología del Esfuerzo (LIBFE), Escuela de Kinesiología, Facultad de Medicina, Universidad de los Andes, Avenida San Carlos de Apoquindo 2200, Santiago, Chile*
[2] *Laboratorio de Fisiología del Ejercicio, Escuela de Kinesiología, Universidad Santo Tomás sede Viña del Mar, Avenida 1 Norte 3041, Viña del Mar, Chile*

Correspondence should be addressed to O. F. Araneda, ofaraneda@miuandes.cl

Received 14 May 2012; Accepted 11 July 2012

Academic Editor: Vincent Pialoux

One of the most important functions of lungs is to maintain an adequate oxygenation in the organism. This organ can be affected by hypoxia facing both physiological and pathological situations. Exposure to this condition favors the increase of reactive oxygen species from mitochondria, as from NADPH oxidase, xanthine oxidase/reductase, and nitric oxide synthase enzymes, as well as establishing an inflammatory process. In lungs, hypoxia also modifies the levels of antioxidant substances causing pulmonary oxidative damage. Imbalance of redox state in lungs induced by hypoxia has been suggested as a participant in the changes observed in lung function in the hypoxic context, such as hypoxic vasoconstriction and pulmonary edema, in addition to vascular remodeling and chronic pulmonary hypertension. In this work, experimental evidence that shows the implied mechanisms in pulmonary redox state by hypoxia is reviewed. Herein, studies of cultures of different lung cells and complete isolated lung and tests conducted *in vivo* in the different forms of hypoxia, conducted in both animal models and humans, are described.

1. Introduction

Lung's main function is the exchange of gases, hence, it is the organ which makes contact with the higher pressure of oxygen in our body. This is especially significant in the light of the known toxic effect of this gas, although there are precedents of lung malfunction under low oxygen pressure conditions or hypoxia.

The generation of hypoxia occurs when staying at high-altitude environments or when receiving mixtures of contaminated gases. Thus, this condition is also a factor in various lung pathological processes like in obstructive sleep apnea (OSA), acute lung injury, asthma attacks, atelectasis, chronic obstructive pulmonary disease, and idiopathic pulmonary hypertension. Lung hypoxia is related, in acute form, to the increase of the pulmonary artery pressure [1], epithelial malfunction [2, 3], edema [4, 5], and lung inflammation [6, 7]. Chronic hypoxia is related to vascular proliferation [8], increase of vascular reactivity [9], chronic pulmonary hypertension, and right heart failure [10–12]. Different lung diseases have identified the participation of reactive oxygen species (ROS) in their pathogenesis [13–16].

Historically, the study of oxidative damage has been linked to the increase of O_2 content in both environment and organs; one of the first times where the oxidative damage of an organ was probably studied may have been when the effect of hyperoxia on the lung tissue was reported [17–19]. Logic tells us that there is a need of O_2 presence for the generation of ROS and oxidative damage; likewise, it would be unlikely that oxidative damage occurs when this element is less available [20]. Currently, this paradigm has been changed by identifying hypoxia as the generator of ROS and oxidative damage for systems as well as specific organs. Although oxidative damage associated with hypoxia on the organism has been the subject of several studies [21–24], the knowledge of the effects on lung tissue is relatively poor, particularly in humans. The latter is probably because of the difficulty in obtaining samples of this organ.

The decrease of pO_2 is monitored by the pulmonary arteries smooth muscle cells (PASMCs); these cells react

to hypoxia favoring bronchial vasoconstriction [25]. An opposite effect occurs in the systemic circulation [26], where hypoxia favors vasodilatation of both arteries and veins [27, 28]. Pulmonary arterial vasoconstriction caused by hypoxia appears to be more effective when hypoxia is present in alveolar lumen regarding this stimulus in the lumen of arterioles and venules. This response to the decrease of alveolar pO_2 in ventilation (V) attempts to improve the diffusion of O_2 into the blood through changes in perfusion (Q), a specific feature of the lungs [29]. Alterations in the V/Q relationship will trigger compensatory hypoxic vasoconstriction, which will attempt to normalize the V/Q relationship [29]. This effect will be accompanied by an increase in alveolar pCO_2 and respiratory acidosis [29]. In this context, the maximum steady state of hypoxic pulmonary vasoconstriction occurs between 25 and 50 mmHg of alveolar pO_2; therefore, the blood redistribution is maximum at these levels. However, the magnitude of hypoxic pulmonary vasoconstriction can decrease with levels <25 mmHg resulting in a reduced blood flow redistribution, deteriorating the V/Q, and worsening hypoxemia [26]. This phenomenon is favored with the existence of a lung disease or exposure to altitude. Now, the increasing of ROS in hypoxic lung has an important role in stimulating PASMC contraction in addition to enhancing inflammation and to establishing oxidative damage as the case may be. This will depend on the severity and duration that hypoxia has with a wide range of effects from the absence of tissue damage to the dysfunction on epithelial and muscle cells associated with vascular remodeling and proliferation (see Figure 1). Current evidence suggests that the primary sensor of hypoxia for the development of pulmonary vasoconstriction is the PASMC mitochondria, which increases the production of ROS at low pressures of O_2, probably in the complex III of the electron transport chain. It is possible that there are secondary sensing mechanisms that contribute to this effect, which will increase the production of ROS during hypoxia such as sarcolemmal NADPH oxidasefrom pulmonary vasculature. Researchers have demonstrated an increase in the mitochondrial ROS generation in various tissues in response to hypoxia, including PASMC. The probability that lower concentrations of oxygen in lungs are sufficient to produce similar effects to those observed in other tissues at higher concentrations is true [26].

In this paper, the proposed mechanisms that explain the redox state changes induced by hypoxia are described (see Figure 1). Hypoxia is herein approached, in its different forms, as for exposure time and its varieties: normobaric, hypobaric, hypercapnic hypoxia or in the ischemia and reperfusion process. Furthermore, pharmacological substances [30–32] and measures (transgenic animals) that have been used to describe the participation of ROS in pulmonary function abnormalities for this condition are included, as well as in the search for strategies to mitigate the effects of the hypoxic pulmonary oxidative damage (see Figure 2). This paper also includes protocols of hypoxia performed in different types of lung cells, homogenized lung cells, pieces of tissue, vascular rings, experimental model of isolated-perfused lung, use of KO animals and noninvasive methods

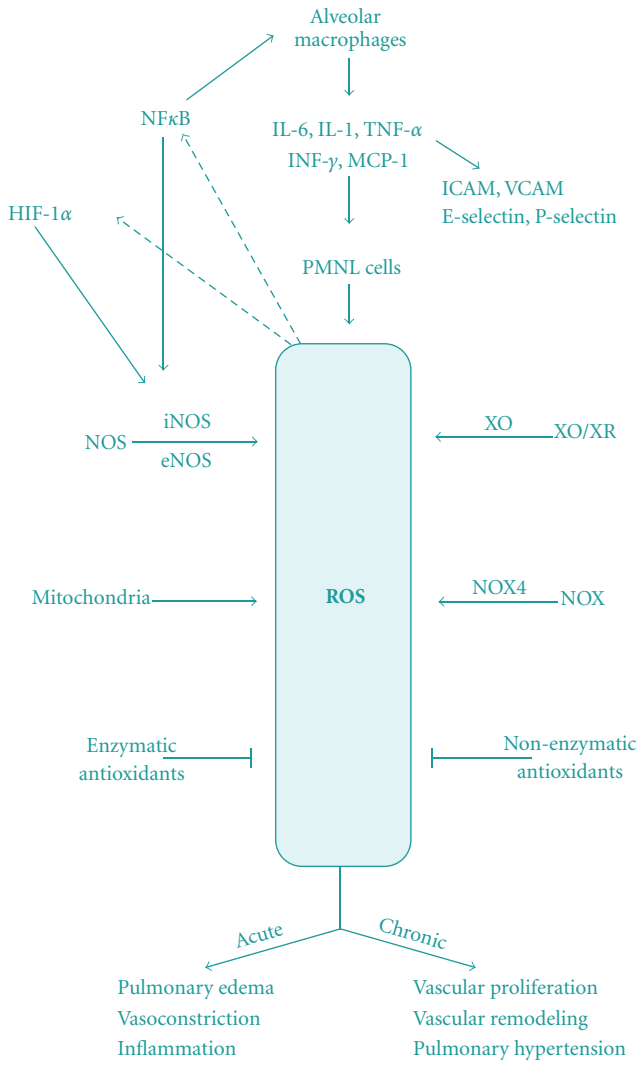

Figure 1: Proposed mechanisms (and effects) for the ROS generation in lungs exposed to hypoxia.

such as the analysis of directly exhaled air and exhaled breath condensate (EBC).

2. ROS

Living under aerobic conditions involves the formation of ROS. These substances have among their more commonly formed constituents superoxide, hydrogen peroxide, hydroxyl, and peroxyl, as well as nitric oxide and peroxynitrite. Some of these ROS contain unpaired electrons (free radicals) and may damage the different types of biomolecules that constitute us: lipids, proteins, carbohydrates, and nucleic acids. The interaction of biomolecules with ROS may occur in the physiological context as well as promoting cellular malfunction, so they have been involved with different diseases [33, 34]. The formation of ROS in the hypoxic context has been a subject of wide-ranging debate in both theory and reported results; the latter is probably influenced by used experimental models and methods by which ROS

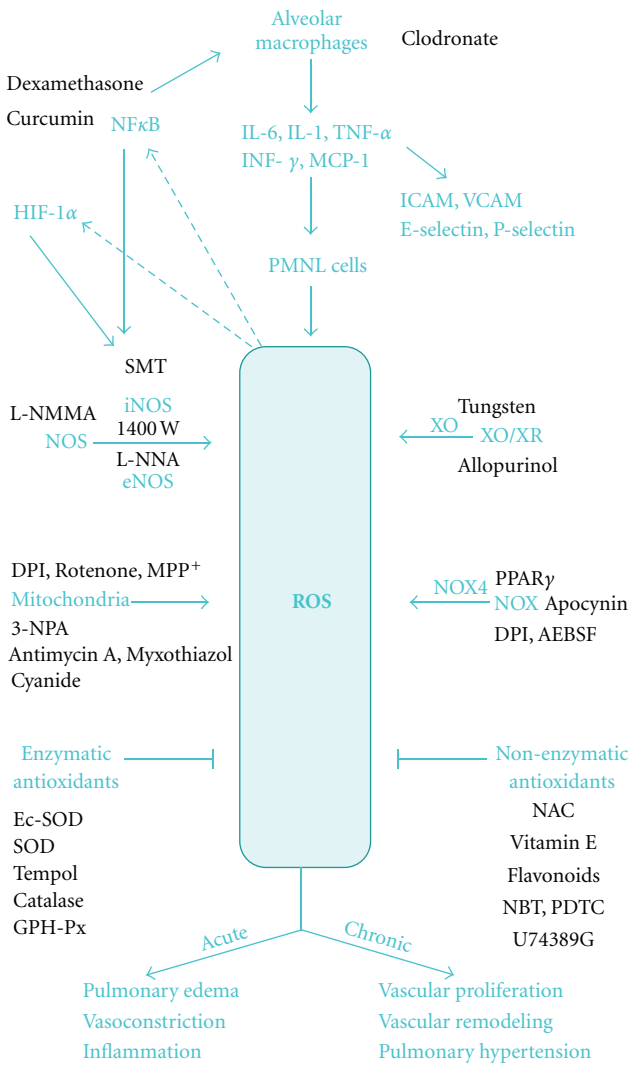

Figure 2: Inhibitors for ROS generation and antioxidants used to study the pulmonary oxidative damage by hypoxia. *NOX inhibitors*: diphenyleneiodonium (DPI) [39, 71–73, 91, 207]; peroxisome proliferator-activated receptor (PPARγ) [79]; 4-(2-Aminoethyl) benzenesulfonyl fluoride (AEBSF) [74]; apocynin [42, 207]. *NOS inhibitors*: N^G-monomethyl-L-arginine (L-NMMA): inhibitor of the three isoforms [31]; N-(3-(aminomethyl)benzyl) acetamidine (1400 W) and S-methylisothiourea sulfate (SMT): inhibitors of iNOS [31, 59]; L-NG-nitroarginine (L-NNA): inhibitor of eNOS and nNOS [59, 182]. *Mitochondrial inhibitors*: complex I: rotenone [38, 40]; diphenyleneiodonium (DPI) [39, 71–73, 91], 1-methyl-4-phenylpyridinium (MPP^+) [75]. Complex II: 3-nitropropionic acid thenoyltrifluoroacetone: (3-NPA) [208]. Complex III: antimycin A [38, 40, 208]; myxothiazol [40, 42]. Complex IV: cyanide [40]. *Enzymatic antioxidants*: Catalase [40, 45, 51, 91, 209]; SOD [37, 49, 91]; Ec-SOD [90]; glutathione peroxidase [49]. *Nonenzymatic antioxidants*: N-acetylcysteine (NAC) [89, 209]; vitamin E [4]; flavonoids [186]; nitro blue tetrazolium (NBT) [30–32]; pyrrolidine dithiocarbamate (PDTC) [42, 209]; U74389G [91]. *XO/XD inhibitors*: allopurinol [89]; tungsten [85]. *Others*: clodronate, acts by decreasing the number of macrophages.

have been determined. Evidence of oxidative damage by hypoxia, in the lungs, is consistent; however, the mechanisms about how it is produced are still being discussed. In this regard, several sources for the generation of ROS in hypoxia have been proposed as detailed below.

3. Mitochondria

This organelle is one of the sites of constant ROS production, particularly superoxide anion. This process specifically occurs in the mitochondrial electron transport chain (ETC), in complexes I and III [35, 36]. Some early reports showed a decrease in intracellular ROS formation by hypoxia, hence, Paky et al. [37], found a decrease in ROS formation and those came from the intracellular environment when the lucigenin assay was applied to a preparation with isolated perfused lung in anoxia (95% N_2 and 5% CO_2). Archer et al. [38] found an ROS decrease in isolated and perfused lungs by using luminol, when 2.5% O_2 during 6 min was applied. The same result was found when rotenone and antimycin A were applied, both ETC inhibitors in complexes I and III, respectively. A similar result was found in rat aortic rings at 36 mmHg pO_2, by using lucigenin [39]. In pulmonary artery rings, endothelium-denuded preparations were carried out and a decrease in ROS formation regarding normoxia (analyzed by lucigenin, 2′,7′-dichlorodihydrofluorescein diacetate (DCF), and Amplex Red) in hypoxia (40 mmHg PO_2) was found when rotenone and antimycin A were applied. This effect was not observed when cyanide was applied, an inhibitor of mitochondrial complex IV [40].

