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Oxidative stress detection: what for?
Part II

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Abstract. – Oxygen-free radicals, more generally known as reactive oxygen species (ROS) along with nitrogen species (RNS) are well recognised for playing a dual role both deleterious and beneficial species. The cumulative generation of ROS/RNS through either endogenous or exogenous insults is termed oxidative stress and is common for many types of diseases that are linked with altered redox regulation of cellular signalling pathways.

Key Words:

Oxidative stress, Free radicals, ROS, RNS, Antioxidants.

Introduction

In the last two decades there has been an explosive interest in the role of oxygen-free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) in experimental and clinical medicine. ROS and RNS:

1. are produced during irradiation by UV light, by X-rays and by γ-rays;
2. are products of metal-catalyzed reactions;
3. are present as pollutants in the atmosphere;
4. are generated by neutrophils and macrophages during inflammation;
5. are by-products of mitochondria-catalyzed electron transport reactions and other mechanisms.

ROS/RNS are known to play a dual role in biological systems, since they can be either harmful or beneficial to living systems. Beneficial effects of ROS involve physiological roles in cellular response to noxia, as for example in defence against infectious agents and in function of a number of cellular signalling systems. One further beneficial example of ROS at low concentrations in the induction of a mitogenic response. In contrast, at high concentrations, ROS can be important mediators of damage to cell structures, including lipids and membranes, proteins and nucleic acids (termed oxidative stress). The harmful effects of ROS are balanced by the antioxidant action of non-enzymatic antioxidants in addition to antioxidant enzymes. Despite the presence of the cell's antioxidant defence system to counteract oxidative damage from ROS, oxidative damage accumulates during the life cycle, and radical-related damage to DNA, to proteins and to lipids has been proposed to play a key role in the development of various disorders.

This paper examines the avilable evidence for the involvement of cellular oxidants in some disorders such as lung, joints, kidney and liver diseases. The role of free radicals is not well known and specified, and commonly there are indirect evidence to suggest oxidative stress as disease-causing. Nevertheless, the importance of free radical can not be understimate anymore.

Respiratory Diseases

The lungs function is to exchange gases between the body tissues and the outside enviroment. In normal conditions (i.e. when an individual is in good health and resides in a relatively clean enviroment) the lung, in comparison with other organs, represents a unique tissue for oxidative stress, continually exposed to relatively-high O_2 tension, inevitable pollutants and the metabolic products derived from the pollutants. Inhalation of airborne irritants and pollutants, including cigarette smoke, ozone, carcinogens (e.g. diesel exhaust) and other chemicals and dust par-

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ticles, will produce excess ROS and RNS in the lung. Additionally, such exposures lead to depletion of endogenous anti-oxidants[1]. Moreover, typical component in most lung diseases and infections is the activation of inflammatory, immune and structural cells of the airways with consequent free radical production. Furthermore, various therapies lead to free-radical-induced tissue damage, i.e. O_2 therapy for the treatment of prematurely-born neonates and acute respiratory distress syndrome (ARDS), and chemotherapy and radiation for cancer patients.

OS is the hallmark of various chronic inflammatory lung diseases. These include, among others, ARDS, bronchopulmonary dysplasia, asthma, chronic obstructive pulmonary disease (COPD), asbestosis and bronchogenic carcinoma. Increased ROS levels in the lungs of such patients are reflected by elevated concentrations of OS markers in the breath, airways, lung tissue and blood.

Lung syndromes like ARDS reflect severe injury leading to dysfunction and compromise of the barrier properties of the pulmonary tissues as a consequence of an up-regulated inflammatory response[2]. In response to an initiating event (sepsis, bacterial infectious, shock, trauma, multiple transfusions, pancreatitis and so on) pulmonary immune cells, namely leukocytes, become activated and release a variety of citotoxic molecules including ROS and other inflammatory mediators. Leukocytes, particularly neutrophils and macrophages, are normally considered to be the most important source of ROS in this setting. In turn, some pro-inflammatory molecules such as cytokines, chemokines, complement fragments and lipid mediators are able to activate neutrophils to produce ROS. It is also evident that non-professional phagocytes including endothelium, epithelium, fibroblasts and smooth muscle cells express oxidases capable of generating physiologically relevant concentrations of ROS. Other sources of ROS include mitochondrial electron transport chain and xanthine oxidase activity. Additionally, during an inflammatory response endogenous antioxidants are rapidly overwhelmed. All these perpetuate a vicious cycle by recruiting additional inflammatory cells that in turn produce more cytotoxic mediators, leading at last to deep injury and respiratory failure.

