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John E. Repine, Aalt Bast, I.L.M. Lankhorst

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Oxidative Stress in Chronic Obstructive Pulmonary Disease

JOHN E. REPINE, AALT BAST, IDA LANKHORST, and The Oxidative Stress Study Group

University of Colorado Health Sciences Center, Denver, Colorado; Free University of Amsterdam, and Zambon Medical Research Department, Amersfoort, The Netherlands

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CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease (COPD) is a major worldwide health problem that has an increasing prevalence and mortality (1, 2). *Oxidative stress*, which can be defined as an increased exposure to oxidants and/or decreased antioxidant capacities, is widely recognized as a central feature of many diseases (3, 4). Considerable evidence now links COPD with increased oxidative stress (5, 6). The purpose of this review is to describe the role and origin of the oxidant–antioxidant disturbances that participate in the development of COPD. Our presentation also addresses ways of assessing the contribution of oxidants and identifies therapeutic approaches that could improve cellular oxidant–antioxidant balance in the lungs of COPD patients.

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Correspondence and requests for reprints should be addressed to John E. Repine, M.D., Webb-Waring Institute for Biomedical Research, 4200 East Ninth Avenue, Box C321, Denver, CO 80262.

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COPD is an obstructive airway disorder characterized by a slowly progressive and irreversible decrease in FEV₁ (1, 2, 7). FEV₁ decreases are caused by a narrowing of airway lumen diameters that develops as a result of varying perturbations in both airway and interstitial lung tissue. Airway abnormalities consist of increased wall thickening, intraluminal mucus accumulation, smooth muscle hypertrophy, and small airway lining fluid changes. Additional early lesions include inflammatory cell infiltration and goblet cell metaplasia.

The tissue component of emphysema is defined anatomically as the permanent destructive enlargement of airspaces distal to the terminal bronchioles with a concomitant loss of alveolar attachments (7). Emphysema is recognized *in vivo* by a decreased diffusing capacity (DL_{CO}) and reduced lung parenchymal density on chest radiograph and high-resolution computerized tomography.

Chronic bronchitis, a frequent feature of COPD, is a persistent recurrent bronchial hypersecretion that causes expectoration on most days for a minimum of 3 mo per year for at least two successive years (7). The pathology of chronic bronchitis is not unequivocal but primarily includes large airway mucus gland hyperplasia and inflammation; however, hypersecretion may occur without airway obstruction.

Patients with COPD often manifest some reversibility of airway obstruction following treatment with bronchodilators and airway hyperresponsiveness when given constrictor stimuli (8). These similar features often cause difficulties in differentiating COPD from asthma, especially in older patients. However, asthma is usually not related to cigarette smoking, and the *reversibility* of the obstructive pattern and airway hyperresponsiveness is a more common and more prominent occurrence in asthma than in COPD. In addition, emphysema and chronic hypoxemia are usually absent in asthma (8, 9).

Prevalence and Clinical Course

COPD is rare before the age of 40, but after that age symptoms of hypersecretion occur with increasing frequency. Persistent airflow obstruction becomes more common at around age 60. The prevalence of COPD then increases progressively until 60–70 yr of age when, in large part due to mortality, it becomes more stable. The distribution of COPD in the United States and western Europe shows a similar pattern with respect to age, with an estimated prevalence of more than 10% in individuals who are at least 50 yr of age. The greater prevalence of COPD in men has diminished recently because of increased smoking by women (10).

The clinical manifestations and progression of COPD are influenced by a number of presumed risk factors, which include alpha₁-antitrypsin deficiency, recurrent bronchopulmonary infections, air pollution, socioeconomic status, lower

birth weight, and a history of severe childhood respiratory infections (11, 12). However, the most striking relationship is between cigarette smoking and COPD. Nearly 90% of all COPD patients are smokers (12, 13). Yet, for unknown reasons, only about 20% of cigarette smokers develop COPD. Smoking may also compound the detrimental effects of inhaled environmental toxins, and vice versa (14).

Because the symptoms and signs of COPD are variable and often attributed mistakenly to increasing age or other conditions, abnormalities in pulmonary function are frequently diagnosed very late or not at all. This is regrettable since earlier diagnosis could be made by measurement of pulmonary function. Assessment of FEV_1/FVC is considered the most sensitive indicator for early airway obstruction, and an accelerated decline in pulmonary function can be reliably detected from repeated yearly measurements. Obviously, a great need exists for more effective smoking cessation approaches and/or a safe drug that could reduce the annual decline in FEV_1 in COPD patients.

OXIDATIVE STRESS AND COPD

Oxygen Radical–Antioxidant Chemistry

The well-described chemistry of oxygen radicals and antioxidants is depicted in Figure 1 (4, 15). Superoxide anion (O_2^-) formation from oxygen is the first step. O_2^- is generated primarily by mitochondrial metabolism, molybdenum hydroxylase (xanthine, sulfite, and aldehyde oxidases) reactions, arachidonic acid metabolism, and NADPH oxidase-dependent processes in phagocytic cells. Reaction of O_2^- and hydrogen peroxide (H_2O_2) in the presence of transition metal, usually ferrous iron (Fe^{++}), produces the hydroxyl radical ($\cdot OH$). When catalyzed by neutrophil myeloperoxidase (MPO), H_2O_2 and a chloride form hypochlorous acid (HOCl). $\cdot OH$ and HOCl are emphasized because both are extremely potent oxidants. H_2O_2 gains significance as a central precursor to both $\cdot OH$ and HOCl (3, 4, 15).

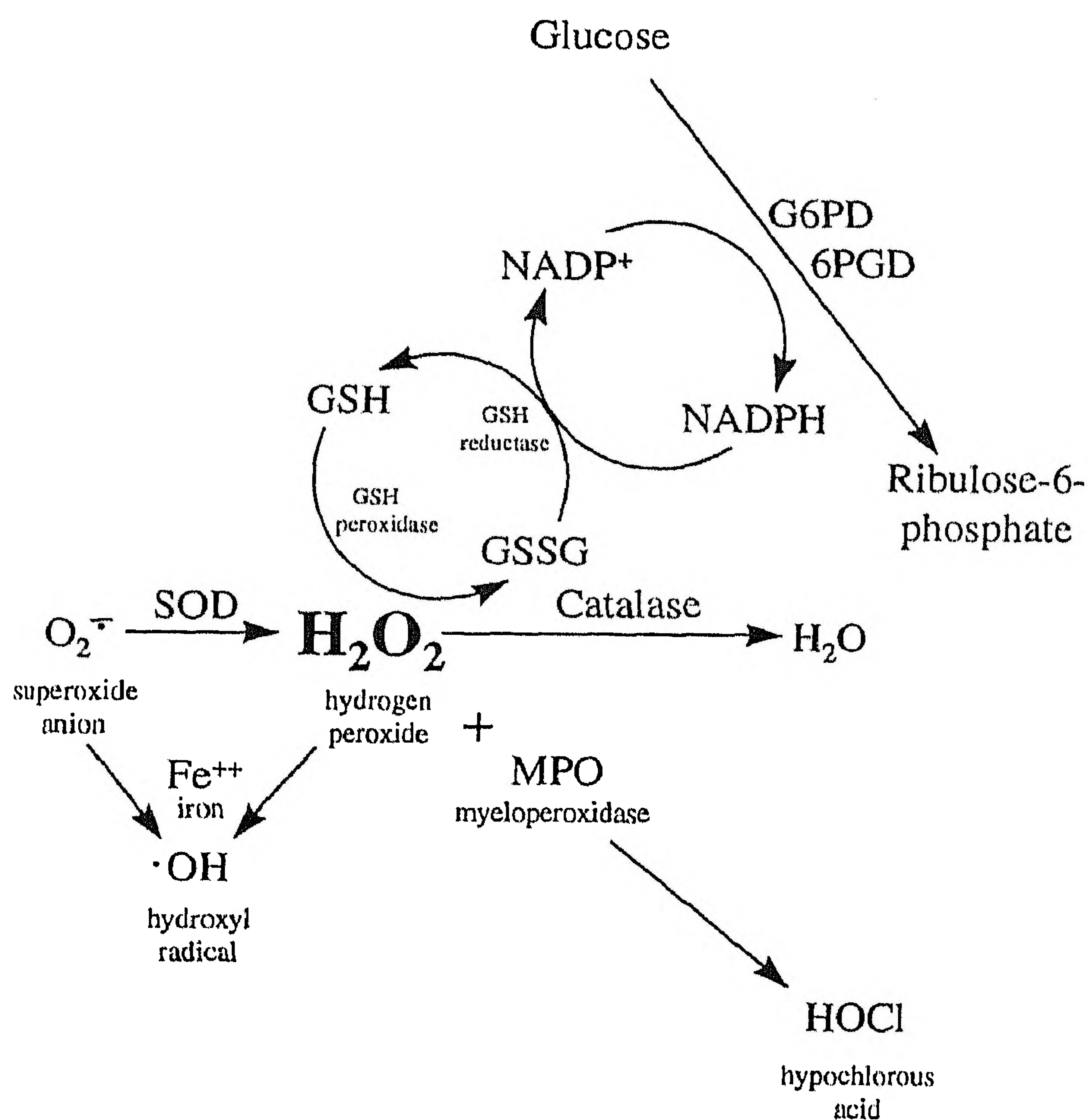


Figure 1. Basic oxygen radical and antioxidant chemistry.

The contribution of iron has become increasingly meaningful in understanding the development of COPD. Iron concentrations were increased in alveolar macrophages (AM) of cigarette smokers, and lung lining fluids obtained from cigarette smokers contained substantially more iron than specimens from nonsmokers (16). Excess iron also appeared to be concentrated in the upper lobes of cigarette smokers (17). The source of the increased iron in lungs of smokers is unknown, but each cigarette contains $0.042 \mu g$ of iron (18). Iron also accumulates progressively with age in men and in postmenopausal women—intriguingly, at the time when COPD worsens in both sexes (Figure 2) (19). AM from cigarette smokers also released more iron than AM from nonsmokers *in vitro* (20). In addition, saturated free fatty acids (mainly stearic and palmitic) concentrated from cigarette smoke bound and transferred ferrous iron into organic phases (21) and enhanced production of HOCl by stimulated polymorphonuclear neutrophils (PMN) *in vitro* (22). Because of its high reactivity, iron is normally bound to various iron-binding compounds, such as transferrin, ceruloplasmin, and ferritin (23). However, this protective mechanism may be disturbed in the lungs of COPD patients, since cigarette smoke and oxidants can release iron from ferritin (24, 25).

Pulmonary antioxidant defenses are widely distributed and include both enzymatic and nonenzymatic systems (3, 4). The major enzymatic antioxidants are superoxide dismutase (SOD), which degrades O_2^- and catalase, and the glutathione (GSH) redox system, which inactivates H_2O_2 and hydroperoxides (Figure 1). Three forms of SOD may be important: manganese SOD, which is located in mitochondria, Cu-Zn SOD, which resides in the cytoplasm, and extracellular SOD, which lines blood vessels. Another important element is glutathione (GSH), which is a water-soluble, low-molecular-weight tripeptide (L- γ -glutamyl-L-cysteinyl glycine) that is present in high concentrations in each cell. GSH is also present extracellularly and is particularly abundant in lung epithelial lining fluids (ELF). Indeed, GSH concentrations in ELF exceed plasma levels by approximately 100-fold (26, 27). In its antioxidant capacity, GSH forms intermolecular disulphide nonradical end-product-oxidized glutathione (GSSG). GSH is also a cofactor for various enzymes that decrease oxidative stress (3, 4, 15). In