Another group of investigators have reported increases in ROS production in hypoxia. Grishko et al. [41], in cultures of pulmonary artery endothelial cells (PAECs), incubated at 25 mmHg O_2 for 15 min and found ROS increases with DCF. Waypa et al. [42] found an ROS increase in pulmonary artery smooth muscle cells (PASMCs) exposed to 2% O_2; this increase was inhibited by myxothiazol (blocker of mitochondrial complex III). Similar results were found by other authors; however, the use of lucigenin and DCF was questioned with regard to its properties to determine ROS properly [43]. Probably the use of more specific methodologies to measure ROS in the last few years, based on proteins that are sensitive to oxidation like HSP-FRET and roGFP, weighed the balance towards hypoxia, as a condition where ROS and oxidation increase [44–48]. Therefore, a decrease of the GSH/GSSG ratio was observed in cultures of PASMCs exposed to 1.5% O_2, which was inhibited by myxothiazol. Moreover, increase of cytoplasmic oxidation by hypoxia was found using HSP-FRET. This change of oxidation by hypoxia was inhibited by nonenzymatic antioxidants like pyrrolidine dithiocarbamate and N-acetylcysteine (NAC). The same effect was gained with the overexpression of mitochondrial catalase through a viral vector. Overexpression of SOD and the use of cyanide did not change the ROS increase by hypoxia [49]. By using the roGFP protein indicator in PASMCs, Waypa et al. [50] after administering 1.5% O_2 for 30 min found ROS increases in the mitochondrial intermembrane space and at a cytoplasm level, and ROS decreases in the mitochondrial matrix. Overexpression in these cells to cytoplasmic catalase

inhibited the ROS increases in this compartment [50]. In a subsequent report using rat lungs slices, Desireddi et al. [51], found an increase in cytosolic ROS by hypoxia (1.5% O_2). This increase was attenuated by the catalase overexpression induced by viral vectors [51]. The complete description of the ROS generation at a mitochondrial level was recently revised by Schumacker [52].

4. Nitric Oxide Synthase (NOS)

This enzyme is capable of forming free radical nitric oxide from L-arginine. This molecule has been involved in multiple processes both physiological (vasodilation and bronchodilation) and pathological (inflammation and oxidative damage) in lungs [15, 53]. In addition, it has been used as a drug [54]. NOS is presented in three isoforms, endothelial (eNOS), neuronal (nNOS), and inducible or iNOS. The expression of the two first ones depends on the activity of the calcium/calmodulin system, whereas the expression of iNOS is mainly stimulated in the context of an inflammatory process. Another recognized stimulating factor to the expression/activity of this enzyme is hypoxia. Xue et al. [10] found the increase of the expression of NOS in endothelium of small pulmonary vessels and vascular smooth muscle after exposing rats during 2 to 4 weeks at a 10% O_2. Shaul et al. [55] found increases of the expression of the nNOS and eNOS after 7 and 21 d of hypoxia, while Fagan et al. [56] described increases of the expression of eNOS and iNOS in lung homogenates of rats exposed during 6 weeks at 5,200 m. Probably in hypoxia the more active isoform that is related to vascular remodeling, observed in pulmonary hypertension, corresponds to the iNOS, which in normoxia has a poor role in the production of NO; however, it is increased in hypoxia as previously suggested [10, 57].

Rus et al. [58] subjected rats to hypobaric hypoxia at a barometric pressure of 225 mmHg during 30 min and subsequently to reoxygenation times from 0 to 5 d, finding in lung homogenates higher oxidative damage to proteins, lipids, and apoptosis increase. The administration of a selective inhibitor of iNOS, the most abundant isoform in the airways, N-(3-(aminomethyl) benzyl) acetamidine (1400 W), resulted in a decrease of nitrite and nitrate, besides decreasing the lipid peroxidation and oxidative damage to proteins. The inhibition of iNOS by 1400 W in a ventilated and isolated perfused lung model of rabbits implicated the decrease in the pulmonary artery pressure and a lower filtration of the pulmonary capillaries in a hypoxic (3% O_2) and hypercapnic (11% CO_2) environment, while the use of L-NG-nitroarginine (L-NNA) inhibitor of the eNOS and nNOS did not produce changes of vascular tone under this conditions [59].

In a model of rats subjected to hypoxia (10% O_2) and hypercapnia (6.5% CO_2) an increase of iNOS (in both mRNA and proteins) was found. In addition, lower quantities of mRNA, proteins and activity of soluble guanylate cyclase (NO effector) were found. This process is probably involved in the development of pulmonary hypertension by hypoxia; this report may be significant in those pathologies where these changes of gases' pressures coexist like in chronic obstructive pulmonary disease and sleep apnea.

5. NADPH Oxidase (NOX)

Its function is to form free radicals, particularly superoxide; the formation of this free radical begins with the transfer of electrons from NADPH to molecular oxygen [60–62]. This enzyme has a great functional relevance since it decreases the levels of nitric oxide when it reacts with it and forms peroxynitrite [63]. There are NOX isoforms, from 1 to 5, and DUOX 1 and 2 [61, 64]. From the structural composition point of view, lung tissue is formed by several cell types that give rise to the presence of more than one variety of NOX in this organ; endothelial cells express NOX1 and NOX5 but predominantly NOX2 and NOX4. Alveolar macrophages express NOX2, fibroblasts and vascular smooth muscles express NOX4, ciliated epithelial cells of the airways express DUOX1 and DUOX2, and type II alveolar cells express NOX1 [65, 66]. The expression of the different NOX isoforms in cells can be modified according to the conditions they are exposed to just as in the case of the increase of NOX4 in some tumor tissues [67]. Notwithstanding to a greater or lesser extent any of the isoforms present in the described cell types can participate by forming free radicals, and, in the case of lung tissue, it is known that the major source of free radicals derived from endothelium is originated in the NOX [68, 69].

PAECs obtained from bovines were exposed to a protocol of ischemia *in vitro* (24 h at a 3% O_2) in which increases of extracellular hydrogen peroxide were found. The production of this prooxidant was inhibited by diphenyleneiodonium (DPI) [70]. Marshall et al. [71] obtained, in rings of pulmonary arteries of cats, that the application of DPI favored the relaxation of hypoxic vasoconstriction induced by three series of gases in hypoxia (95% N_2, 5% CO_2). Thompson et al. [72], in pieces of rats pulmonary arteries, found that in one hour of hypoxia (95% N_2, 5% CO_2) an increase of the pressure within these vessels was observed and that this phenomenon was diminished by the application of DPI. Jones et al. [73] found that a relaxation of arteries was produced by DPI, in a similar preparation of rats arteries, to the same percentages of gases supplied in the previous study.

In preparations of isolated-perfused lungs of rabbits, exposure to series of 10 min at 3% of O_2, application of 4-(2-Aminoethyl) benzenesulfonyl fluoride (AEBSF), an inhibitor of the NADPH oxidase, resulted in a decrease of hypoxic vasoconstriction [74]. Archer et al. [39] found a large decrease of radicals' generation in perfused lungs of rats that lost the $gp91^{phox}$ subunit, after applying 2.5% O_2 during 30 min; however, the maintenance of the vasoconstrictor phenomenon to hypoxia was observed.

Weissmann et al. [75] reported in perfused, ventilated, and isolated lungs a decrease of the release of superoxide radicals measured by spin resonance by using the 1-hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine marker in deficient mice in $p47^{phox}$ subunit; however, when they were exposed for 30 min at 1% of O_2, an increase of superoxide in the perfusion solution was observed.

In models of chronic exposure, it has been reported that in rats exposed at 10% O_2 during 21 d an increase of NOX4 mRNA was found, while no differences for NOX2 mRNA were observed [11].

In a model of rats exposed during 3 weeks at 10% O_2, an increase of the production of superoxide from intrapulmonary artery rings was found, which was related to $gp91^{phox}$ subunit of NADPH oxidase. In addition, there was vascular remodeling and pulmonary hypertension that were not observed in KO rats for $gp91^{phox}$ subunit [76]. Rats were exposed to simulated altitude in hypobaric chamber at 380 mmHg during 21 d where an increase in the ROS' production and an increase in the vascular reactivity of intrapulmonary vessels segments were found; these results were not observed in KO rats for $gp91^{phox}$ subunit [9].

Ismail et al. [8] in culture of human PASMCs found an increase of NOX4, H_2O_2, and cell proliferation that was mediated by the beta type transforming factor when these cells were subjected at 1% O_2 during 72 h. In the same cell types and the same protocol of hypoxia, Lu et al. [77] found similar results in the expression of NOX4 and cell proliferation. Under hypoxia conditions, the expression of the transcription factor NF-κB increased and an increase of the association of this factor to the NOX4 promoter was found. These factors were eliminated when rosiglitazone was added, a pharmacological analogue of peroxisome proliferator-activated receptor (PPARγ), substance that has previously demonstrated to decrease the expression of endothelial NOX [78]. Nisbet et al. [79] demonstrated an increase of NOX4 mRNA and protein, besides an increase in the generation of superoxide anion and vascular remodeling with hypertrophy of the pulmonary artery in rats that were exposed to 10% O_2 during 3 weeks. The administration of PPARγ ligand, rosiglitazone, prevented these changes induced by hypoxia. It is likely that this animal model explains, in part, what happens in humans who suffer idiopathic pulmonary hypertension, since normal human PASMCs increased their NOX4 mRNA levels when they were exposed to 1% O_2 during 24 h and lung tissue of patients who suffer idiopathic pulmonary hypertension showed an increase of the expression of this isoform [11].

Nisbet et al. [80] performed a chronic intermittent hypoxia protocol in rats that simulated the desaturations observed in patients with OSA; for this purpose, the O_2 from 21% to 10% every 90 s during 8 weeks was decreased. After this treatment, an increase of the expression of NOX4 and $p22^{phox}$ in lung homogenates was observed. KO rats to $gp91^{phox}$ which were exposed to the simulated OSA protocol, did not show any changes in the expression of NOX4. These findings support the hypothesis that NOX is involved in vascular remodeling by hypoxia.

6. Xanthine Oxidase/Dehydrogenase

This enzyme is present in all endothelial cells of the organism and also in pulmonary vessels, participating in hypoxanthine and xanthine degradation to uric acid [81, 82]. Throughout this process, superoxide and hydrogen peroxide are formed. Dehydrogenase (XD) form, predominant in normal conditions, can be susceptible to oxidation and proteolysis transforming in oxidase form (XO) [83, 84]. In bovine PAECs exposed to 48 h of hypoxia (95% N_2 and 5% CO_2, balanced with ambient air), XO and XD activity increases was found. Also, an increase in superoxide formation to the medium was found, which was decreased by the use of allopurinol and tungsten, both of them inhibitors of the enzyme. Furthermore, enzymatic activity was inversely related to the O_2 pressure they were exposed to [85]. Hassoun et al. [86] found increases in XO activity, using *in vitro* bovine PAECs at 3% O_2 for 48 h. In the same study, a variation of XO/XD expression between species was reported [86]. Afterwards, the same increase of the XO and XO + XD activity at 3% O_2 for 48 h in PAECs *in vitro* was found. In addition, rats were exposed to 380 mmHg for 5 days, finding an increase in XO activity and the XO/XR ratio. The supplementation of L-arginine decreased the XO activity [87]. Hassoun et al. [88] found an increase in XO activity in lung homogenate in rats exposed to 380 mmHg for 24 h. This change was related to the increase of fluid content in the lungs of the animals. Rats exposed to 10% O_2 for 21 days showed an increase in XO activity from the first day that remained throughout the period. Also, greater lipid peroxidation in homogenates was observed. The use of allopurinol avoided the pulmonary hypertension and right ventricular remodeling [89].

After endothelial cells were exposed to hypoxia for 24 h (1% O_2), they showed a great increase in intracellular ROS production measured with CDF and that was related to an increase in XO concentration. In the same experimental series, transfected cells of the same type of cells overexpressing intracellular SOD did not show any increase in the ROS production or changes in the XO amount [90]. In a *in vivo* study, rats were exposed to 10% O_2 for 10 d and an increase in total leucocyte in BALF and in ROS production and XO amount in lung tissue was found. In transgenic rats overexpressing extracellular SOD, no evidence of this phenomenon was found [90]. In a model of chronic exposure to hypoxia (13% O_2) for up to 14 d in newborn rats, vascular proliferation increased and also vascular reactivity increased oxidative injury to lipids and proteins. The administration of allopurinol decreased at the fourth day the increase of XO, consequently decreasing oxidative tissue injury; the same process happened when administering Tempol, an analogous of SOD and U74389G, and a synthetic nonenzymatic antioxidant [91].