Interestingly, vitamin E seems to play protective effects in lung injury offering a probably new therapeutic approach.

Katsoulis et al[3] wanted to assess serum total antioxidant status (TAS) in patients with community-acquired pneumonia (CAP) and the probable correlation with the severity of the disease. Thirty patients (22 men, 8 women; mean age of 48 ± 21 years) and 10 healthy nonsmokers (mean age 44 ± 16 years) were studied. Clinical, laboratory and radiological findings were recorded on the day of admission and on the 7th day. A severity score was calculated using the Fine scale. Serum TAS was measured at the same time points using a colorimetric method. On admission, TAS (TAS1) was significantly lower than on the 7th day (TAS2) (0.84 ± 0.13 mmo/l vs. 1.00 ± 0.17 mmo/l; $p = 0.0001$) and compared with the healthy subjects (0.84 ± 0.13 vs. 1.19 ± 0.09 mmol/l; $p < 0.001$). TAS change (TAS2-TAS1) was statistically significantly more marked in smokers (0.17 vs. 0.28, $p = 0.001$), in patients with factors predisposing to CAP (0.12 vs. 0.37; $p = 0.000$) and in patients with gram-negative pneumonia (0.16 vs. 0.35; $p = 0.000$). On the other hand, change in TAS was statistically significantly less marked in patients with lobar pneumonia (0.27 vs. 0.17; $p = 0.001$). Additionally, TAS change was positively correlated to white blood count on admission (r = 0.39; $p = 0.029$). It is concluded that serum TAS is decreased in patients with CAP, suggesting the presence of oxidative stress, and that change in TAS seems to be influenced by disease severity. TAS measurement may be useful in estimating the severity of CAP and is a probable indication for the administration of antioxidants in the management of the disease.

A study was undertaken by Guenegou et al[4] to determine whether subjects with low levels of antioxidants (serum beta-carotene, alpha-carotene, vitamins A and E) would be at a higher risk of accelerated decline in forced expiratory volume in 1 sec (FEV_1) as their lungs would be less protected against oxidative stress. 1194 French subjects aged 20-44 years were examined in 1992 as part of the European Community Respiratory Health Survey (ECRHS); 864 were followed up in 2000 and 535 (50% men, 40% life-long non-smokers) had complete data for analysis. During the 8 year study period the mean annual decrease in FEV_1 (adjusted for sex, centre, baseline FEV_1, age, smoking, body mass index and low density lipoprotein cholesterol) was 29.8 ml/year. The rate of decrease was lower for the subjects in tertile I of beta-carotene at baseline than for those in the two other tertiles (–36.5 vs

–27.6 ml/year; $p = 0.004$). An increase in beta-carotene between the two surveys was associated with a slower decline in FEV_1. No association was observed between alpha-carotene, vitamin A, or vitamin E and FEV_1 decline. However, being a heavy smoker (> or = 20 cigarettes/day) in combination with a low level of beta-carotene or vitamin E was associated with the steepest decline in FEV1 (–52.5 ml/year, $p = 0.0002$ and –50.1 ml/year, $p = 0.010$, respectively). These results strongly suggest that beta-carotene protects against the decline in FEV_1 over an 8 year period in the general population, and that beta-carotene and vitamin E are protective in heavy smokers.

Asthma and Allergy

Asthma is a chronic relapsing inflammatory disease of the airways. As inflammatory cells produce and release ROS, asthmatic airways are responsible to oxidative stress. Convincing tests confirm that acute asthma in adult is accompained by oxidative and nitrosative stress[5]. Exhaled air of patients with asthma contains high levels of some markers of OS; a lot of studies have shown that inflammatory cells from peripheral blood and bronchoalveolar lavage fluid of asthmatic subjects produce more superoxide anion radicals than those from controls. Further evidence for an oxidant-antioxidant imbalance is provided by the finding of a decreased anti-oxidant capacity in plasma and bronchoalveolar lavage fluid of patients with asthma[6].

The prevalence of asthma has increased dramatically in many countries during the last few years, suggesting that enviromental factors (for example dietary change) play an important role in the aetiology of the disease. Individuals in Westernised societies have progressively reduced their consumption of fruits and vegetables, and as a result have decreased pulmonary antioxidant defences, making them more sensitive to inhaled irritants and allergens.