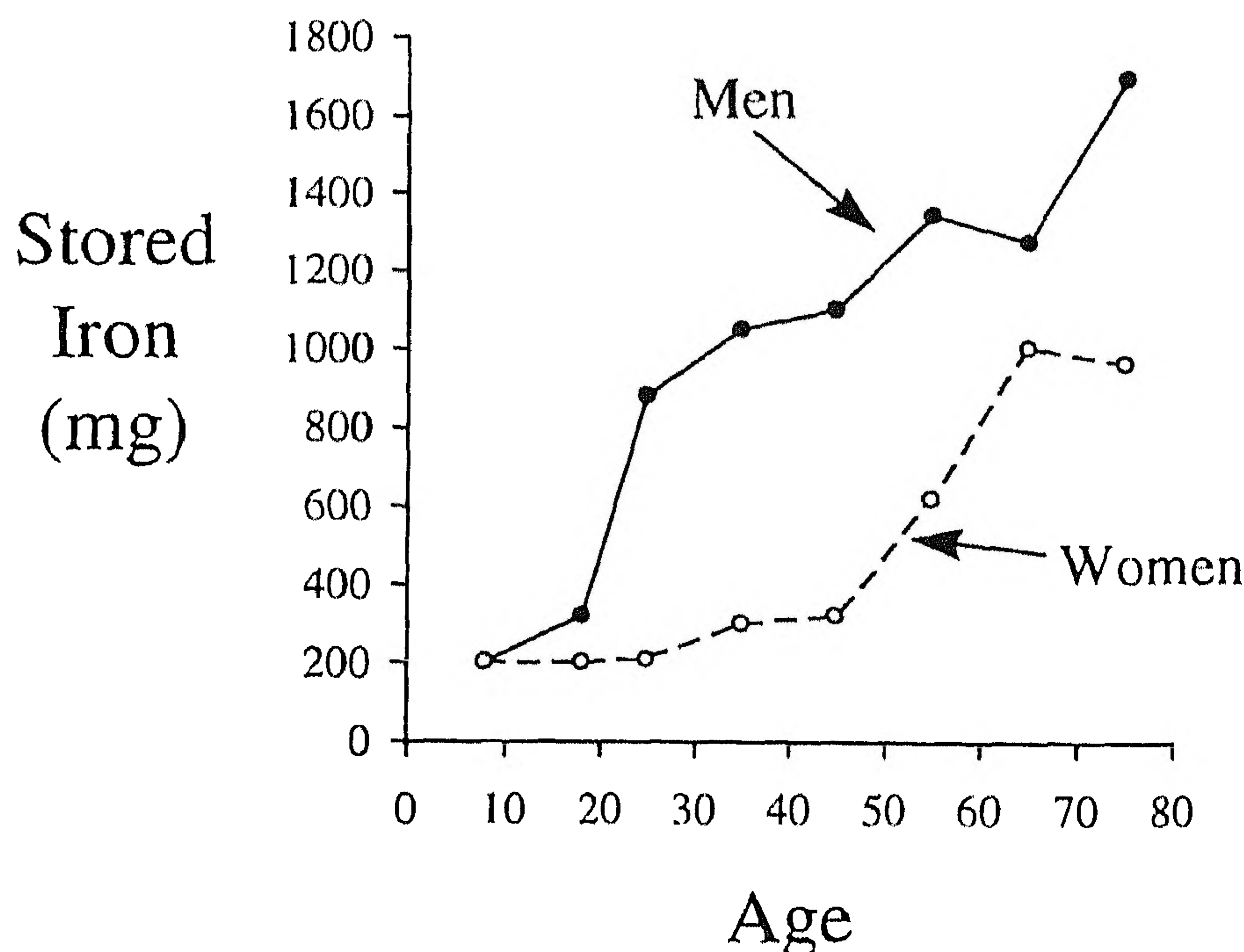


Figure 2. Iron storage as a function of aging. Progressive increases in iron occurred as a function of aging in men and postmenopausally in women. (Adapted by permission from Reference 19.)

contrast, GSSG is either exported from the cell or converted to GSH by a reductase reaction that obtains electrons from NADPH (Figure 1). Vitamin E, β -carotene, vitamin C, uric acid, flavonoids, and bilirubin are some of the nonenzymatic factors that may function as antioxidants (3, 4).

Contribution of Oxidants to COPD

The earliest clue regarding the pathophysiology of COPD was the landmark observation that individuals with congenital α_1 -antitrypsin deficiency developed emphysema prematurely, especially if they smoked cigarettes (28). This finding pointed convincingly not only to a role for elastase but also for oxidants in COPD because α_1 -antitrypsin needed to be inactivated by oxidants for elastase to be toxic (29–32). The latter discovery also explained why emphysema developed in individuals who did not have a genetic defect in α_1 -antitrypsin (30–32). Subsequently, it was shown that cigarette smoke, peroxynitrite, phagocyte, and chemically generated oxidants could inactivate antiproteases *in vitro* (33–35). In addition, stimulated alveolar type II epithelial cells and AM (but not fibroblasts) from guinea pigs inactivated α_1 -proteinase inhibitor in the presence of MPO (36). The susceptibility of the α_1 -antitrypsin methionine site to oxidative injury and the finding that some cigarette smokers had increased levels of α_1 -antitrypsin with oxidized methionine sites in their lung lavages further implicated oxidant inactivation of α_1 -antitrypsin as a precursor of elastase-dependent tissue damage *in vivo* (37, 38). The principle was well demonstrated by studies showing that prior exposure to a small, noninjurious dose of H_2O_2 remarkably increased the susceptibility of isolated lungs to injury caused by perfusion with neutrophil elastase. In contrast, neutrophil elastase was not appreciably toxic in the absence of oxidant preexposure (39). Additional cardinal aspects in the understanding of COPD were the observations that some cigarette smokers had increased elastase in their lung lavages and that intratracheally instilled human neutrophil elastase or proteinase 3 caused emphysema in animals (37, 40–43).

More needs to be done to validate the protease–antiprotease theory of COPD (32), but many aspects of the pathophysiology of COPD are consistent with the potential consequences of increased lung concentrations of elastase (30). For example, elastase can damage airspaces by degrading elastin and a variety of extracellular membrane proteins, proteoglycans, and glycoproteins. Elastase can also stimulate inflammation by increasing interleukin-8 (IL-8) synthesis, impair healing by inactivating cytokines and growth factors, and produce surfactant abnormalities by cleaving surfactant apoproteins. Additionally, elastase can activate or inactivate various other serpins, inhibitors of neutrophil collagenase, and secretory leukoprotease proteinase inhibitor (SLPI)—an inhibitor of neutrophil elastase (44)—and in that way further modulate inflammation. Cigarette smoke and/or elastase-mediated damage to lung connective tissue structural elements and loss of parenchyma produces overly compliant lungs, early airway closure during expiration, and air trapping, which most likely contribute to the distended, hyperlucent lungs of COPD patients (45).

This historically relevant and pioneering mechanism needs to be viewed in combination with the direct toxicity of oxidants to key lung structures, such as lung connective tissue elements. Oxidants can not only damage DNA, lipids, and proteins (3, 4), but also mediate a variety of processes that could foster the development of COPD. For example, oxidants increase high-molecular-weight glycoconjugate (mucus) production by epithelial cells in culture (Figure 3) (46) and impair cilia function (47). Oxidants also stimulate thromboxane for-

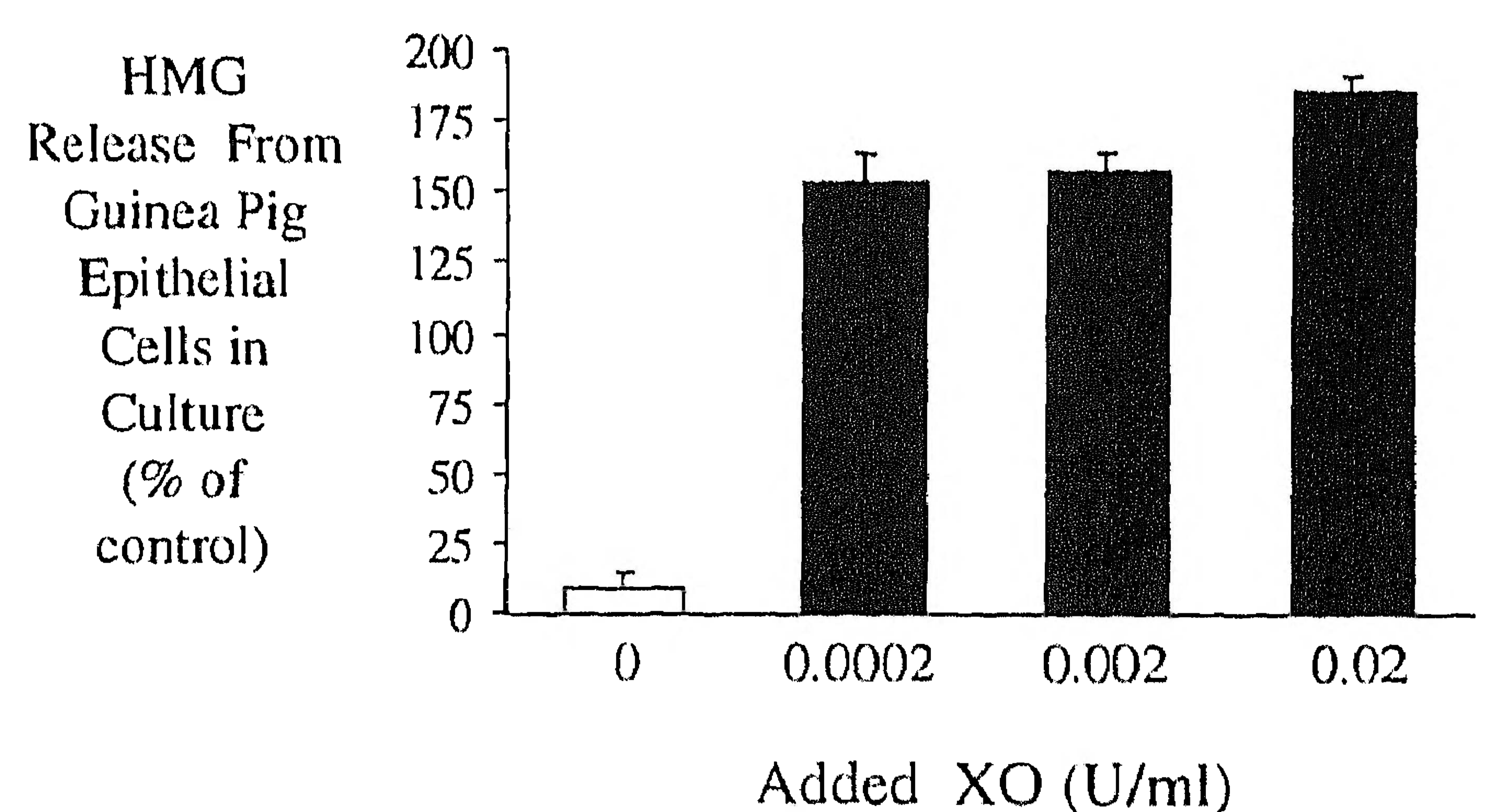


Figure 3. XO-mediated mucus secretion. XO-generated O_2 radicals increased release of mucus (high-molecular-weight glycoconjugates) from guinea pig epithelial cells in culture. (Adapted by permission from Reference 46.)

mation, reduce surfactant activity, injure fibroblasts, and produce numerous other effects that might diminish pulmonary lung mechanics and/or lung repair mechanisms in patients with COPD (48–50). Oxidants also promote epithelial permeability (51, 52). Oxidants in cigarette smoke even reduce O_2^- generation by PMN *in vitro* (53). Treatment of endothelial cells with plasma exposed to cigarette smoke activates the pentose phosphate pathway metabolism, increases GSH extrusion, decreases ATP levels, and releases angiotensin-converting enzyme (ACE) (54). These findings were corroborated by findings showing that airway obstruction, reflected by reduced FEV_1 levels, correlates with GSH, MPO, and eosinophilic cationic protein (ECP) levels in COPD patients (55), and that treatment with manganese SOD reduces cigarette smoke-induced cytotoxicity (56).