7. Lung Inflammation

The inflammatory process is one of the factors involved in the increase of ROS's generation and tissue oxidative damage; in turn, ROS are involved in the NF-κB release, a transcriptional factor that triggers the inflammatory process; HIF1-α has a central role in the response to hypoxia as well [92, 93]. Alveolar hypoxia corresponds to a proven stimulus that triggers inflammation that is first localized and later it becomes systemic; in this point, the alveolar macrophages'

role seems to be essential [6]. It has been previously demonstrated the increase of both the *in vitro* secretion of monocyte chemoattractant protein (MCP-1) and the tumor necrosis factor-alpha (TNF-α) derived from alveolar macrophages of rats exposed to 5% O_2 for 5 h [94]. Gonzalez et al. [95] observed a decrease of the inflammatory response activated by hypoxia after the administration of clodronate liposomes to deplete lung of alveolar macrophages in rats exposed to 10% O_2. In hypobaric hypoxia, an increase in number, proinflammatory activation, and release of ROS by alveolar macrophages has been described [96, 97]. Humans exposed to hypobaric chamber at 4,500 m for one hour during seven days evidenced leukocytosis with a predominance of polymorphonuclear neutrophils leukocytes (PMNLs) on the first day of protocol, while this was not observed on the seventh day. In addition, in *in vitro* PMNLs, which were stimulated by zymosan, the formation of basal superoxide at the end of the seventh day of intermittent altitude was increased and there were no changes in any stage in the formation of ROS by acute exposure [98]. The expression of CD18 and formation of superoxide increased in an exposure to 3,196 m. Training and altitude exposure decreased the capacity to form superoxide by PMNL [99]. One hour at 5% O_2 increased inflammatory cell adhesion to alveolar epithelial cells of rats. Thus, Beck-Schimer et al. [100] found an *in vitro* increase of ICAM-1 and VCAM-1, which was related to a higher PMNL and macrophages adhesion to alveolar cells. In lung homogenates of rats subjected to hypoxia (10% O_2) increases of myeloperoxidase (MPO), NF-κB activity, and mRNA of ICAM-1, VCAM-1, HIF-1α, macrophage inflammatory protein beta (MIP-1β), and MCP-1 were found. In BALF, an increase in both proteins and total number of cells was found. These changes were reduced by the depletion of alveolar macrophages using clodronate [94]. In rats exposed to 9.9% O_2 for 24 h, an increase in the PMNL number and TNF-α, IL-1 beta and IL-6 and MPO was found. At the same time, an increase in the generation of nitrite, nitrate, and hydroxyl radicals in BALF was evidenced. These changes were associated with the increase of the expression of HSP70 in lungs. The hypoxic preconditioning (18.3% O_2 during 5 days) decreased the formation of previously described ROS, nitrites, and, also, pulmonary edema [7]. Shukla et al. [101] exposed rats during 48 h at 7,619 m, observing an increase in ROS formation measured as DCF and lipid peroxidation (TBARs and 4-hydroxynonenal). These changes were associated with increases of TNF-α, interferon gamma and interferon alpha, as well as MCP-1 in BALF. In turn, an increase in mRNA and protein expression of HSP-32 and HSP70 was observed. Furthermore, the expression and activity of HIF-1α increased together with the increase of its targets EPO and GLUT-1. The use of $CoCl_2$, a stabilizer of HIF-1α [102] and an inducer of ischemic preconditioning [103], decreased both oxidative damage and inflammation of this organ by hypoxia.

Exposure of rats during 5 h at 9,142 m favors the generation of pulmonary edema and promotes the release of inflammatory mediators (MCP-1, IL-1, TNF-α, TGF-β, and IFN-γ) and adhesion molecules (ICAM-1, VCAM-1, and P-selectin) in BALF. These changes were associated with increases of NF-κB in lung homogenates. The administration of $CoCl_2$ reduced these effects [5]. Sarada et al. [104] found pulmonary edema and an increase of ROS (DCF), lipid peroxidation (MDA), NF-κB, IL-1, IL-6, and TNF-α and also ICAM, VCAM and P-selectins in lung homogenate of rats exposed at 7,620 m. Preconditioning with curcumin, an inhibitor of the expression of NF-κB, reduced edema and reduced the expression of NF-κB. Rats exposed for 5 h at 9,144 m, in hypobaric chamber, showed an increase of IL-6, IL-10, TNF-α, MCP-1, and VEFG in BALF. The administration of an ethanol extract of *Hippophae rhamnoides L.* leaves, rich in flavonoids, with known antioxidant and anti-inflammatory effect such as quercetin, epicatechin, and flavonols, or the administration of dexamethasone favored inhibition in the formation of proinflammatory factors, reduced edema, and decreased VEGF, the latter, a known promoter of the endothelial permeability increase. An increase of E-selectin after a cicloergometric exercise at 3,810 m has been reported in the BALF analysis in humans [105]. In the same kind of sample, subjects who had pulmonary edema at a moderate altitude (2,600–3,000 m) increased the amount of alveolar macrophages, lymphocytes, PMNL, and total proteins. An increase of IL-1β, IL-6, IL-8, and TNF-α was found; after recovery, these values returned to normal. A similar result was previously reported in four patients for the same cytokines in BALF at a similar height [106].

8. Pulmonary Antioxidant Defenses

The oxidizing effect of ROS can be countered by reducing agents called antioxidants, preventing their formation or removal and, hence, modeling the cellular redox state. Antioxidants can be classified according to their nature as enzymatic or *nonenzymatic* antioxidants. In lungs, the main enzymatic reducers are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and thioredoxin (TRx) [107, 108]. Among the *nonenzymatic* reducers we can find mucin, urate, glutathione (GSH), ascorbate, ceruloplasmin, transferrin, vitamin E, ferritin, and small molecules such as bilirubin [107, 109–113]. Regarding this category, in the central airways in lungs we can find mucopolypeptidic glycoproteins with high molecular weight, released by epithelial cells and glands, which are capable of increasing the amount of mucus. Furthermore, a thin protective film so called epithelial lining fluid (ELF) is deposited on the surface of the airways, which has a significant amount of both enzymatic and nonenzymatic antioxidants among its components [107, 114]. In the ELF, the most important *nonenzymatic* antioxidant contributions are mucin, urate, ascorbate, and GSH; among the enzymatic antioxidants are SOD, CAT, and GSH-Px [114].

9. Enzymatic Antioxidants

SOD has an important role in catalyzing the conversion of superoxide to H_2O_2 and O_2. This enzyme has three isoforms: the copper-zinc SOD (CuZnSOD), located in the nucleus,

cytoplasm, and peroxisomes, the manganese SOD (MnSOD) located in the mitochondria, and the extracellular SOD (Ec-SOD), which is outside of the plasmatic membrane of the lungs [115]. In hypoxia, MnSOD seems to be the target of modifications of this enzymatic variety, unlike the other isoforms. In this regard, Nakanishi et al. [116] found a decrease in the activity of MnSOD in rats' lung homogenates, after 5 d at 5,500 m height in hypobaric chamber. Subsequently, Cantin [117] observed a reduced activity of mitochondrial MnSOD after subjecting rats to 7% O_2 during 60 min. Russell and Jackson [118] observed an increase of the MnSOD protein expression and activity in lung homogenates of rabbits by using an *in vivo* model of hypoxia by lung collapse, during 7 d without subsequent reexpansion. On the other hand, when a group of rats was preconditioned to low concentrations of O_2 simulating a height of 5,500 m during 15 h, an increase of the activity and protein level of MnSOD but not of CuZnSOD was produced [119]. Rusell et al. [120] did not find any changes in MnSOD mRNA in alveolar type II cells after subjecting *in vivo* rabbits' lungs to collapse during 7 d without subsequent reexpansion. Russell et al. [121] did not observe increases in mRNA of mitochondrial MnSOD of the lungs in transgenic rats which overexpressed this enzyme, after being for 7 d at 10% O_2 under normobaria. However, when isolated and ventilated rats' lungs were subjected to subsequent reoxygenation to an anoxia period (10 min to 5% CO_2 and 95% N_2 and then reoxygenated 10 min to 21% O_2), the content of MnSOD mRNA in lung homogenate was increased [122]. These studies confirm the importance of MnSOD as one of the major lines of defense against pulmonary hypoxia. In addition, Ec-SOD isoform is the main extracellular enzymatic antioxidant in lungs [123, 124], with a known protector effect in the lining of the lungs fluids [125, 126] and at interstitial level and a high affinity to the extracellular matrix [127, 128] in rats and humans. In these studies, developed under hyperoxia, fibrosis, and hemorrhage conditions [128–131], the protective effects of Ec-SOD against oxidative damage by hypoxia have also been observed. Both this enzymatic antioxidant and GSH-Px are in charge of protecting the vulnerable targets to hypoxia-induced free radicals, such as alveolar epithelial cells and the endothelium of pulmonary capillaries [125]. Nozik-Grayck et al. [132] studied rats with pulmonary hypertension after chronic hypobaric hypoxia. The group of wild-type rats, after 1 d of exposure at a simulated height of 5,486 m, increased the activity of Ec-SOD and then it decreased at day 35. Transgenic rats that overexpressed Ec-SOD did not show any change in the activity of Ec-SOD and they protected the lung against vascular remodeling and hypertension. In another study, Giles et al. [133] observed a decrease in the expression of Ec-SOD at transcriptional and translational level in lung homogenates of rabbits that were subjected to hypobaric hypoxia (4,572 m for 36 h) at 26 d of gestation. As it happened with Ec-SOD, hypoxia limited the expression of pulmonary MnSOD. A recent study [90] has confirmed the potential inhibitor that the Ec-SOD overexpression has in the processes of pulmonary hypertension induced by hypoxia. According to the authors, the concept of ROS reduction could explain the reversibility of this phenomenon. In vivo, the hypoxic chamber animal model (10 d at 76 mmHg O_2) showed a significant decrease of ROS, right ventricular systolic pressure, and pulmonary vascular wall thickness in the group of transgenic mice that overexpressed Ec-SOD in comparison with the WT adult mice transfected with an empty vector. Other authors have recognized the contribution of Ec-SOD in other oxidative states such as COPD [134], exposure to cigarette smoke [126], and hyperoxia [135]. These results suggest that high concentrations of Ec-SOD present in lung fluids [125] play an essential protective role against the oxidative threat from exposure to external environment.

Catalase is mainly located in peroxisomes of most mammalian cells, but it can also be found in mitochondria and endoplasmic reticulum. This enzyme is responsible for catalyzing the decomposition of H_2O_2 to O_2 and water. In hypobaric hypoxia simulated in chamber, Nakanishi et al. [116] found an increasing trend without significant changes in the activity of CAT in lung homogenates of rats that stayed up to 21 d at 5,500 m. As it happened with MnSOD, Shen et al. [122] demonstrated increases of CAT mRNA subsequent to reoxygenation in a model of isolated lungs of rats subjected to hypoxia and reoxygenation. A recent investigation showed that the activity of CAT was significantly increased with regard to the control in lung homogenate of rats exposed to ischemic preservation. Besides, the increase of CAT was higher as the time of storage increased [136]. Both pregnant and nonpregnant rats exposed to 10% O_2 during 4 d did not show any variation of the activity of CAT [137].

CAT and GSH-Px share the function of controlling the concentrations of H_2O_2; they are the most outstanding cellular mechanisms (to detoxify hydroperoxides) for the neutralization of ROS [138]. Whenever the concentrations of H_2O_2 are in a high level, CAT becomes more effective than GSH-Px and vice versa [117]. Nevertheless, GSH-Px is capable of removing organic peroxides, such as those derived from lipid peroxidation [139]. To control the concentrations of H_2O_2, the enzymatic reaction of GSH-Px uses GSH, which acts as a reducer. Oxidized GSH (GSSG) will be reduced by glutathione reductase (GSH) with the help of NADPH. All glutathione peroxidases described in the body contain selenium, which include the following: the classic cytosolic shape present in all cells (cGSH-Px) [140–143], one associated with membrane phospholipids (PHGSH-Px) [144], other associated with gastro intestine (giGSH-Px) [145] and to extracellular (eGSH-Px) [146]. The latter is part of ELF and it is produced and secreted by alveolar epithelial cells and macrophages increasing lung antioxidant defense against oxidative stress as it occurs during hypoxia [147].

In lungs, antioxidant activity of GSH-Px in ELF is headed by eGSH-Px followed by cGSH-Px. Avissar et al. [147] showed a significant increase of the expression of eGSH-Px and cGSH-Px in a group of bronchial epithelial cell lines and primary human alveolar macrophages when both of them were subjected—*in vitro*—to oxidizing substances such as ozone (O_3). The immediate presence of eGSH-Px in the used medium places these cells and the interstitial cells as potential sources of pulmonary eGSH-Px. In human lungs subjected to oxidizing conditions, as in the case of

smokers, the activity and expression of eGSH-Px in ELF showed some increases [148, 149], the same occurred in asthmatic subjects who were in contact with cigarette smoke [150, 151]. Similar answers have been observed on hypoxia in studies that have quantified pulmonary GSH-Px. In rats placed into a simulated environment at 5,500 m, the activity of GSH-Px in lung homogenate was slightly increased and no significant differences until 21 d of exposure were presented [116]. Likewise, Yeginsu and Ergin [136] published findings about increases of GSH-Px in lungs of rats subjected to 12 h of ischemic preservation in cold solution at 4°C, reaching even lower values than the control group's when preserved at 48 h. On the contrary, Zhao et al. [152] observed a decrease in the activity of GSH-Px in lung homogenate of rabbits exposed to a simulated height at 8,500 m in barochamber during 3 h. Pregnant rats exposed to 10% O_2 during 4 d showed a decrease of the activity of GSH-Px, and no changes in non-pregnant rats were observed [137].

Comhair and Erzurum [146] claim that the transcription of mRNA, under oxidative pathological conditions, protein expression, and release of eGSH-Px to ELF are the most inducible at epithelial level in humans, just as it occurs in the presence of hypoxia.

TRX is a ubiquitous enzyme with a crucial role in controlling cellular redox environment [153]. The active site of this polypeptide has the -Cys-Gly-Pro-Cys-amino acid sequence. Functionally, TRX participates in redox regulation of numerous proteins such as NF-κB, HIF-1α, c-Fos/c-Jun complex, glucocorticoid receptor, estrogen receptor [154]. TRx is induced by the production of H_2O_2 demonstrating a potent cytoprotector effect. Likewise, human TRX (hTRx) protects cells from the increase of superoxide during ischemia-reperfusion (IR) in the reaction that converts hypoxanthine to xanthine by xanthine oxidase [155]. Fukuse et al. [156] observed an increase in the protective capacity of recombinant hTRx *in vivo* in rats of IR in a nonventilated lung during 75 min ischemia. The immediate administration to reperfusion of recombinant hTRx implied higher survival, improved gas exchange, decreased edema, and reduced pulmonary lipid peroxidation [156, 157]. Okubo et al. [158] in a similar protocol of IR administered hTRx, which reduced IR injury in rabbits probably by reduction of ROS. The protective effects in pulmonary IR have been corroborated by several authors. These studies suggest that hTRx could be effective as a radical eliminator in the lung reperfusion treatment, just as it occurs after transplantation [158–160].