Several antioxidant nutrients have been reported to be inversely associated with asthma. A nested case-control study was performed by Patel et al[7] in 515 adults with physician diagnosed asthma and 515 matched controls using dietary data obtained from 7 day food diaries. The main outcome measures were physician diagnosed asthma and current symptomatic asthma (diagnosed asthma and self-reported wheeze within the previous 12 months). Cases were similar to controls in age, sex, social class and daily energy intake but had a lower median intake of fruit (132.1 vs 149.1 g/day, $p <$ or = 0.05). 51.5% of the population reported zero consumption of citrus fruit; relative to these individuals, people who consumed > 46.3 g/day had a reduced risk of diagnosed and symptomatic asthma (OR adjusted for potential confounders 0.59 (95% CI 0.43 to 0.82) and 0.51 (95% CI 0.33 to 0.79), respectively). In nutrient analysis, dietary vitamin C and manganese were inversely and independently associated with symptomatic asthma (adjusted OR per quintile increase 0.88 (95% CI 0.77 to 1.00) for vitamin C and 0.85 (95% CI 0.74 to 0.98) for manganese), but only manganese was independently associated with diagnosed asthma [OR 0.86 (95% CI 0.77 to 0.95)]. Adjusted plasma levels of vitamin C were significantly lower in symptomatic cases than in controls (54.3 vs 58.2 micromol/l, $p = 0.003$). Symptomatic asthma in adults is associated with a low dietary intake of fruit, the antioxidant nutrients vitamin C and manganese, and low plasma vitamin C levels. These findings suggest that diet may be a potentially modifiable risk factor for the development of asthma.

Allergy refer to several kinds of immune reactions in which human body is hypersensitized and develops immunoglobulin E (IgE) type antibodies to typical proteins. Type I hypersensitivity is characterized by excessive activation of mast cells and basophils by IgE resulting in a local or systemic inflammatory response to allergens. Local symptoms comprehend different signs as rhinitis, conjunctivitis, bronchoconstriction, asthma attacks, skin rashes and eczema, besides life-threatening anaphylactic shock and death. Data suggest that blocking ROS should be useful for allergic inflammation improvement in the respiratory and intestinal tracts. Additionally, the modulation of oxidative stress-related compounds, such as inducible nitric oxide synthase and redox-sensitive transcriptional factors, may be useful for the regulation of allergic responses[8].

Studies have shown that vitamin E intake may reduce IgE production. Montano Velazquez et al[9] wanted to evaluate the effects of vitamin E supplementation on the severity of nasal symptoms and the serum levels of specific IgE in patients with perennial allergic rhinitis. Sixty-three patients (mean ± SD age, 12 ± 2.4 years) with a history of perennial allergic rhinitis participated in this study. None of the patients had evidence of

acute infectious disease or used tobacco, corticosteroids, antihistamines, or vitamins. Patients were randomized to receive either vitamin E (400 IU/d) or placebo for 4 weeks, with loratadine-pseudoephedrine (0.2/0.5 mg/kg) during the first 2 weeks of treatment. The severity of nasal symptoms was evaluated using a validated questionnaire, which was administered weekly for 4 weeks. The serum concentrations of specific IgE to 5 common inhalant allergens and lipid peroxides were measured before treatment and at the end of the study. Before, during, and after treatment, the symptom severity scores were similar in the 2 groups; within each group, a significant decrease was observed after the first week of follow-up ($p < .05$), with no further changes. Serum levels of specific IgE and lipid peroxides did not show any significant changes related to vitamin E intake within and between groups. In patients with perennial allergic rhinitis, vitamin E supplementation (400 IU/d) did not have any significant effects on nasal symptom severity or on serum concentrations of specific IgE to 5 common allergens.