SOURCES OF OXIDANTS

Cigarette Smoke

Cigarette smoke is a rich source of oxidants (Figure 4) (57–62). The tar component of cigarette smoke (particulate matter that may be decreased by filters) contains an estimated 10^{18}

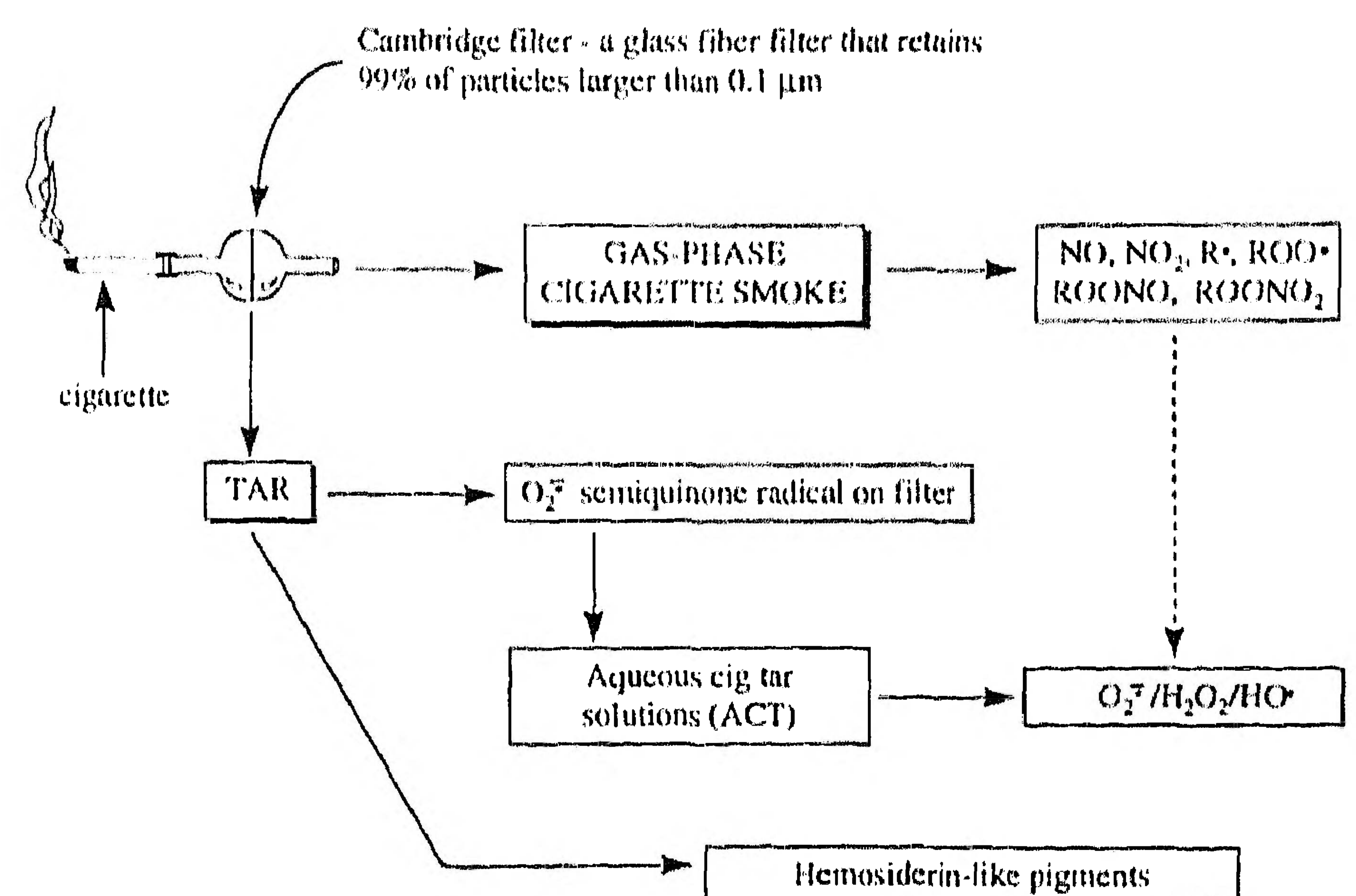


Figure 4. Components of cigarette smoke. Gas-phase smoke contains both carbon-centered and oxygen-centered radicals that are produced from NO/NO₂ reactions with reactive compounds in smoke. (Adapted by permission from Reference 57.)

spins/gram of tar. These cigarette smoke-generated radicals are sufficiently stable to be detected by electron spin resonance. One of these radicals is the semiquinone radical that reduces oxygen to O_2^- . By comparison, the inhaled gas component of cigarette smoke may contain as many as 10^{15} organic radicals per puff. The latter radicals are highly reactive, short-lived (< 1 s), carbon- and nitrogen-centered species. Gas phase smoke also contains high concentrations of reactive olefins and dienes. As much as 500 ppm of nitric oxide (NO) exists in cigarette smoke, and cigarette smoke converts tyrosine to 3-nitrotyrosine and dityrosine in a reaction that can be inhibited by GSH, ascorbic acid, or uric acid (63). Exposure to cigarette smoke also rapidly upregulated lung NO synthase activity in rats (64, 65). Peroxynitrates from the reaction of NO and O_2^- (4) can also be formed under these circumstances, but their fate and role in COPD are uncertain. Finally, oxidants may increase the toxicity of nitrosamines (66).

Inflammation

The coexistence of airway and parenchymal inflammation in most patients with symptomatic chronic airflow limitation effectively connects inflammation with COPD (67). Airway assessments performed by sputum analysis, bronchoscopy, biopsy, and lung lavage, in some cases separating initial from subsequent samples, have all suggested that inflammation contributes to the development of COPD (68–72). Inflammation may not only be responsible for mild airflow limitation and bronchiolar constriction but also may cause fibrosis, gland hypertrophy, and chronically increased smooth muscle tone. Furthermore, by increasing connective tissue deposition and by decreasing the supporting alveolar structure of the outer wall of the small airways, inflammation may further amplify airway limitation by deforming and narrowing the airway lumen (73–78). Most observations relating to inflammatory cells in COPD have focused on PMN and AM, but eosinophils, lymphocytes, and other cells undoubtedly impact the inflammatory process and may alter oxidant–antioxidant balance (79–82).

Neutrophils (PMN). Biopsies from the lungs of COPD patients and specimens from peripheral airway walls of smokers contained increased numbers of neutrophils. Moreover, lungs of smokers with airway obstruction had more PMN than smokers without airway obstruction (56, 83, 84). Since lung lavage and sputum analyses most likely reflect situations in the respiratory bronchioli and alveoli (85), it is not surprising that PMN were increased in lung lavage and sputum specimens from smokers with COPD (86). Furthermore, the degree of airway obstruction and the number of recovered PMN appeared to be related to the amount smoked in most individuals (71, 83).

Many mechanisms could account for the increased numbers of PMN that accumulate in the lungs of cigarette smokers with COPD. For example, PMN transit time was delayed in lungs immediately after smoking (87). In addition, oxidants decreased PMN deformability and, as a result, may enhance PMN sequestration in small blood vessels (88). More PMN may adhere where lung blood vessels are damaged and flow is abnormally low because of lung injury (89). Cigarette smoking also elicited CuZn SOD-inhibitable adhesion of PMN to cultured hamster endothelium (90), and CD18 integrins were increased on the surface of sequestered PMN in the pulmonary vessels of rabbits exposed to cigarette smoke (91). Upregulation of E-selectin expression also occurred in patients with chronic bronchitis, and adhering PMN were associated with E-selectin activity in lung vessels (92). Circulating intercellular

adhesion molecule-1 (ICAM-1) and circulating E-selectin levels were increased in COPD patients and probably altered PMN retention in the lung (93). In addition, neurokinin (NK_1) receptors mediated cigarette smoke-induced adhesion of PMN (and eosinophils) to the endothelium of venules in the rat tracheal mucosa (94).

Recruitment and activation of PMN and other inflammatory cells into the lung may also involve production by AM, epithelial, or other lung cells (95, 96) of interleukin-8 (IL-8). IL-8 levels were increased in sputum recovered from COPD patients compared with nonsmoking control subjects, cigarette smokers, and asthmatic patients (Figure 5) (97). IL-8 is a potent chemotaxin for PMN *in vitro*. Another AM and lung cell-derived molecule that may contribute to PMN recruitment is LTB_4 (98). In addition, nicotine, a chemoattractant for PMN *in vitro*, may attract PMN into the lung (99), prevent the reduction in PMN deformability induced by cigarette smoke (100), and/or even prolong PMN survival by suppressing apoptosis (101).

The O_2^- radical-producing activity of PMN that are lodged within the lungs of COPD patients is unknown, but PMN recovered from the blood of smokers who have elevated peripheral blood leukocyte counts elaborated more O_2^- than PMN recovered from nonsmokers or PMN recovered from smokers who have normal circulating leukocyte counts (Figure 6) (102). In addition, PMN recovered from some COPD patients had enhanced chemotaxis, proteolytic, and MPO activities *in vitro* (103, 104). Similarly, individuals subjected to passive smoking also had increased circulating leukocyte counts and cells that released more oxidants (105). Additionally, in one report (106), circulating O_2^- release from PMN correlated with bronchial hyperactivity in COPD patients, while in another report (107), O_2^- generation from PMN was increased during acute infectious exacerbations. Increased numbers of PMN appear to be making increased amounts of oxidants in the lungs of COPD patients (108).

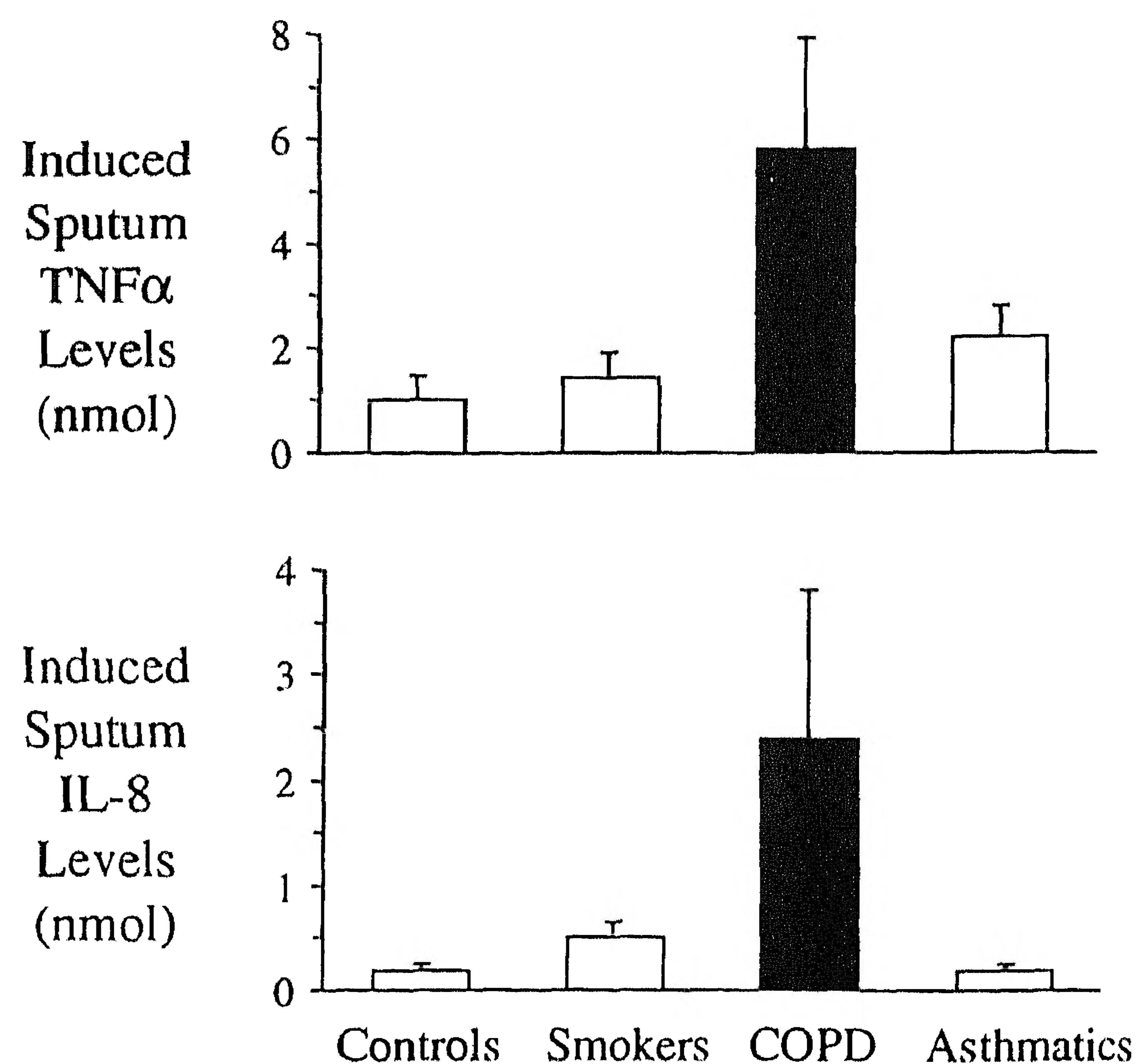


Figure 5. Inflammatory mediators in COPD sputum. COPD patients had increased TNF α and IL-8 levels in their induced sputum compared with control subjects, smokers, and asthmatic patients. (Adapted by permission from Reference 97.)