10. *Nonenzymatic* Antioxidants

As previously mentioned, there are many *nonenzymatic* antioxidants in ELF that are capable of detoxifying ROS [161]. The latter are the first line of defense against inhaled oxidants from environments such as O_3, nitrogen oxides, and tobacco smoke or against changes in O_2 tension such as hypoxia or hyperoxia. One of them is mucin, which besides acting as an antioxidant regulates the viscosity of mucus [162]. It has been demonstrated that oxidative stress increases the expression and production of mucin, improving the antioxidant condition in the surface of the respiratory tract [163, 164]. Likewise, a decrease of GSH in the respiratory epithelium, just like in hypoxia, will favor the increase of viscosity in mucin. Under oxidative-inflammatory pathological conditions, such as cystic fibrosis and chronic bronchitis, excessive increase of mucus viscosity by production of mucin is considered a negative condition for the airways. Therefore, a projection in the therapeutic use of mucin as antioxidant must be carefully considered.

GSH, an abundant antioxidant in lung epithelial cells and in ELF [165, 166], is the key to modulate the development of inflammatory-oxidative pulmonary injury. Reduction of H_2O_2 and lipid hydroperoxides is carried out together with GSH-Px or peroxiredoxin. In both cases, GSH is converted in GSSG and quickly reduced by glutathione reductase and NADPH, or it is used in the process of protein folding in the endoplasmic reticulum. In the latter, GSSG is recycled by the protein disulfide isomerase and converted in GSH.

In hypoxia, Jenkinson et al. [167] measured GSSG in isolated perfused rat lungs. Firstly, a period of anoxia (95% N_2 and 5% CO_2) and subsequent reperfusion (95% O_2 and 5% CO_2) were performed. GSSG only was increased during reoxygenation in both perfusate and lung tissue. This suggested the presence of H_2O_2. In another study, Jackson and Veal [168] used a hypoxia and reoxygenation (collapse and reexpansion) model in lungs of rabbits. In this case, GSSG was significantly increased in the alveolar lavage fluid and lung tissue after 2 h of reexpansion subsequent to collapse; besides, a significant decrease of the total glutathione was observed.

On the other hand, a research carried out by White et al. [169] on isolated lungs of rats previously exposed to hypoxia demonstrated that the activity of the glutathione redox cycle was effective on pulmonary cytoprotection after increasing H_2O_2 by hyperoxia. This effect seems to depend on hexose-monophosphate derivation, since lungs previously exposed to hypoxia and that were perfused with oxidase glycogen had increased the reduction equivalents of H_2O_2 when compared to lungs that were preexposed to normoxia. This was reflected in the increasing of GSH/GSSG and NADPH/NADPH$^+$ ratios. Therefore, White et al. [169] proposed the existence of an adaptive process to previous hypoxia with more tolerance towards H_2O_2 by increasing of GSH and NADPH in lung tissue. In ELF, GSH concentrations in humans are at least 100 times greater than those found in plasma (0.5–5 μM) [165, 170, 171]. Low-molecular-weight antioxidants having greater concentrations in upper and lower respiratory tract are ascorbate and urate. GSH is present in ELF only in a significant manner. The latter does not play down the importance of the GSH function in the pulmonary oxidative control, since this is incorporated to one of the main antioxidant enzymatic systems [172]. Jackson et al. [173] found a decrease in mitochondrial GSH and MnSOD and an increase in the concentrations of lactate dehydrogenase enzyme and H_2O_2 in lung cell lines subjected to 1% O_2. As you can see, the pulmonary antioxidant system of GSH depends on the capacity of all its components, both enzymatic and *nonenzymatic*, in and out of the cell.

It is well known the importance of urate as a *nonenzymatic* antioxidant in human respiratory tract [174–177]. Nasal lining fluid has very similar levels as plasma (200–300 μM). Although there is high plasma concentration levels of urate, the theory of urate consumption by the bronchoalveolar or nasal fluid does not seem solved. In a study carried out by Van der Vliet et al. [172] no significant correlation of urate concentrations between plasma and ELF was obtained, which strengthens the presence of local maintenance mechanisms of urate.

Secretion of urate is carried out together with mucin, hence making evident its role in controlling the attacks of oxidant agents in the respiratory tract. Indeed, its main results in the antioxidant defense have been observed in oxidative control to O_3, where urate concentrations in nasal lining fluid significantly decrease after being exposed to this element [175]. With regard to hypoxia, there is no convincing evidence about its effects in the modification of urate concentrations in the respiratory tract. Deaton et al. [178] using an animal model for recurrent bronchial obstruction without inflammation did not find significant modifications in urate concentrations in EBC samples of horses. The lack of evidence makes it difficult to quantify and determine the antioxidant contribution of urate in airways.

Ascorbate (vitamin C) has a double role: sweeping the neutrophil oxidants away and reducing the oxidation of vitamin E [179]. The latter allows returning the antioxidant capacity to the vitamin E. Researchers have described different ascorbate concentrations in plasma and ELF obtained from both nasal region and bronchoalveolar zone [166, 180, 181]. The effects of hypoxia on ascorbate concentrations in lungs are scarce. Deaton et al. [178] observed an inverse correlation between H_2O_2 concentrations in EBC and ascorbate concentrations in ELF from horses affected by inflammatory recurrent airway obstruction.

11. Oxidative Damage in Lungs by Hypoxia

Oxidative stress to lipids is one of the most studied phenomena regarding oxidative stress by hypoxia. In that regard, a 75% increase in TBARs in rats initially exposed to 12% O_2 for 48 h and to 10% for 12.5 d was found in lung homogenates [183]. Kiliç et al. [182] found an increase in TBARs in lung homogenates in newborn rats exposed to 8% O_2 for 3 h. This process was prevented by administering a nitric oxide synthase inhibitor (NG-nitro-L-arginine) and by administering a precursor for its synthesis (L-arginine). Hoshikawa et al. [89] found an increase in lipid peroxidation measured as phosphatidylcholine hydroperoxide in lung homogenates after rats were exposed to 10% O_2 for four days. The administration of both N-acetylcysteine and allopurinol prevented the increase of lipid peroxidation. Minko et al. [4] found an increase in TBARs and conjugated dienes of lung homogenates in rats exposed to 6% O_2 during 120 min. This process was diminished by the application of intratracheal liposomes charged with α-tocopherol. Wilhelm et al. [184] found an increase of aldehydes in lung homogenates after being in a normobaric chamber with 10% O_2 for 5 d. Jankov et al. [91] exposed rats to 13% O_2 for 4, 7, or 14 d, finding an increase in lipid peroxidation measured as 8-isoprostane in lung homogenates. Rats exposed to a moderate altitude (1,500 m) during 8 weeks had increased MDA in their lung homogenates. The administration of polyphenol extract in another experimental group decreased hypoxia-induced lipid peroxidation [185]. After exposition to high altitude simulated in the hypobaric chamber for 5 h at 9,144 m, an MDA increase in lung homogenates of rats was found. This change was avoided in rats receiving dexamethasone or a leaf extract rich in flavonoids [186].

In regard to oxidation to hypoxia-induced protein, Rus et al. [187], subjected rats to hypobaric hypoxia to a 225 mmHg barometric pressure during 30 min and later to reoxygenation from 0 to 5 d, finding an increase in oxidative stress to proteins in lung homogenates measured as the increase of nitrotyrosine. In rats' lung sections, an increase of nitrotyrosine was also found as from the fourth day at 13% of O_2 [91]. In another report, newborn rats were exposed to the hyperoxia protocol with hypoxic events (65% O_2 with 10 hypoxic events of 8% O_2 for 10 min) during four weeks, finding an increase in carbonyl concentration in lung homogenates [188].

Regarding the oxidative damage to nucleic acids, an increase of oxidative damage to the gene promoter for VEGF and to the DNA recognition area for HIF-1α was reported in culture of PAECs and PASMCs exposed to 2% O_2 from 3 to 48 h [189]. In another similar protocol, PAECs cells remained in a hypoxic medium (2% O_2 and 5% CO_2) from 3 to 48 h. Using the endonuclease fingerprinting and ligation-mediated PCR techniques, the injuries to mitochondrial DNA and nuclear DNA were determined. In nuclear DNA the injury to the gene promoter region for VEGF was found [190].

12. Pulmonary Oxidative Damage by Hypoxia Assessed by Noninvasive Methods

Difficulties to obtain lung tissue samples are the reason of the low number of studies carried out in humans. For this reason, most of the information currently available regarding the effects of hypoxia on lung redox state changes is derived from studies carried out on animal models which have been previously reported. In order to find a solution to these limitations, non-invasive methods are arising and are currently being developed to study pulmonary diseases. By using these methodologies, the effects of hypoxia on ROS formation were studied, as well as the effects this condition generates over lung redox state.

In patients having respiratory distress, an H_2O_2 increase in EBC was found [191]. Similar results were found by Sznajder et al. [192] when describing H_2O_2 increase in patients having hypoxemic respiratory failure. Climbers increased its H_2O_2 concentration in EBC after descending a 6,125 m volcano. Another experience was carried out in biathletes that remained training at 2,800 m, and an increase in H_2O_2 concentration was found in the expired air [193]. Patients with OSA showed an H_2O_2 increase [194]. Malakasioti et al.

[195] found an increase of H_2O_2 concentration in EBC in children with the same disease. Previous studies, although in different contexts, share hypoxia as at least one of the main causes, hence making models with limitations. For this reason, studies developed in more controlled conditions, in animals, are complementary, such as the one carried out by Wilhelm et al. [97] where EBC of rats exposed to normobaric hypoxia for 7 d with 10% O_2 was obtained. In this condition, an increase of H_2O_2 concentration was found. A similar result was reported in subsequent studies with the same species and at the same O_2 fraction but under different exposed times: three days [196] and five days [184]. The measurements of H_2O_2 in EBC show various results that guarantee H_2O_2 increase in hypoxia; however, the origin of this marker has not been determined yet, due to many different sources that might be involved (NOX, mitochondria, inflammatory cells, and XO).

Another *prooxidant* widely determined in humans (at a lung level) is exhaled NO (ENO) [197, 198]. With regard to this marker, results are not even, and there are many studies demonstrating that nitric oxide in exhaled breath increases in relation to hypoxia. Hence, Mansoor et al. [199] found ENO increases with no differences in the concentrations of nitrite plus nitrate in EBC in subjects climbing 4,342 m. The administration of L-arginine did not generate modifications in both types of samples. Macinnis et al. [200] found ENO concentration increases after two hours in subjects remaining with 12% O_2 in normobaria. Furthermore, these increases were related to acute mountain sickness incidence, finding that ENO was lower in subjects developing the disease. In adult patients with OSA, an increase in ENO was found and was directly related to the total sleeping time with saturation of hemoglobin by O_2 below 90%.

There are other results indicating that ENO decreases or does not modify by hypoxia. In subjects breathing 12% O_2, ENO decrease was found after two hours in subjects susceptible to developing high altitude pulmonary edema. Moreover, the percentage change of ENO was correlated inversely with pulmonary artery pressure after 2 h [201]. Under hypobaric hypoxia conditions, Hemmingsson et al. [202] found that subjects had decreased ENO when exposed acutely to 5,000 m in a hypobaric chamber, meanwhile this phenomenon was not observed when carried out in a normobaric hypoxia laboratory (11.3% O_2). Donelly et al. [203] carried out ENO measurements in humans until reaching saturation of hemoglobin for 80% O_2 in the laboratory and then in a climb to 5,050 m in land conditions finding that ENO decreased in hypobaric hypoxia, meanwhile it remained the same in normobaric. In both situations, pulmonary artery pressure increased; therefore, authors came to the conclusion that ENO probably does not participate in tone regulation of pulmonary artery in hypoxia. In longer permanence in hypoxia as the one carried out by Güzel et al. [204] at 2,300 m for seven days, a decrease in ENO was also found. Miners staying sporadically (3 weeks in altitude versus 3 weeks at 800 m) for more than one year in altitude (between 3,600 m and 4,000 m) were measured at 4,000 m, finding a decrease in ENO. Children exposed chronically to altitude did not show differences in ENO—children from Aymara ethnic group versus children having European descent, although the first ones had lower pulmonary artery pressure. The diversity of the results, increased, decreased, or remaining levels of NO, shall be discussed as for the meaning of this parameter in different scenarios where hypoxia participates. All causes of different results are also not known, nevertheless, to a considerable extent, decreases have been attributed to the lack of O_2 (substratum to form NO). Consequently, Schmetterer et al. [20] found that 10 min administration of O_2 from 10% to 100% in humans, oxygen pressure, and ENO were directly related. Another cause is related to the type of hypoxia, hence, the results of hypobaric are not alike to normobaric [202, 203]. Finally, measurements of ENO have variations attributed to the method and equipment, requiring the modification of measurement methods, as it was suggested by Hemmingsson et al. [202].

Oxidative stress in lungs by hypoxia has also been measured in EBC samples; hence, Araneda et al. [205] found MDA increases measured in EBC after 10 d starting from 3,000 m in subjects climbing 6,125 m. In the same work, MDA concentration increases were found in the same type of samples in subjects that carried out a maximal cycloergometer exercise at 2,160 m altitude, meanwhile this same physical effort did not generate changes at 670 m. In a subsequent study, 8-isoprostane was determined in exhaled breath samples in biathletes that trained at 2,800 m for six weeks, finding an increase tendency of this marker [193]. Patients having OSA showed an 8-isoprostane concentration increase in EBC after using oxygen with continuous positive airway pressure. This parameter returned to normal values [194]. Similar results were found by Carpagnano et al. [206], also in patients with this same disease.