Chronic Obstructive Pulmonary Disease (COPD)

COPD is characterised by the progressive decline of lung function and the presence of airflow obstruction as a result of chronic bronchitis or emphysema. The single most important aetiological factor in the pathogenesis of this disease is cigarette smoke. It is estimated that 90% of all patients with COPD are, or have been, smokers but only about 20% of cigarette smokers develop the condition. As oxidants play a relevant role in cigarette smoke-induced lung damage, pulmonary antioxidant status defence mechanisms has a great importance. Plasma antioxidant capacity has been shown to be decreased in smokers and in association with exacerbations of COPD, supporting evidence of a depletion of vitamin C, E and other serum anti-oxidants. However, some studies have revealed increased circulating anti-oxidant concentrations in cigarette smokers. These inconsistencies, together with the great individual variability of anti-oxidant levels in cigarette smokers, has led to suggestions that a protective response mechanism may exist, whereby low-grade oxidative stress can bring about a subsequent adaptive resistance to oxidative stress by increasing anti-oxidant defenses. This system may explain because some cigarette smokers develops COPD while others not. The activation of anti-oxidant enzymes, wheter in erythrocytes, alveolar macrophages and/or lungs, by cigarette smoke exposure are currently unknown; however, it is likely to be a result of anti-oxidant genes induction and probably other intrinsic factors, that control the oxidant/anti-oxidant balance.

A Cerda et al study[10] want to investigate the effect of antioxidant polyphenol-rich pomegranate juice (PJ) supplementation for 5 weeks on patients with stable COPD. A randomized, double-blind, placebo-controlled trial was conducted. A total of 30 patients with stable COPD were randomly distributed in two groups (15 patients each). Both groups consumed either 400 ml PJ daily or matched placebo (synthetic orange-flavoured drink) for 5 weeks. Trolox Equivalent Antioxidant Capacity (TEAC) of PJ, blood parameters (14 haematological and 18 serobiochemical), respiratory function variables, bioavailability of PJ polyphenols (plasma and urine) and urinary isoprostane (8-iso-PGF(2alpha)) were evaluated. The daily dose of PJ (containing 2.66 g polyphenols) provided 4 mmol/l TEAC. None of the polyphenols present in PJ were detected in plasma or in urine of volunteers. The most abundant PJ polyphenols, ellagitannins, were metabolized by the colonic microflora of COPD patients to yield two major metabolites in both plasma and urine (dibenzopyranone derivatives) with no TEAC. No differences were found ($p > 0.05$) between PJ and placebo groups for any of the parameters evaluated (serobiochemical and haematological), urinary 8-iso-PGF(2alpha), respiratory function variables and clinical symptoms of COPD patients. These results suggest that PJ supplementation adds no benefit to the current standard therapy in patients with stable COPD. The high TEAC of PJ cannot be extrapolated in vivo probably due to the metabolism of its polyphenols by the colonic microflora. The understanding of the different bioavailability of dietary polyphenols is critical before claiming any antioxidant-related health benefit.

Inflammatory Diseases and Infections

Inflammation is the first response of the immune system to infection or irritation and represents an innate cascade of events including ROS

and RNS generation. Consequently, OS is a feature of many viral infections. ROS and RNS modulate the cells permissiveness to viral replication, regulate host inflammatory and immune responses and cause oxidative damage to both host tissue and progeny virus. Even if the metabolic role of oxidants in viral infections is still not fully understood, a role for them in viruses activation is generally recognized.

Low-grade inflammation enhances oxidative stress and lipid peroxidation in several clinical settings. Since the inflammation response is non-specific, it can, infact, be also dangerous. As a result, chronic inflammatory and/or autoimmune diseases such as lupus erythematosus and reumathoid arthritis can develop. Increased generation of free radicals also plays an important role in some infections and in several inflammatory diseases, such as hepatitis, gastritis and colitis, as well as in chronic renal failure. Also hepatic metabolism of biological toxins and drugs is related to augmented ROS production. The subsequent redox imbalance and OS causes tissue damage ranging from subclinical anicteric hepatitis to necroinflammatory hepatitis (acute, recurrent, or chronic), cirrhosis and carcinoma. OS plays a relevant pathogenetic role in viral hepatitis and human immunodeficiency virus (HIV) infection with which it's frequently associated.

Poor nutrition is inherently associated with decreased immunity and increased sensitivity to infection. A relevant recent advance is the recognition that OS is the major pathogenetic factor in inflammatory, toxic, ischemic and infectious diseases. Xenobiotics disturb the cellular metabolism and also affect other organs. Plasma anti-oxidant depletion affects the target organ most severely and involves other organs secondarily, rendering them vulnerable to toxins and co-infections[11].