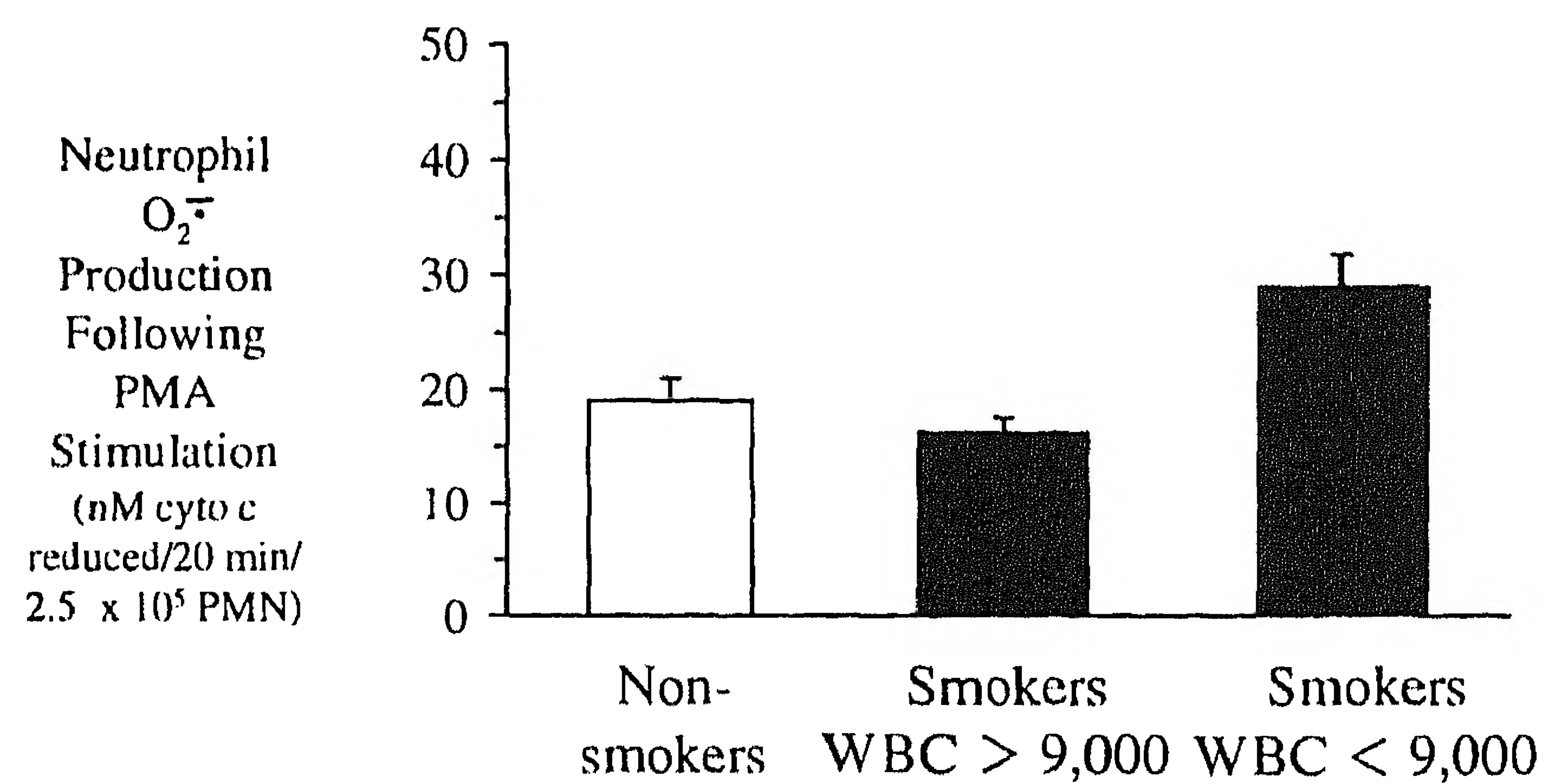


Figure 6. Superoxide production by neutrophils from cigarette smokers. Neutrophils from cigarette smokers with circulating leukocytes exceeding 9,000 made more O_2^- than neutrophils from smokers with circulating leukocytes of less than 9,000 or non-smokers. (Adapted by permission from Reference 102.)

Alveolar macrophages. Because of their strategic location and robust effector capabilities, AM may be pivotal in the development of COPD. More AM were recovered from the lungs of smokers, and these AM appeared to be activated since they were larger, stickier, and contained more pigmented cytoplasmic inclusions than AM from nonsmokers (109, 110). Increased AM pigmentation has also been associated with poor lung function (111).

Numerous mechanisms, such as those described for PMN, probably contribute to the recruitment of monocytes and their maturation into AM in lungs of patients with COPD (112–115). In addition, elastin fragments were increased substantially in lungs of cigarette smokers and are selective chemotaxins for monocytes *in vitro* (116). Prostaglandin $F_{2\alpha}$ and thromboxane B_2 , most likely derived from activated AM, were also increased in lung lavages of smokers and may facilitate inflammatory responses (117).

AM recovered by lung lavage from healthy young cigarette smokers released more O_2^- than AM from nonsmoking control subjects *in vitro* (Figure 7) (118–120). In addition, subpopulations of higher density AM were increased in smokers compared with nonsmokers and were responsible for the increased CD11/CD18 positivity and enhanced O_2^- production of AM from smokers (121). Spontaneous release of increased amounts of H_2O_2 from smoker monocytes has also been observed and related to accelerated maturation and activation

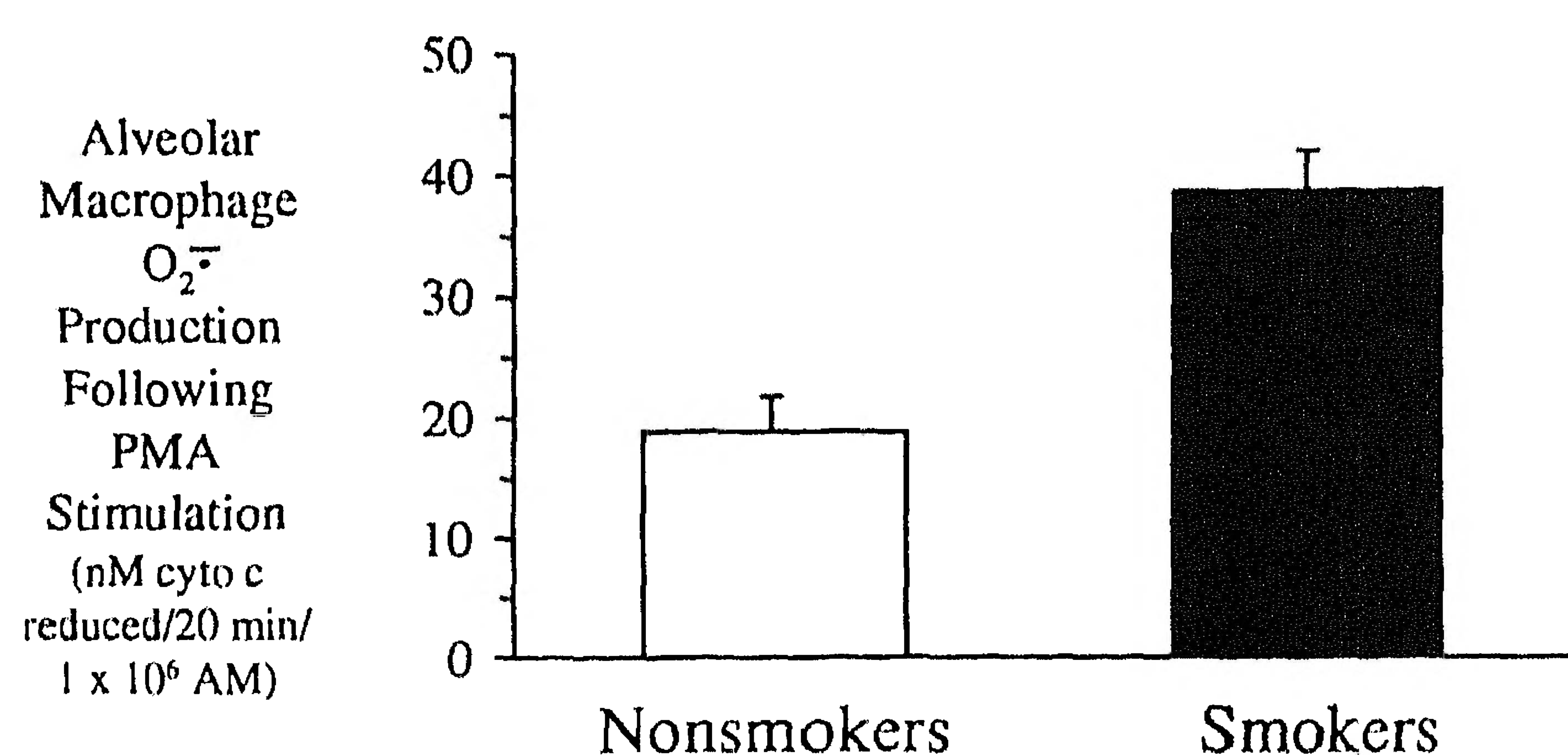


Figure 7. Superoxide production by alveolar macrophages from cigarette smokers. AM recovered by lung lavage from asymptomatic smokers made more O_2^- than AM from nonsmokers. (Adapted by permission from Reference 118.)

(122). Exposure to tobacco smoke *in vitro* also increased AM oxidative metabolism (123).

Eosinophils. Peripheral blood eosinophilia has been identified as a risk factor for the development of airway obstruction and a negative prognostic sign in newly diagnosed patients with chronic bronchitis (124, 125). Moreover, airway wall biopsies of patients with COPD contained increased numbers of eosinophils (79), and lung lavages from COPD patients had increased levels of eosinophilic cationic proteins (ECP), a marker of eosinophil activation (126). Furthermore, reversibility of airway obstruction correlated with bronchial eosinophilia in patients with very severe airflow limitation and emphysema, suggesting that eosinophils may contribute to pulmonary oxidative stress. This impression is also supported by observations that eosinophils make much more O_2^- than PMN or AM *in vitro* (127–130). For example, in response to phorbol myristate acetate (PMA), normal human eosinophils generated significantly more O_2^- (14.1 ± 3.3 nmol of cytochrome C/10 min/ 5×10^5 cells) than matched neutrophil fractions (5.9 ± 0.9 nmol/20 min/ 5×10^5 cells) (127). By comparison, PMA-stimulated human neutrophils (15 ± 0.8 nmol/20 min/ 5×10^6 cells) made more O_2^- than PMA-stimulated human AM (8.6 ± 1.0 nmol/20 min/ 5×10^6 cells). Although the relationship that eosinophils generate more O_2^- than PMN, which generate more O_2^- than AM, generally holds true, the pattern is dependent on the stimulus (127).

Xanthine oxidase. Xanthine oxidase (XO), which generates O_2^- and H_2O_2 , was increased in lungs of rats exposed to cigarette smoke (131). Lung XO increases might reflect conversion of xanthine dehydrogenase (XD) to XO by elastase or oxidants and/or increased synthesis of XD (132). XO was also increased and associated with increased leukocyte adhesion and erythrocyte (RBC) hemolysis in hamsters exposed to cigarette smoke (90). Reaction of XO-derived O_2^- metabolites with serum forms chemotaxins for PMN and thereby might be another mechanism responsible for recruiting PMN to the lungs of cigarette smokers (133). Moreover, XO activity was increased in cell-free lung lavages from COPD patients compared with nonsmoking normal subjects and associated with increased O_2^- generation, clastogenic (DNA damaging) activity, and uric acid production (Figure 8) (134). Increased lung XO activity could contribute to the increased exhaled H_2O_2 levels of cigarette smokers (135).

Other sources. Increased numbers of lymphocytes, epithelial, mast, and other metabolically active lung cells that consume oxygen probably release O_2^- radicals that could alter oxidant-antioxidant balance in the lungs of patients with COPD (Figure 9) (3, 4, 36). Mitochondrial and arachidonic acid metabolism also can generate oxidants that might participate in the development of COPD (34).