13. Conclusions

Early studies on hypoxia have reported the decrease of ROS. Nowadays, with the development of methodologies based on oxidation-sensitive proteins, it is known that ROS and oxidative damage increase in hypoxia. In lungs, ROS sources by hypoxia are multiple: in the mitochondrial ETC they are mainly formed in the complexes I and III, which increases ROS in the intermembrane space and cell cytoplasm. The enzyme XO/XD has a proven role as ROS generator by hypoxia in lungs, being, probably, one of the most important ROS sources. Another source is NOx, which has a large representation in lungs. Current records point out that, from the various isoforms, the ROS formation from this system is mainly originated in NOX4, this isoform being also associated with vascular remodeling and proliferation. From the three NOS isoforms, the inducible variety, activated in this context by NF-κB, HIF-1α, and ROS, has a clearer role in the ROS formation under hypoxic conditions. The inflammatory process, which is developed by hypoxia, provides alveolar macrophages that secrete proinflammatory substances and ROS, which promote the infiltration of PMNL, ROS secretor activators.

In regard to antioxidant systems, from all the works that have studied this compound, it is possible to conclude that there is great variety of results. SOD is the most studied antioxidant, showing modifications of mRNA, amounts and activity in both mitochondrial and extracellular varieties. Studies on animals overexpressing Ec-SOD are probably the ones that make the role of this isoform in hypoxia clearer for being a protector of the development of pulmonary hypertension by chronic hypoxia. Catalase and glutathione peroxidase have demonstrated a great variety of results, from nonshowing any variation to increases and decreases; although, in cultured cells transfected with these enzymes, a decrease in the formation of ROS by hypoxia has been observed.

In relation to TRx, available results do not allow directly knowing their modifications by hypoxia. We do know that their administration as a recombinant protein has a protector effect against ischemia and reperfusion of this organ. Regarding nonenzymatic antioxidants against hypoxia, there is almost exclusively information about glutathione and the decrease of the relation GSH/GSGG against hypoxia; besides, on the basis of its concentration, its protector role as part of the ELF is speculated.

It is a fact that the great difficulty for studying oxidative damage by hypoxia in lungs, in all species including humans, is because of the difficulties to obtain tissue, the current evidence being mostly obtained from studies conducted whether in cells and pieces of organs or isolated complete organs. In this sense, it is of utmost importance to check and transfer many of the results presented herein to whole bodies and humans, too. In order to accomplish this task, it is necessary to move forward in using and developing methodologies, minimally invasive for the study of this phenomenon, as well as developing new ways that allow direct studies on humans.

References

[1] J. T. Sylvester, L. A. Shimoda, P. I. Aaronson, and J. P. T. Ward, "Hypoxic pulmonary vasoconstriction," *Physiological Reviews*, vol. 92, no. 1, pp. 367–520, 2012.

[2] D. Bouvry, C. Planès, L. Malbert-Colas, V. Escabasse, and C. Clerici, "Hypoxia-induced cytoskeleton disruption in alveolar epithelial cells," *American Journal of Respiratory Cell and Molecular Biology*, vol. 35, no. 5, pp. 519–527, 2006.

[3] L. A. Dada and J. I. Sznajder, "Hypoxic inhibition of alveolar fluid reabsorption," *Advances in Experimental Medicine and Biology*, vol. 618, pp. 159–168, 2007.

[4] T. Minko, A. Stefanov, and V. Pozharov, "Selected contribution: lung hypoxia: antioxidant and antiapoptotic effects of liposomal α-tocopherol," *Journal of Applied Physiology*, vol. 93, no. 4, pp. 1550–1560, 2002.

[5] D. Shukla, S. Saxena, J. Purushothaman et al., "Hypoxic preconditioning with cobalt ameliorates hypobaric hypoxia induced pulmonary edema in rat," *European Journal of Pharmacology*, vol. 656, no. 1-3, pp. 101–109, 2011.

[6] N. C. Gonzalez and J. G. Wood, "Alveolar hypoxia-induced systemic inflammation: what low PO_2 does and does not do," *Advances in Experimental Medicine and Biology*, vol. 662, pp. 27–32, 2010.

[7] H. J. Lin, C. T. Wang, K. C. Niu et al., "Hypobaric hypoxia preconditioning attenuates acute lung injury during high-altitude exposure in rats via up-regulating heat-shock protein 70," *Clinical Science*, vol. 121, no. 5, pp. 223–231, 2011.

[8] S. Ismail, A. Sturrock, P. Wu et al., "NOX4 mediates hypoxia-induced proliferation of human pulmonary artery smooth muscle cells: the role of autocrine production of transforming growth factor-β1 and insulin-like growth factor binding protein-3," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 296, no. 3, pp. L489–L499, 2009.

[9] F. Fresquet, F. Pourageaud, V. Leblais et al., "Role of reactive oxygen species and gp91*phox* in endothelial dysfunction of pulmonary arteries induced by chronic hypoxia," *British Journal of Pharmacology*, vol. 148, no. 5, pp. 714–723, 2006.

[10] C. Xue, A. Rengasamy, T. D. Le Cras, P. A. Koberna, G. C. Dailey, and R. A. Johns, "Distribution of NOS in normoxic vs. hypoxic rat lung: upregulation of NOS by chronic hypoxia," *AM.J.PHYSIOL.*, vol. 267, no. 6, pp. L667–L678, 1994.

[11] M. Mittal, M. Roth, P. König et al., "Hypoxia-dependent regulation of nonphagocytic NADPH oxidase subunit NOX4 in the pulmonary vasculature," *Circulation Research*, vol. 101, no. 3, pp. 258–267, 2007.

[12] T. C. Resta, B. R. S. Broughton, and N. L. Jernigan, "Reactive oxygen species and RhoA signaling in vascular smooth muscle: role in chronic hypoxia-induced pulmonary hypertension," *Advances in Experimental Medicine and Biology*, vol. 661, pp. 355–373, 2010.

[13] I. Rahman and W. MacNee, "Oxidative stress and regulation of glutathione in lung inflammation," *European Respiratory Journal*, vol. 16, no. 3, pp. 534–554, 2000.

[14] P. A. Ward, "Oxidative stress: acute and progressive lung injury," *Annals of the New York Academy of Sciences*, vol. 1203, pp. 53–59, 2010.

[15] H. Sugiura and M. Ichinose, "Nitrative stress in inflammatory lung diseases," *Nitric Oxide*, vol. 25, pp. 138–144, 2011.

[16] B. M. Fischer, E. Pavlisko, and J. A. Voynow, "Pathogenic triad in COPD: oxidative stress, protease-antiprotease imbalance, and inflammation," *International Journal of Chronic Obstructive Pulmonary Disease*, vol. 6, pp. 413–421, 2011.

[17] B. A. Freeman and J. D. Crapo, "Hyperoxia increases oxygen radical production in rat lungs and lung mitochondria," *Journal of Biological Chemistry*, vol. 256, no. 21, pp. 10986–10992, 1981.

[18] L. L. Mantell, S. Horowitz, J. M. Davis, and J. A. Kazzaz, "Hyperoxia-induced cell death in the lung—the correlation of apoptosis, necrosis, and inflammation," *Annals of the New York Academy of Sciences*, vol. 887, pp. 171–180, 1999.

[19] A. Pagano and C. Barazzone-Argiroffo, "Alveolar cell death in hyperoxia-induced lung injury," *Annals of the New York Academy of Sciences*, vol. 1010, pp. 405–416, 2003.

[20] L. Schmetterer, K. Strenn, J. Kastner, H. G. Eichler, and M. Wolzt, "Exhaled NO during graded changes in inhaled oxygen in man," *Thorax*, vol. 52, no. 8, pp. 736–738, 1997.

[21] G. Celedón, G. González, C. P. Sotomayor, and C. Behn, "Membrane lipid diffusion and band 3 protein changes in human erythrocytes due to acute hypobaric hypoxia," *American Journal of Physiology. Cell Physiology*, vol. 275, no. 6, pp. C1429–C1431, 1998.

[22] D. M. Bailey, B. Davies, and I. S. Young, "Intermittent hypoxic training: implications for lipid peroxidation induced by acute normoxic exercise in active men," *Clinical Science*, vol. 101, no. 5, pp. 465–475, 2001.

[23] D. M. Bailey, G. R. Kleger, M. Holzgraefe, P. E. Ballmer, and P. Bärtsch, "Pathophysiological significance of peroxidative stress, neuronal damage, and membrane permeability in acute mountain sickness," *Journal of Applied Physiology*, vol. 96, no. 4, pp. 1459–1463, 2004.

[24] V. Pialoux, R. Mounier, E. Rock et al., "Effects of acute hypoxic exposure on prooxidant/antioxidant balance in elite endurance athletes," *International Journal of Sports Medicine*, vol. 30, no. 2, pp. 87–93, 2009.

[25] B. E. Marshall, C. Marshall, F. Frasch, and C. W. Hanson, "Role of hypoxic pulmonary vasoconstriction in pulmonary gas exchange and blood flow distribution. Physiologic concepts," *Intensive Care Medicine*, vol. 20, no. 4, pp. 291–297, 1994.

[26] J. T. Sylvester, L. A. Shimoda, P. I. Aaronson, and J. P. T. Ward, "Hypoxic pulmonary vasoconstriction," *Physiological Reviews*, vol. 92, no. 1, pp. 367–520, 2012.

[27] C. J. Ray, A. R. Abbas, A. M. Coney, and J. M. Marshall, "Interactions of adenosine, prostaglandins and nitric oxide in hypoxia-induced vasodilatation: in vivo and in vitro studies," *Journal of Physiology*, vol. 544, no. 1, pp. 195–209, 2002.

[28] A. H. M. Van Mil, A. Spilt, M. A. Van Buchem et al., "Nitric oxide mediates hypoxia-induced cerebral vasodilation in humans," *Journal of Applied Physiology*, vol. 92, no. 3, pp. 962–966, 2002.

[29] W. F. Boron and E. L. Boulpaep, *Medical Physiology: A Cellular and Molecular Approach*, Saunders/Elsevier, Philadelphia, Pa, USA, 2009.

[30] N. Weissmann, F. Grimminger, R. Voswinckel, J. Conzen, and W. Seeger, "Nitro blue tetrazolium inhibits but does not mimic hypoxic vasoconstriction in isolated rabbit lungs," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 274, no. 5, pp. L721–L727, 1998.

[31] N. Weissmann, S. Winterhalder, M. Nollen et al., "NO and reactive oxygen species are involved in biphasic hypoxic vasoconstriction of isolated rabbit lungs," *American Journal of Physiology*, vol. 280, no. 4, pp. L638–L645, 2001.

[32] R. Paddenberg, P. König, P. Faulhammer, A. Goldenberg, U. Pfeil, and W. Kummer, "Hypoxic vasoconstriction of partial muscular intra-acinar pulmonary arteries in murine precision cut lung slices," *Respiratory Research*, vol. 7, article no. 93, 2006.

[33] B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford, UK, 1999.

[34] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, and J. Telser, "Free radicals and antioxidants in normal physiological functions and human disease," *International Journal of Biochemistry and Cell Biology*, vol. 39, no. 1, pp. 44–84, 2007.

[35] M. R. Duchen, "Contributions of mitochondria to animal physiology: from homeostatic sensor to calcium signalling and cell death," *Journal of Physiology*, vol. 516, no. 1, pp. 1–17, 1999.

[36] J. F. Turrens, "Mitochondrial formation of reactive oxygen species," *Journal of Physiology*, vol. 552, no. 2, pp. 335–344, 2003.

[37] A. Paky, J. R. Michael, T. M. Burke-Wolin, M. S. Wolin, and G. H. Gurtner, "Endogenous production of superoxide by rabbit lungs: effects of hypoxia or metabolic inhibitors," *Journal of Applied Physiology*, vol. 74, no. 6, pp. 2868–2874, 1993.

[38] S. L. Archer, J. Huang, T. Henry, D. Peterson, and E. K. Weir, "A redox-based O_2 sensor in rat pulmonary vasculature," *Circulation Research*, vol. 73, no. 6, pp. 1100–1112, 1993.

[39] S. L. Archer, H. L. Reeve, E. Michelakis et al., "O_2 sensing is preserved in mice lacking the gp91*phox* subunit of NADPH oxidase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 14, pp. 7944–7949, 1999.

[40] E. D. Michelakis, V. Hampl, A. Nsair et al., "Diversity in mitochondrial function explains differences in vascular oxygen sensing," *Circulation Research*, vol. 90, no. 12, pp. 1307–1315, 2002.

[41] V. Grishko, M. Solomon, J. F. Breit et al., "Hypoxia promotes oxidative base modifications in the pulmonary artery endothelial cell VEGF gene," *The FASEB Journal*, vol. 15, no. 7, pp. 1267–1269, 2001.

[42] G. B. Waypa, N. S. Chandel, and P. T. Schumacker, "Model for hypoxic pulmonary vasoconstriction involving mitochondrial oxygen sensing," *Circulation Research*, vol. 88, no. 12, pp. 1259–1266, 2001.

[43] C. Rota, Y. C. Fann, and R. P. Mason, "Phenoxyl free radical formation during the oxidation of the fluorescent dye 2',7'-dichlorofluorescein by horseradish peroxidase. Possible consequences for oxidative stress measurements," *Journal of Biological Chemistry*, vol. 274, no. 40, pp. 28161–28168, 1999.

[44] C. T. Dooley, T. M. Dore, G. T. Hanson, W. C. Jackson, S. J. Remington, and R. Y. Tsien, "Imaging dynamic redox changes in mammalian cells with green fluorescent protein indicators," *Journal of Biological Chemistry*, vol. 279, no. 21, pp. 22284–22293, 2004.

[45] R. D. Guzy, B. Hoyos, E. Robin et al., "Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing," *Cell Metabolism*, vol. 1, no. 6, pp. 401–408, 2005.

[46] E. Robin, R. D. Guzy, G. Loor et al., "Oxidant stress during simulated ischemia primes cardiomyocytes for cell death during reperfusion," *Journal of Biological Chemistry*, vol. 282, no. 26, pp. 19133–19143, 2007.

[47] J. R. Lohman and S. J. Remington, "Development of a family of redox-sensitive green fluorescent protein indicators for use in relatively oxidizing subcellular environments," *Biochemistry*, vol. 47, no. 33, pp. 8678–8688, 2008.

[48] M. B. Cannon and S. James Remington, "Redox-sensitive green fluorescent protein: probes for dynamic intracellular redox responses. A review," *Methods in Molecular Biology*, vol. 476, pp. 50–64, 2009.

[49] G. B. Waypa, R. Guzy, P. T. Mungai et al., "Increases in mitochondrial reactive oxygen species trigger hypoxia-induced calcium responses in pulmonary artery smooth muscle cells," *Circulation Research*, vol. 99, no. 9, pp. 970–978, 2006.

[50] G. B. Waypa, J. D. Marks, R. Guzy et al., "Hypoxia triggers subcellular compartmental redox signaling in vascular smooth muscle cells," *Circulation Research*, vol. 106, no. 3, pp. 526–535, 2010.

[51] J. R. Desireddi, K. N. Farrow, J. D. Marks, G. B. Waypa, and P. T. Schumacker, "Hypoxia increases ros signaling and cytosolic Ca2+ in pulmonary artery smooth muscle cells of mouse lungs slices," *Antioxidants and Redox Signaling*, vol. 12, no. 5, pp. 595–602, 2010.

[52] P. T. Schumacker, "Lung cell hypoxia: role of mitochondrial reactive oxygen species signaling in triggering responses," *Proceedings of the American Thoracic Society*, vol. 8, no. 6, pp. 477–484, 2011.

[53] F. García-Río, R. Casitas, and D. Romero, "Utility of two-compartment models of exhaled nitric oxide in patients with asthma," *Journal of Asthma*, vol. 48, no. 4, pp. 329–334, 2011.

[54] A. Afshari, J. Brok, A. M. Møller, and J. Wetterslev, "Inhaled nitric oxide for acute respiratory distress syndrome and acute lung injury in adults and children: a systematic review with meta-analysis and trial sequential analysis," *Anesthesia and Analgesia*, vol. 112, no. 6, pp. 1411–1421, 2011.

[55] P. W. Shaul, A. J. North, T. S. Brannon et al., "Prolonged in vivo hypoxia enhances nitric oxide synthase type I and type III gene expression in adult rat lung," *American Journal of Respiratory Cell and Molecular Biology*, vol. 13, no. 2, pp. 167–174, 1995.

[56] K. A. Fagan, B. Morrissey, B. W. Fouty et al., "Upregulation of nitric oxide synthase in mice with severe hypoxia-induced pulmonary hypertension," *Respiratory Research*, vol. 2, no. 5, pp. 306–313, 2001.

[57] C. Xue and R. A. Johns, "Upregulation of nitric oxide synthase correlates temporally with onset of pulmonary vascular remodeling in the hypoxic rat," *Hypertension*, vol. 28, no. 5, pp. 743–753, 1996.

[58] A. Rus, F. Molina, M. A. Peinado, and M. L. Del, "Endothelial NOS-derived nitric oxide prevents injury resulting from reoxygenation in the hypoxic lung," *Free Radical Research*, vol. 44, no. 9, pp. 1027–1035, 2010.

[59] F. Ketabchi, H. A. Ghofrani, R. T. Schermuly et al., "Effects of hypercapnia and NO synthase inhibition in sustained hypoxic pulmonary vasoconstriction," *Respiratory Research*, vol. 13, Article ID 7, 2012.

[60] G. Cheng, Z. Cao, X. Xu, E. G. V. Meir, and J. D. Lambeth, "Homologs of gp91*phox*: cloning and tissue expression of Nox3, Nox4, and Nox5," *Gene*, vol. 269, no. 1-2, pp. 131–140, 2001.

[61] K. Bedard and K. H. Krause, "The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology," *Physiological Reviews*, vol. 87, no. 1, pp. 245–313, 2007.

[62] S. Pendyala and V. Natarajan, "Redox regulation of Nox proteins," *Respiratory Physiology and Neurobiology*, vol. 174, no. 3, pp. 265–271, 2010.

[63] J. S. Beckman and W. H. Koppenol, "Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly," *American Journal of Physiology. Cell Physiology*, vol. 271, no. 5, pp. C1424–C1437, 1996.

[64] M. Geiszt and T. L. Leto, "The Nox family of NAD(P)H oxidases: host defense and beyond," *Journal of Biological Chemistry*, vol. 279, no. 50, pp. 51715–51718, 2004.

[65] J. D. Lambeth, "Nox enzymes, ROS, and chronic disease: an example of antagonistic pleiotropy," *Free Radical Biology and Medicine*, vol. 43, no. 3, pp. 332–347, 2007.

[66] R. Damico, J. J. Zulueta, and P. M. Hassoun, "Pulmonary endothelial cell NOX," *American Journal of Respiratory Cell and Molecular Biology*, vol. 47, no. 2, pp. 129–139, 2012.

[67] K. Shimada, T. Fujii, S. Anai, K. Fujimoto, and N. Konishi, "ROS generation via NOX4 and its utility in the cytological diagnosis of urothelial carcinoma of the urinary bladder," *BMC Urology*, vol. 11, Article ID 22, 2011.

[68] B. M. Babior, "The NADPH oxidase of endothelial cells," *IUBMB Life*, vol. 50, no. 4-5, pp. 267–269, 2000.

[69] R. S. Frey, M. Ushio-Fukai, and A. B. Malik, "NADPH oxidase-dependent signaling in endothelial cells: role in physiology and pathophysiology," *Antioxidants and Redox Signaling*, vol. 11, no. 4, pp. 791–810, 2009.

[70] J. J. Zulueta, F. S. Yu, I. A. Hertig, V. J. Thannickal, and P. M. Hassoun, "Release of hydrogen peroxide in response to hypoxia-reoxygenation: role of an NAD(P)H oxidase-like enzyme in endothelial cell plasma membrane," *American Journal of Respiratory Cell and Molecular Biology*, vol. 12, no. 1, pp. 41–49, 1995.

[71] C. Marshall, A. J. Mamary, A. J. Verhoeven, and B. E. Marshall, "Pulmonary artery NADPH-oxidase is activated in hypoxic pulmonary vasoconstriction," *American Journal of Respiratory Cell and Molecular Biology*, vol. 15, no. 5, pp. 633–644, 1996.

[72] J. S. Thompson, R. D. Jones, T. K. Rogers, J. Hancock, and A. H. Morice, "Inhibition of hypoxic pulmonary vasoconstriction in isolated rat pulmonary arteries by diphengleneiodonium (DPI)," *Pulmonary Pharmacology and Therapeutics*, vol. 11, no. 1, pp. 71–75, 1998.

[73] R. D. Jones, J. S. Thompson, and A. H. Morice, "The NADPH oxidase inhibitors iodonium diphenyl and cadmium sulphate inhibit hypoxic pulmonary vasoconstriction in isolated rat pulmonary arteries," *Physiological Research*, vol. 49, no. 5, pp. 587–596, 2000.

[74] N. Weissmann, A. Tadić, J. Hänze et al., "Hypoxic vasoconstriction in intact lungs: a role for NADPH oxidase-derived H_2O_2?" *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 279, no. 4, pp. L683–L690, 2000.

[75] N. Weissmann, S. Zeller, R. U. Schäfer et al., "Impact of mitochondria and NADPH oxidases on acute and sustained hypoxic pulmonary vasoconstriction," *American Journal of Respiratory Cell and Molecular Biology*, vol. 34, no. 4, pp. 505–513, 2006.

[76] J. Q. Liu, I. N. Zelko, E. M. Erbynn, J. S. K. Sham, and R. J. Folz, "Hypoxic pulmonary hypertension: role of superoxide and NADPH oxidase (gp91*phox*)," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 290, no. 1, pp. L2–L10, 2006.

[77] X. Lu, T. C. Murphy, M. S. Nanes, and C. M. Hart, "PPARγ regulates hypoxia-induced Nox4 expression in human pulmonary artery smooth muscle cells through NF-κB," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 299, no. 4, pp. L559–L566, 2010.

[78] J. Hwang, D. J. Kleinhenz, H. L. Rupnow et al., "The PPARγ ligand, rosiglitazone, reduces vascular oxidative stress and NADPH oxidase expression in diabetic mice," *Vascular Pharmacology*, vol. 46, no. 6, pp. 456–462, 2007.

[79] R. E. Nisbet, J. M. Bland, D. J. Kleinhenz et al., "Rosiglitazone attenuates chronic hypoxia-induced pulmonary hypertension in a mouse model," *American Journal of Respiratory Cell and Molecular Biology*, vol. 42, no. 4, pp. 482–490, 2010.

[80] R. E. Nisbet, A. S. Graves, D. J. Kleinhenz et al., "The role of NADPH oxidase in chronic intermittent hypoxia-induced pulmonary hypertension in mice," *American Journal of Respiratory Cell and Molecular Biology*, vol. 40, no. 5, pp. 601–609, 2009.

[81] C. A. Partridge, F. A. Blumenstock, and A. B. Malik, "Pulmonary microvascular endothelial cells constitutively release xanthine oxidase," *Archives of Biochemistry and Biophysics*, vol. 294, no. 1, pp. 184–187, 1992.

[82] E. Garattini, R. Mendel, M. J. Romão, R. Wright, and M. Terao, "Mammalian molybdo-flavoenzymes, an expanding family of proteins: structure, genetics, regulation, function and pathophysiology," *Biochemical Journal*, vol. 372, no. 1, pp. 15–32, 2003.

[83] T. D. Engerson, G. McKelvey, and D. B. Rhyne, "Conversion of xanthine dehydrogenase to oxidase in ischemic rat tissues," *Journal of Clinical Investigation*, vol. 79, no. 6, pp. 1564–1570, 1987.

[84] A. Boueiz, M. Damarla, and P. M. Hassoun, "Xanthine oxidoreductase in respiratory and cardiovascular disorders,"

American Journal of Physiology. Lung Cellular and Molecular Physiology, vol. 294, no. 5, pp. L830–L840, 2008.

[85] L. S. Terada, D. M. Guidot, J. A. Leff et al., "Hypoxia injures endothelial cells by increasing endogenous xanthine oxidase activity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 8, pp. 3362–3366, 1992.

[86] P. M. Hassoun, F. S. Yu, A. L. Shedd et al., "Regulation of endothelial cell xanthine dehydrogenase/xanthine oxidase gene expression by oxygen tension," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 266, no. 2, pp. L163–L171, 1994.

[87] C. G. Cote, F. S. Yu, J. J. Zulueta, R. J. Vosatka, and P. M. Hassoun, "Regulation of intracellular xanthine oxidase by endothelial-derived nitric oxide," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 271, no. 5, pp. L869–L874, 1996.

[88] P. M. Hassoun, F. S. Yu, C. G. Cote et al., "Upregulation of xanthine oxidase by lipopolysaccharide, interleukin-1, and hypoxia: role in acute lung injury," *American Journal of Respiratory and Critical Care Medicine*, vol. 158, no. 1, pp. 299–305, 1998.

[89] Y. Hoshikawa, S. Ono, S. Suzuki et al., "Generation of oxidative stress contributes to the development of pulmonary hypertension induced by hypoxia," *Journal of Applied Physiology*, vol. 90, no. 4, pp. 1299–1306, 2001.

[90] M. N. Ahmed, Y. Zhang, C. Codipilly et al., "Extracellular superoxide dismutase overexpression can reverse the course of hypoxia-induced pulmonary hypertension," *Molecular Medicine*, vol. 18, no. 1, pp. 38–46, 2012.

[91] R. P. Jankov, C. Kantores, J. Pan, and J. Belik, "Contribution of xanthine oxidase-derived superoxide to chronic hypoxic pulmonary hypertension in neonatal rats," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 294, no. 2, pp. L233–L245, 2008.

[92] C. K. Sen and L. Packer, "Antioxidant and redox regulation of gene transcription," *FASEB Journal*, vol. 10, no. 7, pp. 709–720, 1996.

[93] Q. Li, N. Y. Spencer, F. D. Oakley, G. R. Buettner, and J. F. Engelhardt, "Endosomal Nox2 facilitates redox-dependent induction of NF-kB by TNF α," *Antioxidants and Redox Signaling*, vol. 11, no. 6, pp. 1249–1263, 2009.

[94] C. Madjdpour, U. R. Jewell, S. Kneller et al., "Decreased alveolar oxygen induces lung inflammation," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 284, no. 2, pp. L360–L367, 2003.

[95] N. C. Gonzalez, J. Allen, V. G. Blanco, E. J. Schmidt, N. Van Rooijen, and J. G. Wood, "Alveolar macrophages are necessary for the systemic inflammation of acute alveolar hypoxia," *Journal of Applied Physiology*, vol. 103, no. 4, pp. 1386–1394, 2007.

[96] F. Richards, P. Smith, and D. Heath, "The influence of hypoxia on the population density of alveolar macrophages in the lungs of rats," *Investigative and Cell Pathology*, vol. 3, no. 4, pp. 409–413, 1980.

[97] J. Wilhelm, M. Frydrychová, and M. Vízek, "Hydrogen peroxide in the breath of rats: the effects of hypoxia and paraquat," *Physiological Research*, vol. 48, no. 6, pp. 445–449, 1999.

[98] Y. Hitomi, M. Miyamura, S. Mori et al., "Intermittent hypobaric hypoxia increases the ability of neutrophils to generate superoxide anion in humans," *Clinical and Experimental Pharmacology and Physiology*, vol. 30, no. 9, pp. 659–664, 2003.

[99] A. Choukèr, F. Demetz, A. Martignoni et al., "Strenuous physical exercise inhibits granulocyte activation induced by high altitude," *Journal of Applied Physiology*, vol. 98, no. 2, pp. 640–647, 2005.

[100] B. Beck-Schimmer, R. C. Schimmer, C. Madjdpour, J. M. Bonvini, T. Pasch, and P. A. Ward, "Hypoxia mediates increased neutrophil and macrophage adhesiveness to alveolar epithelial cells," *American Journal of Respiratory Cell and Molecular Biology*, vol. 25, no. 6, pp. 780–787, 2001.