Infections

Infections may contribute to oxidative stress in patients with COPD by facilitating the recruitment and activation of phagocytic cells in the lung (136). *Streptococcus pneumoniae* and nonencapsulated *Haemophilus influenzae* emerge during exacerbations and remissions of COPD (137–140), and O_2^- production by blood neutrophils was increased in COPD patients during acute exacerbations and then returned to normal during recovery (141). Even clinically stable COPD patients are colonized with bacteria that might stimulate phagocytic cell oxidant production. The bronchi of 50% of COPD patients are colonized with bacteria belonging to the normal oropharyngeal flora (138, 142). Bacterial adherence may favor bacterial persistence and colonization of the respiratory tract (143,

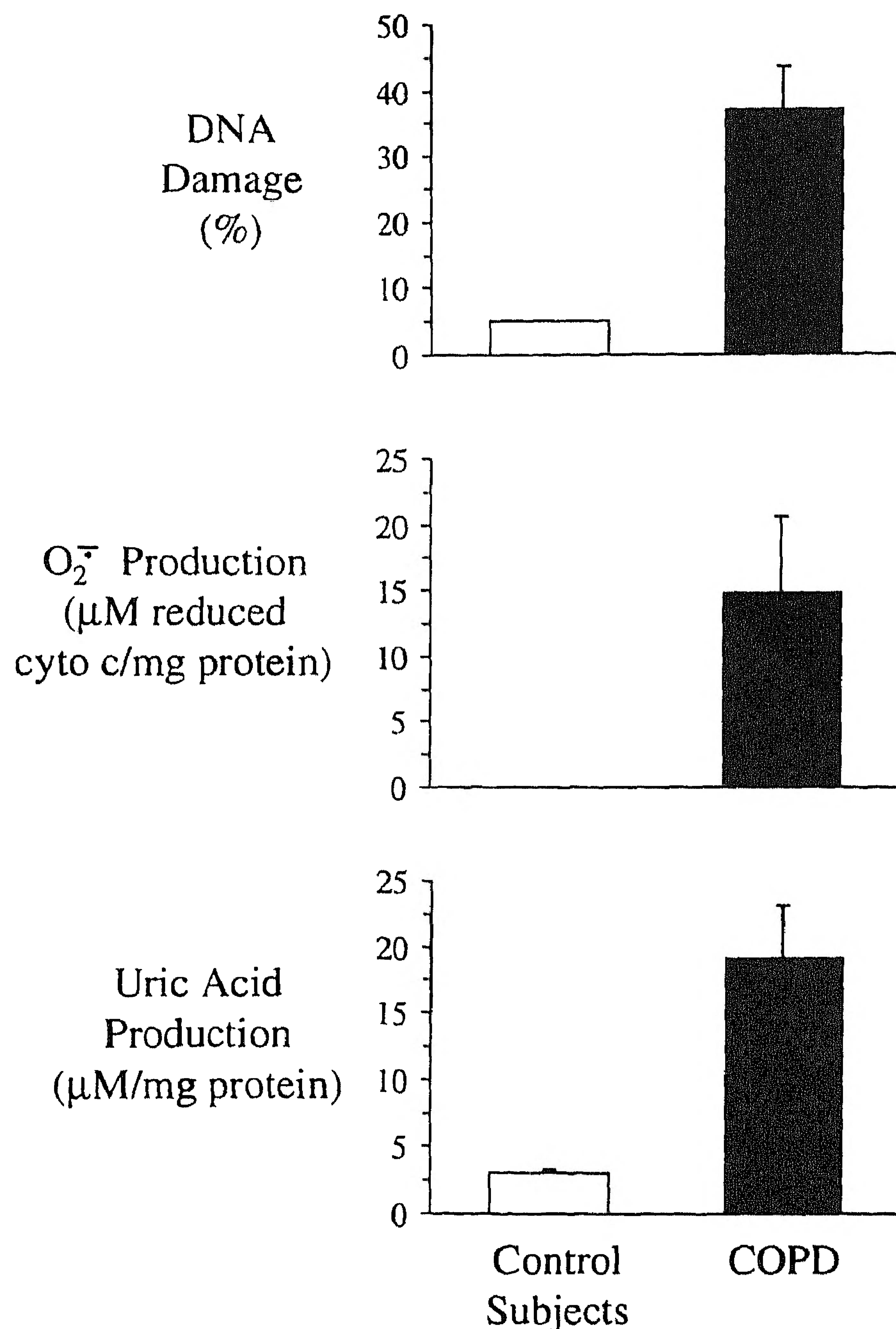


Figure 8. Factors associated with increased lung lavage XO activity in COPD patients. Cell-free lung lavages from COPD patients had increased XO levels and manifested increased lavage clastogenic activity, O₂⁻ production, and uric acid production compared with lavages obtained from control subjects. (Adapted by permission from Reference 134.)

144), and smokers had an enhanced adherence of *S. pneumoniae* to their buccal cells *in vitro* (145). In addition, *H. influenzae* adhered more to the pharyngeal cells of smokers with chronic bronchitis than healthy subjects (146).

ANTIOXIDANT DISTURBANCES

Because oxidants play such a pronounced role in cigarette smoke-induced lung damage, the status of pulmonary antioxidant defense mechanisms assumes paramount importance (3, 4, 15). In 1986, Taylor raised the question, "Is antioxidant deficiency related to chronic obstructive pulmonary disease?" and reported a relationship between a deficiency in plasma antioxidant activity and an abnormal FEV₁/FVC ratio in patients with COPD (147). Subsequently, a number of antioxidant disturbances have been observed in COPD patients, but a notable inconsistency exists, and the findings are difficult to compare because of the different designs of the various studies. Some examples of both antioxidant decreases and increases include the following observations.

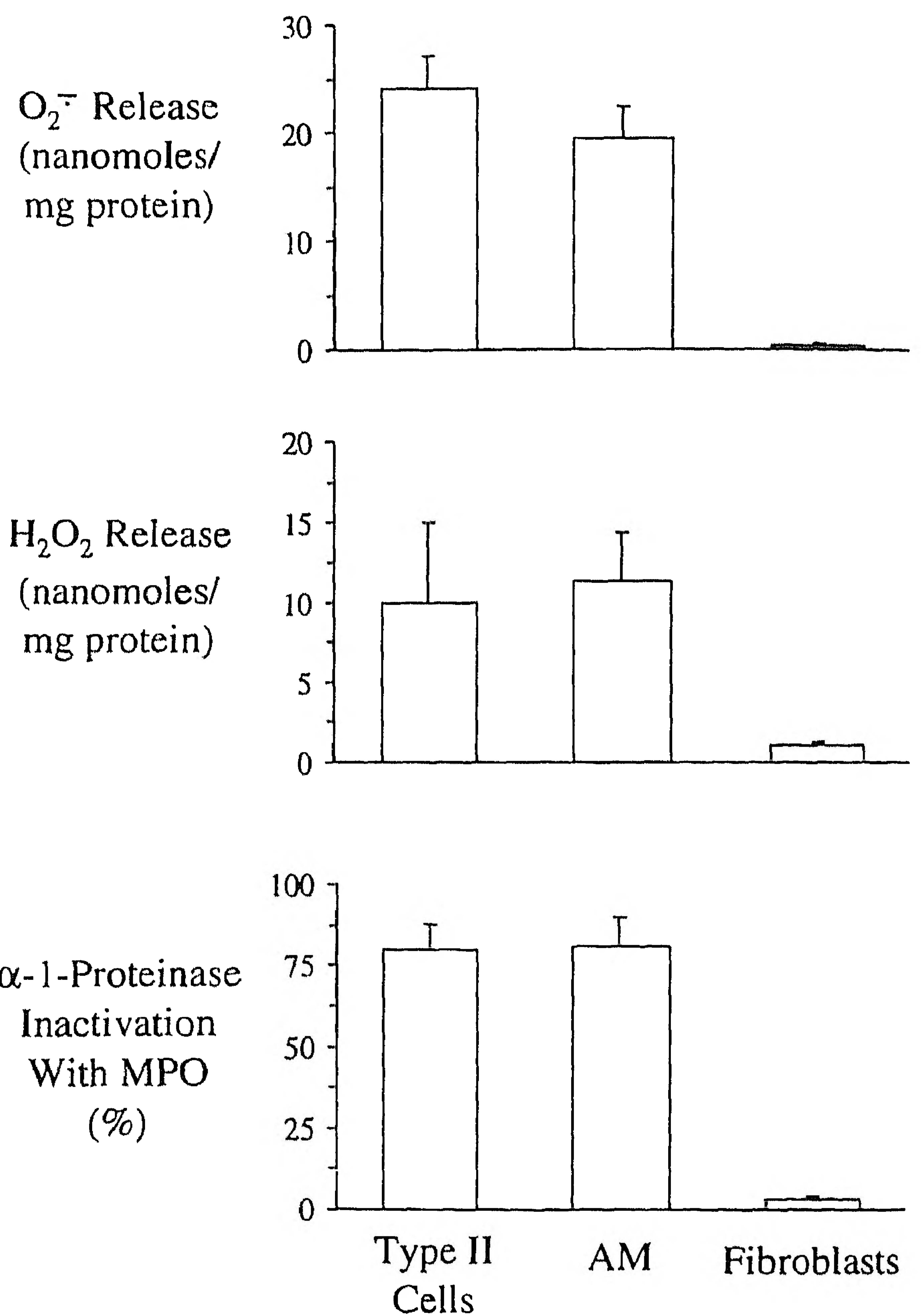


Figure 9. Oxidant production by cultured lung cells. Type II cells and AM released more O₂⁻ and H₂O₂ and inhibited α₁-proteinase activity (with MPO) better than fibroblasts. (Adapted by permission from Reference 36.)

Antioxidant Decreases

One study found that erythrocytes (RBC) from some smokers had decreased G6PD and GPX activity and were more susceptible to lipid peroxidation *in vitro* than RBCs from nonsmokers (148). Similarly, RBCs from the children of smoking parents were peroxidized more readily *in vitro* than RBCs from the children of nonsmokers, and this tendency was reversed by vitamin E treatment (149). RBCs from children with smoking parents also had decreased G6PD, GPX, and SOD activities compared with RBCs recovered from the children of nonsmoking parents (149). In addition, cigarette smoking has been associated with decreased plasma ascorbate, plasma β-carotene, and vitamin C levels (150–160). Additionally, vitamin E levels were lower in the lung lavages of young asymptomatic smokers, and this deficiency was linked with enhanced AM cytotoxicity (Figure 10) (161–165). Establishing the relationship between decreased antioxidant capacity and smoking remains difficult because many confounding variables, such as life-style, diet, and social class, may alter both smoking and changes in antioxidant levels.

Antioxidant Increases

A number of studies have revealed increased antioxidants in cigarette smokers. For example, vitamin E and C levels were

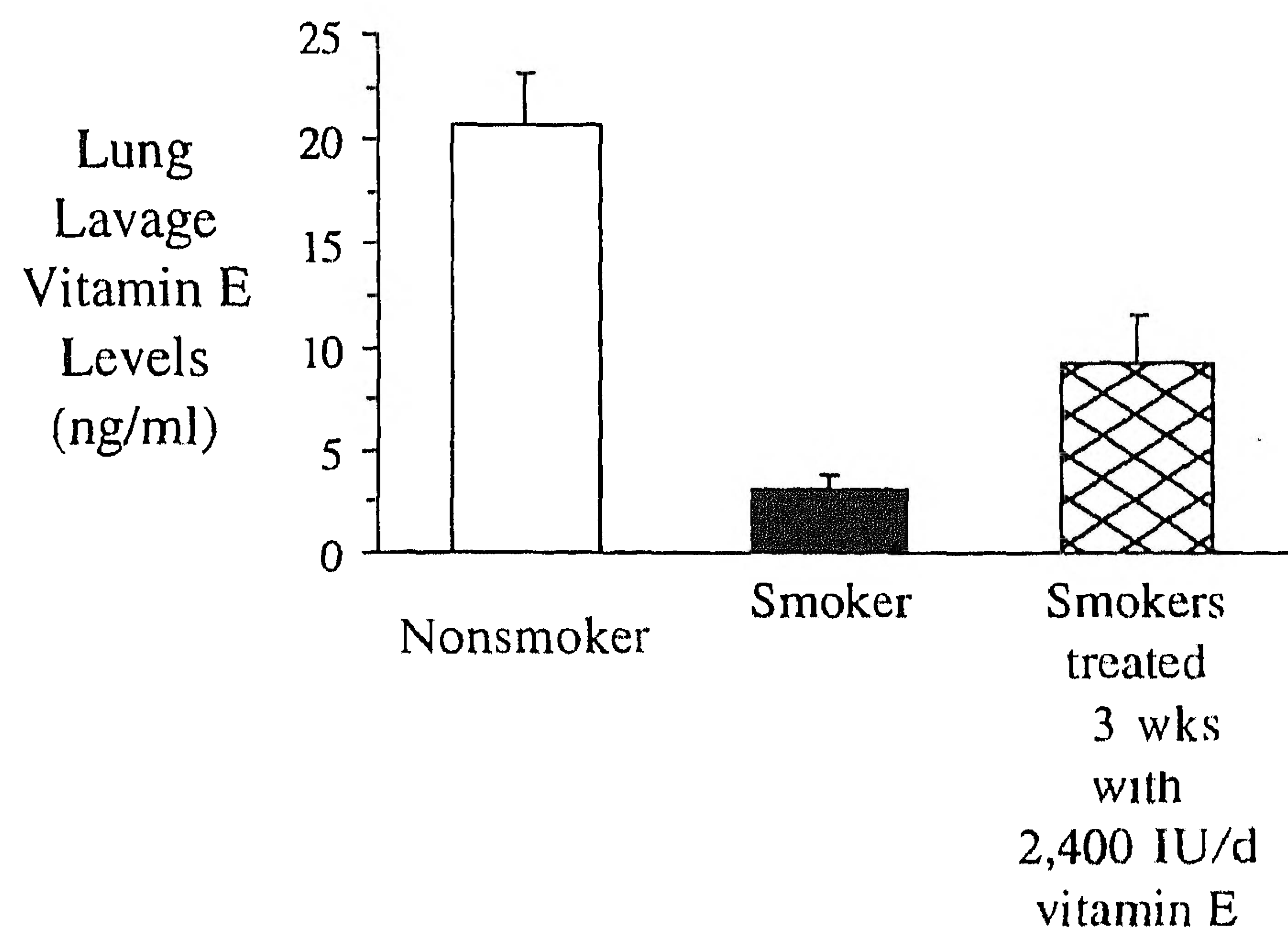


Figure 10. Vitamin E levels in lung lavages of cigarette smokers. Cigarette smokers have decreased lung lavage vitamin E levels compared with nonsmokers. Lung lavage vitamin E levels increased following oral therapy with vitamin E for 3 wk. (Adapted by permission from Reference 164.)

increased in the plasma and internal mammary arteries of cigarette smokers compared with nonsmokers, and the smokers with higher vitamin C levels had lower levels of lipid peroxidation (166, 167). In certain smokers, oxidatively stressed AM appeared to accumulate vitamin C and perhaps other antioxidants (168). This may be beneficial since treatment with vitamin C prevented cigarette smoke-induced leukocyte aggregation and adhesion to hamster endothelium in skinfolds (169) and altered biochemical responses in rats (170). In another study, vitamin C intake improved the lung function of cigarette smokers, asthmatics, and bronchitics (171). Nonetheless, in a recent trial, β -carotene treatment may have accelerated the development of lung cancer in cigarette smokers (172, 173).