[101] D. Shukla, S. Saxena, P. Jayamurthy et al., "Hypoxic preconditioning with cobalt attenuates hypobaric hypoxia-induced oxidative damage in rat lungs," *High Altitude Medicine and Biology*, vol. 10, no. 1, pp. 57–69, 2009.

[102] L. Xi, M. Taher, C. Yin, F. Salloum, and R. C. Kukreja, "Cobalt chloride induces delayed cardiac preconditioning in mice through selective activation of HIF-1α and AP-1 and iNOS signaling," *American Journal of Physiology, Heart and Circulatory Physiology*, vol. 287, no. 6, pp. H2369–H2375, 2004.

[103] M. Matsumoto, Y. Makino, T. Tanaka et al., "Induction of renoprotective gene expression by cobalt ameliorates ischemic injury of the kidney in rats," *Journal of the American Society of Nephrology*, vol. 14, no. 7, pp. 1825–1832, 2003.

[104] S. Sarada, P. Himadri, C. Mishra, P. Geetali, M. S. Ram, and G. Ilavazhagan, "Role of oxidative stress and NFkB in hypoxia-induced pulmonary edema," *Experimental Biology and Medicine*, vol. 233, no. 9, pp. 1088–1098, 2008.

[105] M. W. Eldridge, R. K. Braun, K. Y. Yoneda, W. F. Walby, S. Bennett, and D. M. Hyde, "Lung injury after heavy exercise at altitude," *Chest*, vol. 114, no. 1, pp. S66–S67, 1998.

[106] K. Kubo, M. Hanaoka, S. Yamaguchi et al., "Cytokines in bronchoalveolar lavage fluid in patients with high altitude pulmonary oedema at moderate altitude in Japan," *Thorax*, vol. 51, no. 7, pp. 739–742, 1996.

[107] J. E. Heffner and J. E. Repine, "Pulmonary strategies of antioxidant defense," *American Review of Respiratory Disease*, vol. 140, no. 2 I, pp. 531–554, 1989.

[108] E. M. Bulger and R. V. Maier, "Antioxidants in critical illness," *Archives of Surgery*, vol. 136, no. 10, pp. 1201–1207, 2001.

[109] C. E. Cross, B. Halliwell, and A. Allen, "Antioxidant protection: a function of tracheobronchial and gastrointestinal mucus," *Lancet*, vol. 1, no. 8390, pp. 1328–1330, 1984.

[110] P. M. Finglas, A. Bailey, A. Walker, J. M. Loughridge, A. J. A. Wright, and S. Southon, "Vitamin C intake and plasma ascorbic acid concentration in adolescents," *British Journal of Nutrition*, vol. 69, no. 2, pp. 563–576, 1993.

[111] J. P. Eiserich, A. Van der Vliet, G. J. Handelman, B. Halliwell, and C. E. Cross, "Dietary antioxidants and cigarette smoke-induced biomolecular damage: a complex interaction," *American Journal of Clinical Nutrition*, vol. 62, no. 6, pp. 1490S–1500S, 1995.

[112] S. A. A. Comhair and S. C. Erzurum, "Antioxidant responses to oxidant-mediated lung diseases," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 283, no. 2, pp. L246–L255, 2002.

[113] I. Rahman and W. MacNee, "Lung glutathione and oxidative stress: implications in cigarette smoke-induced airway disease," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 277, no. 6, pp. L1067–L1088, 1999.

[114] A. M. Cantin, G. A. Fells, R. C. Hubbard, and R. G. Crystal, "Antioxidant macromolecules in the epithelial lining fluid of the normal human lower respiratory tract," *Journal of Clinical Investigation*, vol. 86, no. 3, pp. 962–971, 1990.

[115] R. J. Folz, J. Guan, M. F. Seldin, T. D. Oury, J. J. Enghild, and J. D. Crapo, "Mouse extracellular superoxide dismutase: primary structure, tissue-specific gene expression, chromosomal localization, and lung in situ hybridization," *American Journal of Respiratory Cell and Molecular Biology*, vol. 17, no. 4, pp. 393–403, 1997.

[116] K. Nakanishi, F. Tajima, A. Nakamura et al., "Effects of hypobaric hypoxia on antioxidant enzymes in rats," *Journal of Physiology*, vol. 489, no. 3, pp. 869–876, 1995.

[117] P. A. Cantin, "Oxidant and antioxidants in lung injury," in *Lam and Other Diseases Characterized By Smooth Muscle Proliferation*, J. Moss, Ed., pp. 519–531, Dekker, New York, NY, USA, 1999.

[118] W. J. Russell and R. M. Jackson, "MnSOD protein content changes in hypoxic/hypoperfused lung tissue," *American Journal of Respiratory Cell and Molecular Biology*, vol. 9, no. 6, pp. 610–616, 1993.

[119] T. F. Chou, M. C. Ma, C. P. Tsai, and C. F. Chen, "Enhancement of superoxide dismutase activity in rat lungs after hypoxic preconditioning," *Chinese Journal of Physiology*, vol. 52, no. 5, pp. 376–383, 2009.

[120] W. J. Russell, S. Matalon, and R. M. Jackson, "Manganese superoxide dismutase expression in alveolar type II epithelial cells from nonventilated and hypoperfused lungs," *American Journal of Respiratory Cell and Molecular Biology*, vol. 11, no. 3, pp. 366–371, 1994.

[121] W. J. Russell, Y. S. Ho, G. Parish, and R. M. Jackson, "Effects of hypoxia on MnSOD expression in mouse lung," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 269, no. 2, pp. L221–L226, 1995.

[122] C. Y. Shen, J. F. Lee, C. L. Su, D. Wang, and C. F. Chen, "Hypoxia and reoxygenation of the lung tissues induced mRNA expressions of superoxide dismutase and catalase and interventions from different antioxidants," *Transplantation Proceedings*, vol. 40, no. 7, pp. 2182–2184, 2008.

[123] C. L. Fattman, L. M. Schaefer, and T. D. Oury, "Extracellular superoxide dismutase in biology and medicine," *Free Radical Biology and Medicine*, vol. 35, no. 3, pp. 236–256, 2003.

[124] R. J. Folz and J. D. Crapo, "Extracellular superoxide dismutase (SOD3): tissue-specific expression, genomic characterization, and computer-assisted sequence analysis of the human EC SOD gene," *Genomics*, vol. 22, no. 1, pp. 162–171, 1994.

[125] I. Rahman, S. K. Biswas, and A. Kode, "Oxidant and antioxidant balance in the airways and airway diseases," *European Journal of Pharmacology*, vol. 533, no. 1-3, pp. 222–239, 2006.

[126] E. A. Regan, W. Mazur, E. Meoni et al., "Smoking and COPD increase sputum levels of extracellular superoxide dismutase," *Free Radical Biology and Medicine*, vol. 51, no. 3, pp. 726–732, 2011.

[127] T. D. Oury, L. Y. Chang, S. L. Marklund, B. J. Day, and J. D. Crapo, "Immunocytochemical localization of extracellular superoxide dismutase in human lung," *Laboratory Investigation*, vol. 70, no. 6, pp. 889–898, 1994.

[128] R. P. Bowler, J. Arcaroli, J. D. Crapo, A. Ross, J. W. Slot, and E. Abraham, "Extracellular superoxide dismutase attenuates lung injury after hemorrhage," *American Journal of Respiratory and Critical Care Medicine*, vol. 164, no. 2, pp. 290–294, 2001.

[129] R. J. Folz, A. M. Abushamaa, and H. B. Suliman, "Extracellular superoxide dismutase in the airways of transgenic mice reduces inflammation and attenuates lung toxicity following hyperoxia," *Journal of Clinical Investigation*, vol. 103, no. 7, pp. 1055–1066, 1999.

[130] E. Nozik-Grayck, H. B. Suliman, and C. A. Piantadosi, "Extracellular superoxide dismutase," *International Journal of Biochemistry and Cell Biology*, vol. 37, no. 12, pp. 2466–2471, 2005.

[131] F. Gao, V. L. Kinnula, M. Myllärniemi, and T. D. Oury, "Extracellular superoxide dismutase in pulmonary fibrosis," *Antioxidants and Redox Signaling*, vol. 10, no. 2, pp. 343–354, 2008.

[132] E. Nozik-Grayck, H. B. Suliman, S. Majka et al., "Lung EC-SOD overexpression attenuates hypoxic induction of Egr-1 and chronic hypoxic pulmonary vascular remodeling," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 295, no. 3, pp. L422–L430, 2008.

[133] B. L. Giles, H. Suliman, L. B. Mamo, C. A. Piantadosi, T. D. Oury, and E. Nozik-Grayck, "Prenatal hypoxia decreases lung extracellular superoxide dismutase expression and activity," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 283, no. 3, pp. L549–L554, 2002.

[134] R. E. Oberley-Deegan, E. A. Regan, V. L. Kinnula, and J. D. Crapo, "Extracellular superoxide dismutase and risk of COPD," *Journal of Chronic Obstructive Pulmonary Disease*, vol. 6, no. 4, pp. 307–312, 2009.

[135] R. J. Folz, A. M. Abushamaa, and H. B. Suliman, "Extracellular superoxide dismutase in the airways of transgenic mice reduces inflammation and attenuates lung toxicity following hyperoxia," *Journal of Clinical Investigation*, vol. 103, no. 7, pp. 1055–1066, 1999.

[136] A. Yeginsu and M. Ergin, "Antioxidant enzymes and lipid peroxidation in cold ischemic lung preservation," *Experimental and Clinical Transplantation*, vol. 7, no. 2, pp. 94–98, 2009.

[137] H. Mover-Lev and A. Ar, "Changes in enzymatic antioxidant activity in pregnant rats exposed to hyperoxia or hypoxia," *Comparative Biochemistry and Physiology C*, vol. 118, no. 3, pp. 353–359, 1997.

[138] B. P. Yu, "Cellular defenses against damage from reactive oxygen species," *Physiological Reviews*, vol. 74, no. 1, pp. 139–162, 1994.

[139] D. Ross, K. Norbeck, and P. Moldeus, "The generation and subsequent fate of glutathionyl radicals in biological systems," *Journal of Biological Chemistry*, vol. 260, no. 28, pp. 15028–15032, 1985.

[140] G. C. MILLS, "Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown," *The Journal of Biological Chemistry*, vol. 229, no. 1, pp. 189–197, 1957.

[141] L. Flohe, W. A. Gunzler, and H. H. Schock, "Glutathione peroxidase: a selenoenzyme," *FEBS Letters*, vol. 32, no. 1, pp. 132–134, 1973.

[142] E. Beutler and F. Matsumoto, "Ethnic variation in red cell glutathione peroxidase activity," *Blood*, vol. 46, no. 1, pp. 103–110, 1975.

[143] J. T. Rotruck, A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman, and W. G. Hoekstra, "Selenium: biochemical role as a component of glatathione peroxidase," *Science*, vol. 179, no. 4073, pp. 588–590, 1973.

[144] F. Ursini, M. Maiorino, and M. Valente, "Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides," *Biochimica et Biophysica Acta*, vol. 710, no. 2, pp. 197–211, 1982.

[145] F. F. Chu, J. H. Doroshow, and R. S. Esworthy, "Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI," *Journal of Biological Chemistry*, vol. 268, no. 4, pp. 2571–2576, 1993.

[146] S. A. A. Comhair and S. C. Erzurum, "The regulation and role of extracellular glutathione peroxidase," *Antioxidants and Redox Signaling*, vol. 7, no. 1-2, pp. 72–79, 2005.

[147] N. Avissar, J. N. Finkelstein, S. Horowitz et al., "Extracellular glutathione peroxidase in human lung epithelial lining fluid and in lung cells," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 270, no. 2, pp. L173–L182, 1996.

[148] J. E. Repine, A. Bast, and I. Lankhorst, "Oxidative stress in chronic obstructive pulmonary disease," *American Journal of Respiratory and Critical Care Medicine*, vol. 156, no. 2 I, pp. 341–357, 1997.

[149] S. A. A. Comhair, M. J. Lewis, P. R. Bhathena, J. P. Hammel, and S. C. Erzurum, "Increased glutathione and glutathione peroxidase in lungs of individuals with chronic beryllium disease," *American Journal of Respiratory and Critical Care Medicine*, vol. 159, no. 6, pp. 1824–1829, 1999.

[150] S. A. A. Comhair, P. R. Bhathena, R. A. Dweik, M. Kavuru, and S. C. Erzurum, "Rapid loss of superoxide dismutase activity during antigen-induced asthmatic response," *Lancet*, vol. 355, no. 9204, p. 624, 2000.

[151] S. A. A. Comhair, P. R. Bhathena, C. Farver, F. B. J. M. Thunnissen, and S. C. Erzurum, "Extracellular glutathione peroxidase induction in asthmatic lungs: evidence for redox regulation of expression in human airway epithelial cells," *FASEB Journal*, vol. 15, no. 1, pp. 70–78, 2001.

[152] H. Zhao, W. Chai, W. Gao, L. Xu, H. Zhang, and Y. Yang, "Hyperoxygenated solution: effects on acute hypobaric hypoxia-induced oxidative damage in rabbits," *High Altitude Medicine and Biology*, vol. 10, no. 3, pp. 283–291, 2009.

[153] A. Holmgren, "Thioredoxin and glutaredoxin systems," *Journal of Biological Chemistry*, vol. 264, no. 24, pp. 13963–13966, 1989.

[154] A. Holmgren, "Thioredoxin," *Annual Review of Biochemistry*, vol. 54, pp. 237–271, 1985.

[155] A. Spector, R. R. C. Huang, G. Z. Yan, and R. R. Wang, "Thioredoxin fragment 31-36 is reduced by dihydrolipoamide and reduces oxidized protein," *Biochemical and Biophysical Research Communications*, vol. 150, no. 1, pp. 156–162, 1988.

[156] T. Fukuse, T. Hirata, H. Yokomise et al., "Attenuation of ischaemia reperfusion injury by human thioredoxin," *Thorax*, vol. 50, no. 4, pp. 387–391, 1995.