Additional endogenous mechanisms may increase antioxidant levels in certain smokers. Certain cigarette smokers had increased GSH and glutathione peroxidase activities in their ELF compared with nonsmokers (26, 27, 174, 175). These increased GSH levels in ELF from human smokers are consistent with the high GSH levels observed in animals exposed to cigarette smoke and may be functionally important since reducing lung GSH increased lung epithelial permeability (175–179). For example, cigarette smoke and its condensates caused an oxidant-induced injury to A549 human type II alveolar epithelial cells (reflected by impaired attachment, decreased proliferation, and lysis), which was reversed by adding GSH extracellularly and worsened by depleting GSH intracellularly with buthionine sulfoxamine (177). Moreover, reduced FEV₁ levels correlated with decreased lung lavage GSH levels in smokers with chronic bronchitis, underscoring the importance of adequate GSH levels in COPD. GSH levels were also increased in RBCs from certain cigarette smokers compared with nonsmokers. Adding RBCs from cigarette smokers protected cultured endothelial cells against damage by H₂O₂ better than adding RBCs from nonsmokers (180). Catalase activity in RBCs can decrease oxidative inactivation of α_1 -antitrypsin by cigarette smoke, further indicating that alterations in RBC antioxidants may be meaningful (181). In related observations, cigarette smoking increased lung SOD, catalase, and glutathione peroxidase activities, but these responses did not protect the rats against cigarette smoke (182, 183). mRNA for gamma-

glutamylcysteine synthetase—the rate-limiting enzyme in GSH synthesis—was also increased in human alveolar epithelial cells following exposure to cigarette smoke (184).

The increased antioxidant activity in RBCs and lungs of cigarette smokers and the increased activity of SOD and catalase activities in lungs of rats and hamsters exposed to cigarette smoke are both reminiscent of protective antioxidant responses that occur in oxidative “tolerance” models (185). Tolerance is not a well understood phenomenon, but it appears that an antecedent low-grade oxidative stress can confer a subsequent adaptive resistance to oxidative stress, ostensibly by increasing antioxidant defenses (3, 4, 186). Thus, if oxidant stress contributes to COPD, then adaptive increases in antioxidants may be protective and explain why some cigarette smokers do not develop COPD. Indeed, certain cigarette smokers, for genetic or other reasons, may respond by increasing their antioxidant enzymes (*Antioxidant Responsive*), while other smokers, for unknown reasons, do not increase their lung antioxidants (*Antioxidant Unresponsive*).

Consistent with the tolerance premise was the finding that individual measurements of GSH levels in RBC and ELF of cigarette smokers showed great individual variability. Therefore, it is possible that individuals with absolutely or relatively lower GSH and other compensating responses are more susceptible to COPD, while individuals with enhanced antioxidant responses are less susceptible to COPD (187–189). A case in point exists in patients with tobacco smoke-induced optic neuropathy who did not develop the increased GSH levels found in cigarette smokers who do not develop the ocular disorder (190). Thus, adaptive increases in antioxidants in certain individuals may be a valuable protective mechanism against COPD induced by cigarette smoke (186, 191).

INDICATORS OF OXIDATIVE STRESS

A number of abnormalities and measurement of biomarkers have suggested that increased oxidative stress is occurring and is detrimental in cigarette smokers with COPD. The most convincing way to determine the involvement of oxidative stress in COPD is to directly measure oxygen radicals in lung tissue or exhaled air. However, direct measurement is difficult since oxygen radicals are highly reactive, short-lived species, and electron spin resonance and other direct techniques cannot be easily applied to the lung. The alternative has been to measure damage inflicted by oxygen radicals upon various lung biomolecules, usually lipids, proteins, or DNA. Some of the approaches that have been used which indicate that oxidative stress is occurring in COPD are described below.

H₂O₂ Exhalation

During acute exacerbations, patients with COPD exhaled more H₂O₂ by 100% than stable ex-smokers with COPD or normal subjects (Figure 11) (135). The source of the exhaled H₂O₂ was unknown (192), but AM from smokers released significantly more O₂⁻ than AM from nonsmokers (118), and AM recovered by lung lavage from subjects with a recent lower respiratory tract infection released more H₂O₂ *in vitro* (122). Additionally, smokers had increased lung lavage XO levels (134).

Lipid Peroxidation

Free radicals trigger lipid peroxidation chain reactions by abstracting a hydrogen atom from a side-chain methylene carbon (193–198). The resulting carbon-centered lipid radical then reacts with O₂ in aerobic cells to give a peroxy radical

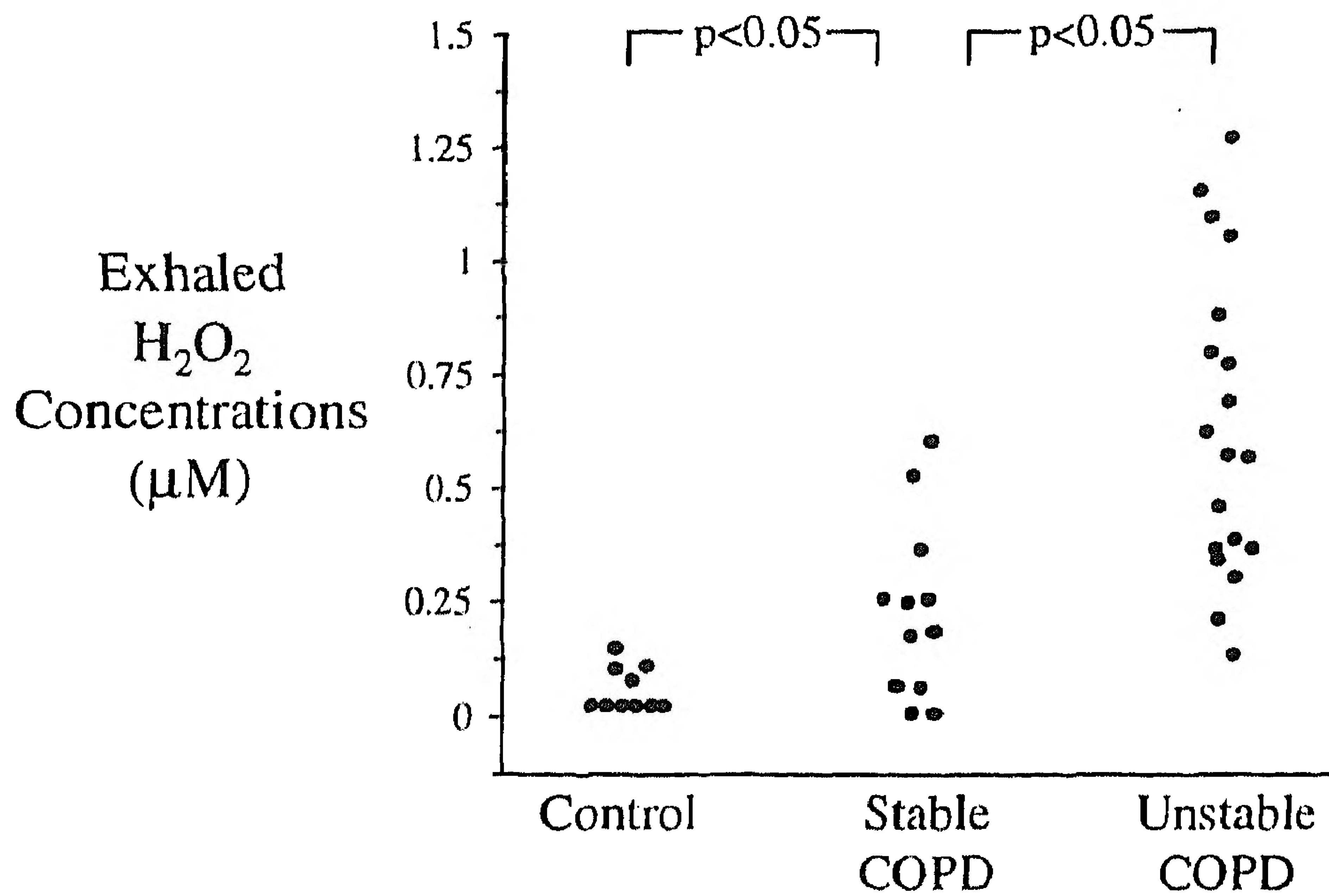


Figure 11. Exhaled H_2O_2 levels from patients with COPD. Patients with stable COPD exhaled more H_2O_2 than control subjects. Patients with unstable COPD exhaled more H_2O_2 than stable COPD patients or control subjects. (Adapted by permission from Reference 135.)

that subsequently propagates a chain reaction which transforms polyunsaturated fatty acids (either as free acids or as part of lipids) into lipid hydroperoxides. Lipid peroxidation (LPO) can impair membrane function, inactivate membrane-bound receptors and enzymes, disturb membrane fluidity, and increase permeability (193). Lipid hydroperoxides can also interact with antioxidants (such as α -tocopherol) or decompose after reacting with metal ions (such as iron or copper) or iron proteins (such as hemoglobin), leaving hydrocarbon gases (ethane, pentane) and unsaturated aldehydes (malondialdehyde) as by-products (194). Methods for detecting and quantifying LPO *in vitro* and *in vivo* usually examine lipid peroxides or derived radicals directly or else detect lipid peroxide conjugates or decomposition products indirectly (194–198).

Lipid peroxidation products (assessed as thiobarbituric acid-reacting substances) were increased in the plasma and lung lavages of healthy cigarette smokers (24, 166, 199–206), and patients with emphysema, chronic bronchitis, and asthma (203–205). In addition, increased LPO products correlated inversely with the time elapsed from the last exposure to tobacco smoke and the degree of small airway obstruction in COPD patients (205). Cigarette smoke exposure produced lipid peroxidation in plasma *in vitro* (201). Lipid peroxidation occurred in cigarette smoke-exposed rat tracheal epithelium along with histochemical evidence of continuing production of both H_2O_2 and $O_2^{\cdot-}$ at the apical cell membrane (207). LPO also occurred in lungs of animals exposed to cigarette smoke and sonicates of AM exposed to cigarette smoke *in vitro* (208). Pentane and ethane exhalation were increased in cigarette smokers (209, 210), and ethane exhalation was decreased by antioxidant treatment (210). Notwithstanding these observations, some concern has persisted because of the difficulty in accurately measuring lipid peroxidation *in vivo* (211). This worry was mitigated recently by findings that plasma levels of free and esterified F_2 -isoprostanes (a series of bioactive prostaglandin F_2 -like compounds that are made by free radical catalyzed peroxidation of arachidonic acid) were increased in smokers compared with nonsmokers (Figure 12) (212). Moreover, free and esterified F_2 -isoprostane levels decreased following smoking cessation for 2 wk. Additionally, plasma F_2 -isoprostanes were normal or only slightly increased in some cigarette

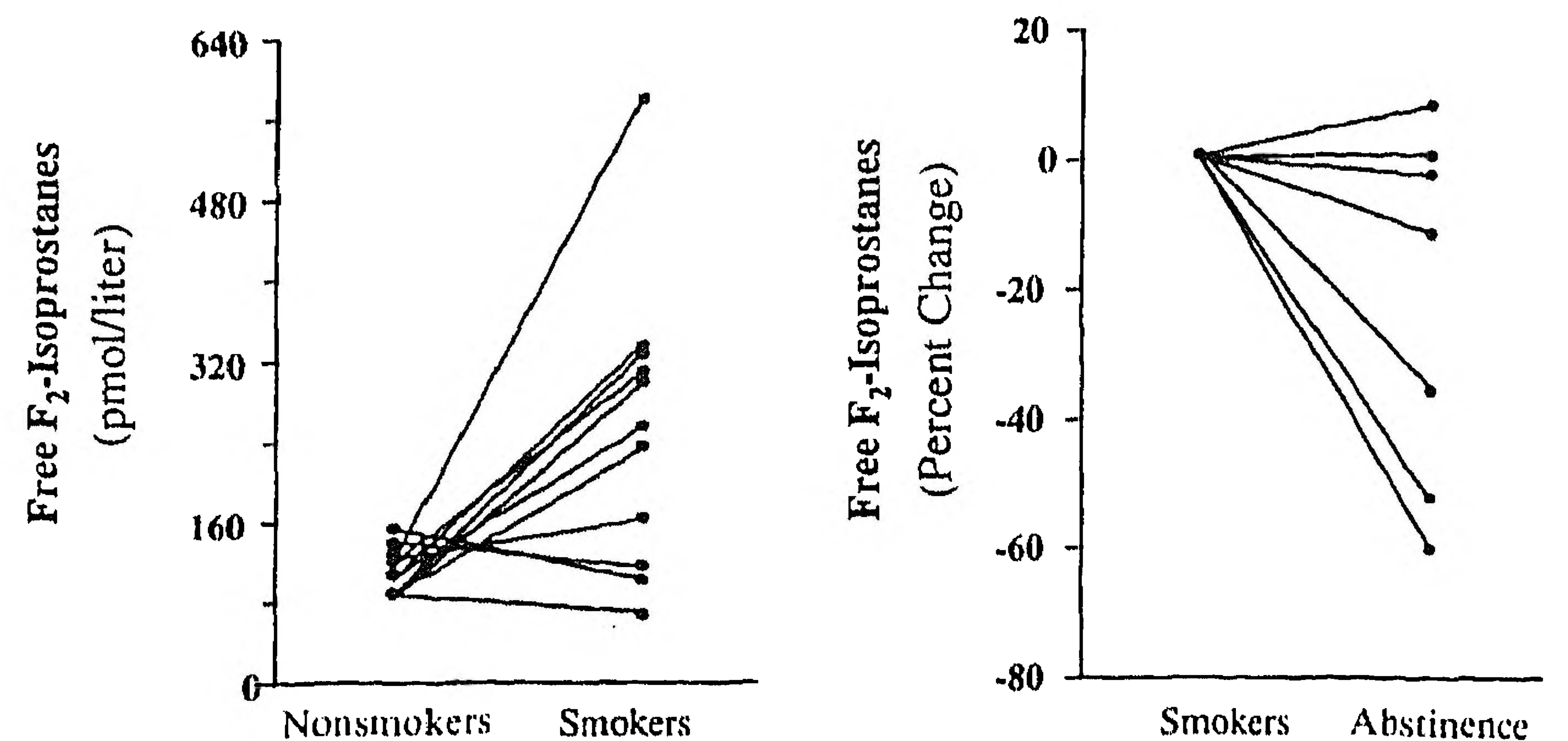


Figure 12. Free F_2 -isoprostanes in cigarette smokers. F_2 -isoprostanes were increased in cigarette smokers compared with nonsmokers. F_2 -isoprostane levels decreased following smoking cessation. (Adapted by permission from Reference 212.)

smokers, consistent with the possibility that certain individuals are more resistant to oxidative stress, perhaps as a consequence of their enhanced antioxidant defenses (212).

DNA Damage

Many different compounds in cigarette smoke can readily react directly to form radicals, while other substances (procarcinogens) must be activated by one or more of the p-450 cytochromes before becoming electrophilic species that enter into damaging interactions which produce single-strand breaks in DNA (213–215). For example, treating human respiratory tract tracheobronchial epithelial cells with gas-phase cigarette smoke produced DNA strand breakage and the formation of double-stranded DNA (216). Moreover, multiple chemical modifications (including guanine and adenine base deamination) occurred in all four DNA bases in a pattern suggestive of reaction with $\cdot OH$ or deaminating species, such as HNO_2 , NO_2 , N_2O_3 , and $ONOO^-$ in cigarette smoke (216). Hydroquinone and semiquinone radicals in cigarette smoke can also produce oxyradicals that may nick DNA, causing mutations and, ultimately, carcinogenesis (46, 217).

Increased 8-hydroxy-2'-deoxyguanosine activity, a product of the reaction of oxidants and DNA, has been detected in the peripheral blood leukocytes of cigarette smokers and in lung epithelial cells exposed to cigarette smoke *in vitro* (218, 219). Likewise, oxidant-mediated DNA strand breaks occurred more frequently in mononuclear leukocytes exposed to activated PMN from cigarette smokers (220).

Other "molecular dosimeters" may indicate indirectly the level of biologically relevant exposure to oxidants. Typical examples include polycyclic aromatic hydrocarbon (PAH)-DNA adducts, which reflect exposure to tobacco smoke (221, 222). These compounds exist in pulmonary tissue, circulating blood cells, and AM. 4-Hydroxy-1-(3-pyridyl)-1-butanone (HPB)-DNA adducts derived from metabolism of nicotine similarly reflect the amount of oxidative stress induced by cigarette smoke. Alkyl-DNA adducts, which may be formed during oxidation processes in target cells, have been detected in lung tissue and AM of cigarette smokers (223, 224).

Finally, certain gene mutations might be regarded as unique "fingerprints" of oxidative stress to DNA. Mutations of the p53 and K-ras genes are both associated with cigarette smoking, and the higher rate of lung cancer in cigarette smokers suggests the damaging effects of oxidants on DNA (225, 226). Although it has been suggested that benzo(a)pyrenes in

tobacco smoke were related to certain mutations (GC → TA transversion), oxidation reactions may be involved, since similar mutations develop following exposure to hyperoxia.

Carbonyl Proteins

Oxygen radicals can modify amino acid side chains, form protein aggregates, cleave peptide bonds, and make proteins more susceptible to proteolytic degradation (227). In the process, some amino acid residues are converted to carbonyl derivatives. Exposure to gas-phase cigarette smoke also modified human plasma proteins, producing carbonyl proteins with lost sulfhydryl groups (228, 229). In one study, the content of oxidized proteins recovered by lung lavage was 0.59 ± 0.14 nmol carbonyl/ml bronchoalveolar lavage fluid in asymptomatic smokers compared with 0.30 ± 0.07 nmol carbonyl/ml bronchoalveolar lavage fluid in nonsmoking control subjects (230). Plasma protein sulfhydryls were also depleted following exposure to cigarette smoke *in vitro*. Reaction of proteins with nitric oxide or its derivatives may also lead to protein degradation (63). Furthermore, using a Trolox (vitamin E analog)-related assay, plasma antioxidant activity was decreased acutely in cigarette smokers, following acute exacerbations in COPD patients, and associated with protein sulfhydryl oxidation (107).

CONVENTIONAL TREATMENT AND OXIDATIVE STRESS IN COPD

Improving the quality and duration of life of COPD patients is a distinct challenge. The specific therapeutic goals are to reduce symptoms, preserve lung function, optimize gas exchange, and limit and/or treat acute exacerbations rapidly (231). Because pulmonary function declines with aging, everything must be done to prevent any additional functional loss caused by COPD. Indeed, elderly cigarette smokers who develop COPD may lose as much as 80 ml of FEV₁/yr compared with 33 ml/yr for nonsmokers (232).

The treatment for cigarette smoke-induced COPD has been reviewed extensively (233, 234). In general, the accepted strategies encompass approaches that limit initiating and aggravating triggers, such as tobacco smoke (smoking cessation) and inhalation of environmental and work-related irritants. More specific treatment consists of administering bronchodilators, antiinflammatory agents, and/or antibiotics. Additional approaches include nutritional supplementation, immunization, breathing exercises, pulmonary rehabilitation, and ultimately supplemental oxygen, given nocturnally or continuously. Conventional therapies for COPD are discussed below, focusing on their potential effect on oxidant-antioxidant balance.

Smoking Cessation

It is not surprising that the rates of decline in lung function in smokers with mild COPD were reduced by smoking cessation, since smoking cessation potentially decreases most sources of oxidative stress. A prime example of this possibility was the finding that smoking cessation for 6 mo reduced the numbers of AM and PMN recoverable by lung lavage (235–237).

Sympathomimetics/Anticholinergics/Methylxanthines

While the primary effect of β_2 agonists is to relax airway smooth muscle, some antioxidant benefits may be provided as well (238–240). For example, O₂⁻ production by AM was decreased in chronic bronchitis patients treated with formoterol, and terbutaline reduced O₂⁻ generation by AM *in vitro* (241,

242). However, the effects of theophylline on oxygen radical generation by PMN remain controversial (243–245). Incubating blood PMN from healthy volunteers with increasing concentrations of theophylline inhibited their oxygen radical production in a dose-dependent fashion. Importantly, inhibition was reached at clinically achievable concentrations of theophylline (244, 245). However, in other studies theophylline treatment enhanced O₂⁻ production by PMN (246, 247). Theophylline treatment also increased O₂⁻ release from eosinophils from patients with peripheral eosinophilia (248).

Corticosteroids

It is still unclear whether inhaled corticosteroids attenuate COPD, even though several placebo-controlled clinical studies have addressed the question (249–255). However, steroids may have an antioxidant effect by decreasing the numbers as well as the oxidative and chemotactic responses of neutrophils. In one study, prolonged daily oral corticosteroid treatment decreased the O₂⁻ production and the chemotactic responsiveness of unstimulated, but not stimulated, peripheral blood PMN (256). In another study, O₂⁻ generation by PMN was decreased after *in vivo* prednisolone treatment in patients with emphysema (257). In another study, dexamethasone did not alter unstimulated O₂⁻ production by PMN either *in vitro* or *in vivo* (258). Likewise, inhaled corticosteroids did not change PMN numbers or IL-8 levels in the peripheral blood but did decrease the number of PMN in the sputum of COPD patients (259). Steroid therapy did not reduce eosinophil numbers or ECP levels in the sputum of COPD patients (260).

Antibiotics

Antibiotics used for treating chronic bronchitis would seem to have a certain role in reducing oxidative stress in COPD by reducing infection and thereby lung inflammation. In addition, tetracycline and other anti-infectives may have independent antioxidant properties.

Miscellaneous Drugs

Nedocromil sodium inhibited O₂⁻ production by PMN *in vitro* (261). Ambroxol is an expectorant with antioxidant properties that stimulates the formation and release of surfactant by type II pneumocytes (262, 263). Inhaled NO (40 ppm) had no effect on respiratory system resistance in patients with COPD or healthy subjects (264).

ANTIOXIDANT THERAPY

N-acetylcysteine (NAC) is the most widely investigated drug with antioxidant properties that has been used in both experimental and clinical settings which are relevant to COPD (265–267). Although given initially because of its mucolytic properties, NAC is a thiol-containing compound that may act as an antioxidant by providing cysteine intracellularly for the enhanced production of GSH (268). This potentially beneficial antioxidant effect is suggested because NAC decreased H₂O₂-induced damage to epithelial cells *in vitro* (269) and NF κ B activation in some cells (270). In addition, NAC treatment reduced cigarette smoke-induced abnormalities in PMN (271), AM, fibroblasts, and epithelial cells *in vitro* (272–275). NAC treatment also attenuated rat secretory cell hyperplasia induced by tobacco smoke (276) and prevented HOCl-mediated inactivation of α_1 -proteinase inhibitor *in vitro* (277). NAC treatment may alter lung oxidant-antioxidant imbalance in