[157] H. Yokomise, T. Fukuse, T. Hirata et al., "Effect of recombinant human adult T cell leukemia-derived factor on rat lung reperfusion injury," *Respiration*, vol. 61, no. 2, pp. 99–104, 1994.

[158] K. Okubo, S. Kosaka, N. Isowa et al., "Amelioration of ischemia-reperfusion injury by human thioredoxin in rabbit lung," *Journal of Thoracic and Cardiovascular Surgery*, vol. 113, no. 1, pp. 1–9, 1997.

[159] K. Yagi, H. Wada, T. Fukuse et al., "Inhibition of reperfusion injury by rADF/human thioredoxin in canine lung transplantation," *Transplantation Proceedings*, vol. 26, no. 2, pp. 878–879, 1994.

[160] H. Wada, T. Hirata, K. N. Decampos, S. Hitomi, and A. S. Slutsky, "Effect of the combination of human thioredoxin and L-cysteine on ischemia-reperfusion injury in isolated rat lungs," *European Surgical Research*, vol. 27, no. 6, pp. 363–370, 1995.

[161] C. A. Ballinger, R. Cueto, G. Squadrito et al., "Antioxidant-mediated augmentation of ozone-induced membrane oxidation," *Free Radical Biology and Medicine*, vol. 38, no. 4, pp. 515–526, 2005.

[162] M. Litt, "Rheological aspects of mucociliary flow," *Annals of the New York Academy of Sciences*, vol. 221, pp. 212–213, 1974.

[163] D. T. Wright, B. M. Fischer, C. Li, L. G. Rochelle, N. J. Akley, and K. B. Adler, "Oxidant stress stimulates mucin secretion and PLC in airway epithelium via a nitric oxide-dependent mechanism," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 271, no. 5, pp. L854–L861, 1996.

[164] K. Takeyama, K. Dabbagh, J. J. Shim, T. Dao-Pick, I. F. Ueki, and J. A. Nadel, "Oxidative stress causes mucin synthesis via transactivation of epidermal growth factor receptor: role of neutrophils," *Journal of Immunology*, vol. 164, no. 3, pp. 1546–1552, 2000.

[165] A. M. Cantin, S. L. North, R. C. Hubbard, and R. G. Crystal, "Normal alveolar epithelial lining fluid contains high levels of glutathione," *Journal of Applied Physiology*, vol. 63, no. 1, pp. 152–157, 1987.

[166] R. Slade, K. Crissman, J. Norwood, and G. Hatch, "Comparison of antioxidant substances in bronchoalveolar lavage cells and fluid from humans, guinea pigs, and rats," *Experimental Lung Research*, vol. 19, no. 4, pp. 469–484, 1993.

[167] S. G. Jenkinson, R. D. Black, and R. A. Lawrence, "Glutathione concentrations in rat lung bronchoalveolar lavage fluid: effects of hyperoxia," *Journal of Laboratory and Clinical Medicine*, vol. 112, no. 3, pp. 345–351, 1988.

[168] R. M. Jackson and C. F. Veal, "Effects of hypoxia and reoxygenation on lung glutathione system," *American Journal of Physiology, Heart and Circulatory Physiology*, vol. 259, no. 2, pp. H518–H524, 1990.

[169] C. W. White, J. H. Jackson, I. F. McMurtry, and J. E. Repine, "Hypoxia increases glutathione redox cycle and protects rat lungs against oxidants," *Journal of Applied Physiology*, vol. 65, no. 6, pp. 2607–2616, 1988.

[170] J. H. Roum, R. Buhl, N. G. McElvaney, Z. Borok, and R. G. Crystal, "Systemic deficiency of glutathione in cystic fibrosis," *Journal of Applied Physiology*, vol. 75, no. 6, pp. 2419–2424, 1993.

[171] A. Meyer, R. Buhl, S. Kampf, and H. Magnussen, "Intravenous N-acetylcysteine and lung glutathione of patients with pulmonary fibrosis and normals," *American Journal of Respiratory and Critical Care Medicine*, vol. 152, no. 3, pp. 1055–1060, 1995.

[172] A. Van Der Vliet, C. A. O'Neill, C. E. Cross et al., "Determination of low-molecular-mass antioxidant concentrations in human respiratory tract lining fluids," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 276, no. 2, pp. L289–L296, 1999.

[173] R. M. Jackson, A. L. Brannen, C. F. Veal, and J. D. Fulmer, "Superoxide dismutase and cytochrome oxidase in collapsed lungs: possible role in reexpansion edema," *Journal of Applied Physiology*, vol. 65, no. 1, pp. 235–241, 1988.

[174] D. B. Peden, R. Hohman, M. E. Brown et al., "Uric acid is a major antioxidant in human nasal airway secretions," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 87, no. 19, pp. 7638–7642, 1990.

[175] D. B. Peden, M. Swiersz, K. Ohkubo, B. Hahn, B. Emery, and M. A. Kaliner, "Nasal secretion of the ozone scavenger uric acid," *American Review of Respiratory Disease*, vol. 148, no. 2, pp. 455–461, 1993.

[176] C. E. Cross, A. Van Der Vliet, C. A. O'Neill, and J. P. Eiserich, "Reactive oxygen species and the lung," *Lancet*, vol. 344, no. 8927, pp. 930–933, 1994.

[177] C. E. Cross, A. Van der Vliet, C. A. O'Neill, S. Louie, and B. Halliwell, "Oxidants, antioxidants, and respiratory tract lining fluids," *Environmental Health Perspectives*, vol. 102, no. 10, pp. 185–191, 1994.

[178] C. M. Deaton, D. J. Marlin, N. C. Smith et al., "Breath condensate hydrogen peroxide correlates with both airway cytology and epithelial lining fluid ascorbic acid concentration in the horse," *Free Radical Research*, vol. 38, no. 2, pp. 201–208, 2004.

[179] J. E. Packer, T. F. Slater, and R. L. Willson, "Direct observation of a free radical interaction between vitamin E and vitamin C," *Nature*, vol. 278, no. 5706, pp. 737–738, 1979.

[180] M. H. Bui, A. Sauty, F. Collet, and P. Leuenberger, "Dietary vitamin C intake and concentrations in the body fluids and cells of male smokers and nonsmokers," *Journal of Nutrition*, vol. 122, no. 2, pp. 312–316, 1992.

[181] F. J. Kelly, M. Cotgrove, and I. S. Mudway, "Respiratory tract lining fluid antioxidants: the first line of defence against gaseous pollutants," *Central European journal of public health*, vol. 4, pp. 11–14, 1996.

[182] I. Kiliç, B. A. Kiliç, C. Güven, E. Demirpençe, and M. A. Akşit, "Role of nitric oxide in hypoxia-induced changes in newborn rats," *Biology of the Neonate*, vol. 78, no. 3, pp. 191–197, 2000.

[183] A. K. Reddy, R. E. Kimball, and S. T. Omaye, "Selected pulmonary biochemical and hematological changes produced by prolonged hypoxia in the rat," *Experimental and Molecular Pathology*, vol. 45, no. 3, pp. 336–342, 1986.

[184] J. Wilhelm, M. Vaňková, H. Maxová, and A. Šišková, "Hydrogen peroxide production by alveolar macrophages is increased and its concentration is elevated in the breath of rats exposed to hypoxia: relationship to lung lipid peroxidation," *Physiological Research*, vol. 52, no. 3, pp. 327–332, 2003.

[185] H. Zhan, J. Y. Lu, Q. J. Zhang, T. Li, and Y. M. Xin, "Protective effects of tea polyphenols on mild hypobaric hypoxia induced pulmonary free radical metabolic disorder in mice," *Hangtian Yixue Yu Yixue Gongcheng/Space Medicine and Medical Engineering*, vol. 14, no. 2, pp. 79–83, 2001.

[186] G. Suryakumar, J. Purushothaman, D. Shukla et al., "Modulation of hypoxia-induced pulmonary vascular leakage in rats by seabuckthorn (*Hippophae rhamnoides* L.)," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 574524, 13 pages, 2011.

[187] A. Rus, L. Castro, M. L. Del Moral, and A. Peinado, "Inducible NOS inhibitor 1400W reduces hypoxia/reoxygenation injury in rat lung," *Redox Report*, vol. 15, no. 4, pp. 169–178, 2010.

[188] V. Ratner, S. Slinko, I. Utkina-Sosunova, A. Starkov, R. A. Polin, and V. S. Ten, "Hypoxic stress exacerbates hyperoxia-induced lung injury in a neonatal mouse model of bronchopulmonary dysplasia," *Neonatology*, vol. 95, no. 4, pp. 299–305, 2009.

[189] K. A. Ziel, V. Grishko, C. C. Campbell, J. F. Breit, G. L. Wilson, and M. N. Gillespie, "Oxidants in signal transduction: impact on DNA integrity and gene expression," *FASEB Journal*, vol. 19, no. 3, pp. 387–394, 2005.

[190] V. Grishko, M. Solomon, G. L. Wilson, S. P. LeDoux, and M. N. Gillespie, "Oxygen radical-induced mitochondrial DNA damage and repair in pulmonary vascular endothelial cell phenotypes," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 280, no. 6, pp. L1300–L1308, 2001.

[191] S. R. Baldwin, C. M. Grum, and L. A. Boxer, "Oxidant activity in expired breath of patients with adult respiratory distress syndrome," *Lancet*, vol. 1, no. 8471, pp. 11–13, 1986.

[192] J. I. Sznajder, A. Fraiman, J. B. Hall et al., "Increased hydrogen peroxide in the expired breath of patients with acute hypoxemic respiratory failure," *Chest*, vol. 96, no. 3, pp. 606–612, 1989.

[193] I. Heinicke, A. Boehler, T. Rechsteiner et al., "Moderate altitude but not additional endurance training increases markers of oxidative stress in exhaled breath condensate," *European Journal of Applied Physiology*, vol. 106, no. 4, pp. 599–604, 2009.

[194] M. Petrosyan, E. Perraki, D. Simoes et al., "Exhaled breath markers in patients with obstructive sleep apnoea," *Sleep and Breathing*, vol. 12, no. 3, pp. 207–215, 2008.

[195] G. Malakasioti, E. Alexopoulos, C. Befani et al., "Oxidative stress and inflammatory markers in the exhaled breath condensate of children with OSA," *Sleep and Breathing*. In press.

[196] P. Hitka, M. Vízek, and J. Wilhelm, "Hypoxia and reoxygenation increase H_2O_2 production in rats," *Experimental Lung Research*, vol. 29, no. 8, pp. 585–592, 2003.

[197] L. E. Gustafsson, A. M. Leone, M. G. Persson, N. P. Wiklund, and S. Moncada, "Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans," *Biochemical and Biophysical Research Communications*, vol. 181, no. 2, pp. 852–857, 1991.

[198] C. Borland, Y. Cox, and T. Higenbottam, "Measurement of exhaled nitric oxide in man," *Thorax*, vol. 48, no. 11, pp. 1160–1162, 1993.

[199] J. K. Mansoor, B. M. Morrissey, W. F. Walby et al., "L-arginine supplementation enhances exhaled NO, breath condensate VEGF, and headache at 4342 m," *High Altitude Medicine and Biology*, vol. 6, no. 4, pp. 289–300, 2005.

[200] M. J. MacInnis, E. A. Carter, M. S. Koehle, and J. L. Rupert, "Exhaled nitric oxide is associated with acute mountain sickness susceptibility during exposure to normobaric hypoxia," *Respiratory Physiology and Neurobiology*, vol. 180, no. 1, pp. 40–44, 2012.

[201] T. Busch, P. Bärtsch, D. Pappert et al., "Hypoxia decreases exhaled nitric oxide in mountaineers susceptible to high-altitude pulmonary edema," *American Journal of Respiratory and Critical Care Medicine*, vol. 163, no. 2, pp. 368–373, 2001.

[202] T. Hemmingsson, A. Horn, and D. Linnarsson, "Measuring exhaled nitric oxide at high altitude," *Respiratory Physiology and Neurobiology*, vol. 167, no. 3, pp. 292–298, 2009.

[203] J. Donnelly, D. C. Cowan, D. J. Yeoman et al., "Exhaled nitric oxide and pulmonary artery pressures during graded ascent to high altitude," *Respiratory Physiology and Neurobiology*, vol. 177, no. 3, pp. 213–217, 2011.

[204] N. A. Güzel, H. Sayan, and D. Erbas, "Effects of moderate altitude on exhaled nitric oxide, erythrocytes lipid peroxidation and superoxide dismutase levels," *Japanese Journal of Physiology*, vol. 50, no. 2, pp. 187–190, 2000.

[205] O. F. Araneda, C. García, N. Lagos et al., "Lung oxidative stress as related to exercise and altitude. Lipid peroxidation evidence in exhaled breath condensate: a possible predictor

of acute mountain sickness," *European Journal of Applied Physiology*, vol. 95, no. 5-6, pp. 383–390, 2005.

[206] G. E. Carpagnano, S. A. Kharitonov, O. Resta, M. P. Foschino-Barbaro, E. Gramiccioni, and P. J. Barnes, "8-Isoprostane, a marker of oxidative stress, is increased in exhaled breath condensate of patients with obstructive sleep Apnea after night and is reduced by continuous positive airway pressure therapy," *Chest*, vol. 124, no. 4, pp. 1386–1392, 2003.

[207] F. Grimminger, N. Weissmann, R. Spriestersbach, E. Becker, S. Rosseau, and W. Seeger, "Effects of NADPH oxidase inhibitors on hypoxic vasoconstriction in buffer- perfused rabbit lungs," *American Journal of Physiology. Lung Cellular and Molecular Physiology*, vol. 268, no. 5, pp. L747–L752, 1995.

[208] N. Weissmann, N. Kuzkaya, B. Fuchs et al., "Detection of reactive oxygen species in isolated, perfused lungs by electron spin resonance spectroscopy," *Respiratory Research*, vol. 6, article no. 86, 2005.

[209] G. B. Waypa and P. T. Schumacker, "Role for mitochondrial reactive oxygen species in hypoxic pulmonary vasoconstriction," *Novartis Foundation Symposium*, vol. 272, pp. 176–192, 2006.