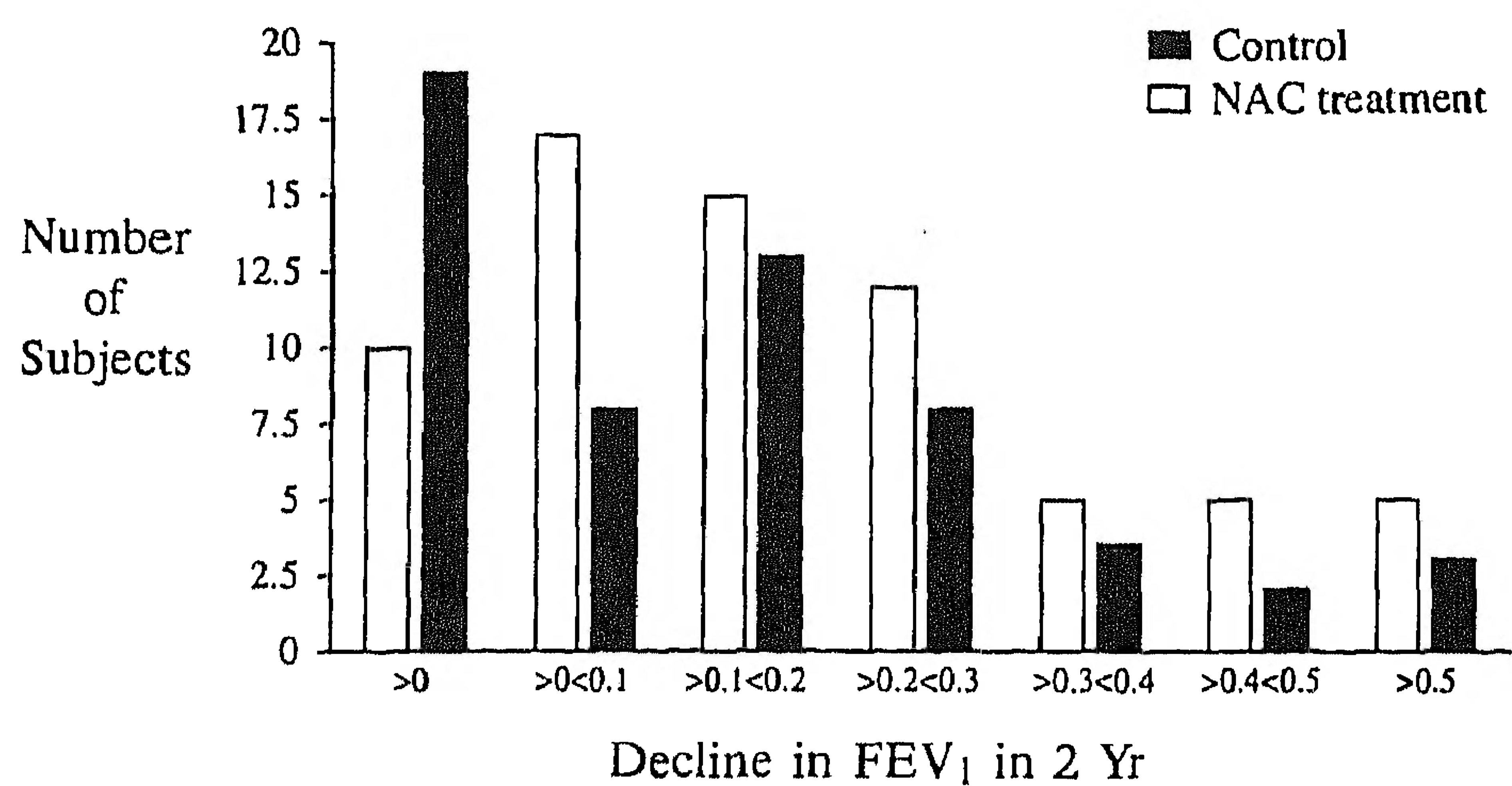


Figure 13. Effect of N-acetylcysteine on FEV₁ decline in COPD patients. NAC-treated patients had relatively less decline in FEV₁ compared with untreated patients during a 2-yr period of observation. (Adapted by permission from Reference 290.)

humans (278). NAC given orally increased lung lavage GSH levels (279), reduced O₂⁻ production by AM recovered by lavage (273), and decreased lung lavage PMN chemiluminescence *in vitro* (280). However, peripheral blood PMN obtained from healthy nonsmokers treated with NAC produced normal amounts of H₂O₂ (273).

The clinical efficacy of NAC has been investigated in a number of studies of patients with chronic bronchitis, with and without COPD. Treatment with NAC caused symptomatic improvement in COPD patients, reflected by decreased sputum viscosity and purulence and improved sputum expectoration (281). In addition, NAC treatment reduced the number of exacerbations and sick-leave days in some (282, 283), but not other (284–286), investigations of COPD patients. Parenthetically, NAC treatment also decreased the number of viral infections (287, 288) and airway bacterial colonization (289) in patients with COPD. In a recent investigation in Sweden, the decline in FEV₁ in COPD patients who took NAC for 2 yr was less than in a reference group (Figure 13) (290). This favorable effect of NAC was particularly apparent in COPD patients over 50 yr of age (yearly decline of 30 ml in FEV₁) compared with the reference group (yearly decline of 54 ml in FEV₁).

CONCLUSIONS

COPD is a costly health problem (291). As reviewed herein, a myriad of evidence suggests that oxidative stress contributes to COPD (Figure 14) (292). Nonetheless, obtaining definitive proof that oxidants contribute to COPD and/or that antioxidant therapy is beneficial in COPD remains problematic. Indeed, because COPD is a chronic, progressive disease, long-term study of many patients would be required. Furthermore, evaluation is complicated because of coincident decreases in pulmonary function related to aging and other maladies. Clinical investigations must also account for variations in the duration of smoking, daily smoking consumption, and smoking cessation, as well as for other confounding factors, such as cardiovascular disease and cancer. Suitable endpoints are also difficult because objective decreases in FEV₁ and mortality do not change sufficiently in the short term. Other changes, such as exacerbations and sick days, which occur more rapidly, are not as convincing because of their subjectivity. Not surprisingly, for the aforementioned reasons, none of the presently used short-term therapies, such as antibiotics or steroids, has been shown to unequivocally improve FEV₁ or survival in COPD. So far, appreciable improvement in declines in FEV₁ has been found in COPD patients only after smoking cessation (293, 294).

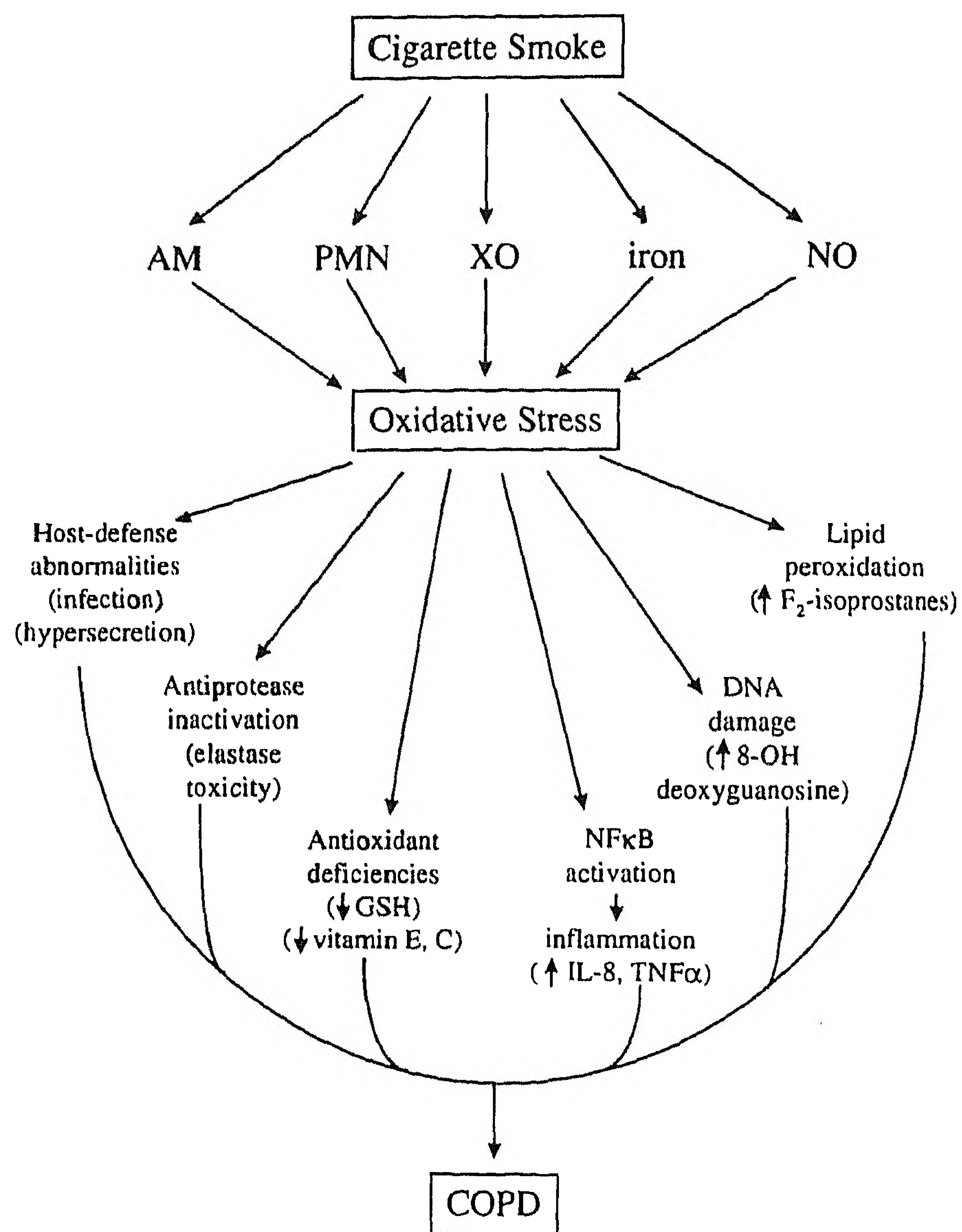


Figure 14. Cigarette smoke, oxidative stress, and COPD. Numerous processes increase lung oxidative stress and contribute to a variety of abnormalities that contribute to COPD.

It should be possible to gain meaningful information from studies using surrogate markers that reflect oxidative status (295). If an acceptable marker of oxidative stress increased in COPD patients and correlated with either an increased rate of pulmonary dysfunction and/or the severity of COPD, then the association between oxidants and COPD would be strengthened. Likewise, if antioxidant therapy decreased both the marker and meaningful endpoints, then the role of oxidants and the value of antioxidant therapy in COPD would be further supported, even if there was no significant effect on mortality or FEV₁ during the short-term analyses. Finally, if these approaches could be applied to subpopulations of individuals who have rapid declines in FEV₁, then a definitive answer to this important premise might be secured even more rapidly.

It is obvious from this review that lung oxidant-antioxidant balance is abnormal in cigarette smokers (Figure 14). However, it remains unclear why only certain cigarette smokers (actually a minority) develop COPD and, for that matter, lung cancer and atherosclerosis. Exposure to inhaled oxidants from cigarette smoke would seem to be fairly consistent among individuals with comparable smoking histories. The answer to this intriguing question lies in an improved understanding of the nature of the oxidant-antioxidant balance and the genetic factors and other intrinsic factors, including dietary factors, that control this balance (296). Unfortunately, because of the great variability that exists in the individuals who smoke and difficulties in measuring oxidative status, many challenges remain in understanding, treating, and preventing COPD.

THE OXIDATIVE STRESS STUDY GROUP

WILLIAM DE BACKER, M.D., PH.D.

*Professor of Pulmonary Medicine
University Hospital of Antwerp, Belgium*

RICHARD DEKHUIZEN, M.D., PH.D.

*Department of Pulmonary Medicine
University Hospital of Nijmegen, The Netherlands*

MAURICE DEMEDTS, M.D., PH.D.

*Professor of Pulmonary Medicine
University Hospital of Leuven, Belgium*

CEES VAN HERWAARDEN, M.D., PH.D.

*Professor of Pulmonary Medicine
University Hospital of Nijmegen, The Netherlands*

ROBERT VAN KLAVEREN, M.D., PH.D.

*Department of Pulmonary Medicine
University Hospital of Antwerp, Belgium*

JAN-WILLEM LAMMERS, M.D., PH.D.

*Professor of Pulmonary Medicine
University Hospital of Utrecht, The Netherlands*

SVEN LARSSON, M.D., PH.D.

*Professor of Pulmonary Medicine
Sahlgrenska University Hospital
Gothenburg, Sweden*

BO LUNDBACK, M.D., PH.D.

*National Institute of Occupational Health
Umea, Sweden*

STEFANO PETRUZZELLI, M.D., PH.D.

*Department of Respiratory Physiology
University of Pisa, Italy*

DIRKJE POSTMA, M.D., PH.D.

*Professor of Pulmonary Medicine
University Hospital of Groningen, The Netherlands*

GERDT RIISE, M.D., PH.D.

*Department of Pulmonary Medicine
Sahlgrenska University Hospital
Gothenburg, Sweden*

PAUL VERMEIRE, M.D., PH.D.

*Professor of Pulmonary Medicine
University Hospital of Antwerp, Belgium*

EMIEL WOUTERS, M.D., PH.D.

*Professor of Pulmonary Medicine
University Hospital Maastricht, The Netherlands*

JEAN CLAUDE YERNAULT, M.D., PH.D.

*Professor of Pulmonary Medicine
Erasmus University Brussels, Belgium*

NICO VAN ZANDWIJK, M.D., PH.D.

*The Netherlands Cancer Institute
Antoni van Leeuwenhoek Huis Amsterdam, The Netherlands*

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