Vitamin C concentrations in the plasma and leukocytes rapidly decline during infections and stress. Supplementation of vitamin C was found to improve components of the human immune system such as antimicrobial and natural killer cell activities, lymphocyte proliferation, chemotaxis, and delayed-type hypersensitivity. Vitamin C contributes to maintaining the redox integrity of cells and thereby protects them against reactive oxygen species generated during the respiratory burst and in the inflammatory response. Likewise, zinc undernutrition or deficiency was shown to impair cellular mediators of innate immunity such as phagocytosis, natural killer cell activity, and the generation of oxidative burst. Therefore, both nutrients play important roles in immune function and the modulation of host resistance to infectious agents, reducing the risk, severity, and duration of infectious diseases: this is of special importance in populations in which insufficient intake of these nutrients is prevalent. A large number of randomized controlled intervention trials with intakes of up to 1 g of vitamin C and up to 30 mg of zinc are available. These trials document that adequate intakes of vitamin C and zinc ameliorate symptoms and shorten the duration of respiratory tract infections including the common cold. Furthermore, vitamin C and zinc reduce the incidence and improve the outcome of pneumonia, malaria, and diarrhea infections, especially in children in developing countries[12].

Viral infections and Hepatitis

OS occurs following different viral infections and may enhance viral replication. It has long been thought that the nutritional status can influence an individual's sensitivity to infection as well as the infection severity. The basis for this condition was initially thought to be a decreased level of immune function secondary to the underlying nutritional deficiency. An anti-oxidant deficiency not only decreased specific immune functions, but also led to specific mutational changes in the viral genome such that a normally avirulent virus became virulent. Patients who developed clinical sequelae from viral exposure had lower serum levels of several nutrients, including vitamin B2, selenium, carotenes and lycopene, than a control, non-infected group. Furtehrmore, researches of influenza virus have confirmed that oxidative stress influenced both the infectivity as well as the infection severity.

Beyond to the acute infections, chronic viral infection has been correlated with increased OS and subsequent increased probability of progression to viral-associated carcinoma.

Of the three dominant types of viral hepatitis (A, B and C), B and C are increasing in percentage. Old age, male sex, recurrent blood transfusions and alcholism are tipically related to chronic hepatiits which is one of the most serious causes of liver disease.

In HCV (chronic hepatitis C) patients, plasma and hepatic Zn levels are low and hyperzincuria is pronounced. Some HCV patients exhibit unusually high concentrations of serum ferritin and hepatic iron and since iron catalyzes production of highly ROS.

Even if OS favours viral replication, it is inhibited by anti-oxidants so in hepatitis progression there are periodic exacerbations. The fluctuating redox balance reflects the nutritional status, concurrent illness, xenobiotics and drugs.

Melhem et al[13] wanted to determine the safety and efficacy of treatment of chronic HCV patients via a combination of antioxidants. Fifty chronic HCV patients were treated orally on a daily basis for 20 weeks with seven antioxidative oral preparations (glycyrrhizin, schisandra, silymarin, ascorbic acid, lipoic acid, L-glutathione, and alpha-tocopherol), along with four different intravenous preparations (glycyrrhizin, ascorbic acid, L-glutathione, B-complex) twice weekly for the first 10 weeks, and followed up for an additional 20 weeks. Patients were monitored for HCV-RNA levels, liver enzymes, and liver histology. Assessment of quality of life was performed using the SF-36 questionnaire. In one of the tested parameters (e.g., liver enzymes, HCV RNA levels, or liver biopsy score), a combination of antioxidants induced a favorable response in 48% of the patients (24). Normalization of liver enzymes occurred in 44% of patients who had elevated pretreatment ALT levels (15 of 34). ALT levels remained normal throughout follow-up period in 72.7% (8 of 11). A decrease in viral load (one long or more) was observed in 25% of the patients (12). Histologic improvement (2-point reduction in the HAI score) was noted in 36.1% of the patients. The SF-36 score improved in 26 of 45 patients throughout the course of the trial (58% of the patients). Treatment was well tolerated by all patients. No major adverse reactions were noted. These data suggest that multi antioxidative treatment in chronic HCV patients is well tolerated and may have a beneficial effect on necro-inflammatory variables. A combination of antiviral and antioxidative therapies may enhance the overall response rate of these patients.

Liver Diseases

Many studies suggest a synergetic hepatotoxic effect between alcohol and ischemia-reperfusion injury. Even if other toxic mechanisms may be involved in these experimental conditions, the events induced by both alcohol metabolism and long-term anoxia lead to a final perturbation of oxygen free radical (OFR)[14,15]. It is well known that chronic administration of high doses of ethanol (EtOH) is related to an increase in the generation of superoxide, hydroxyl radical, hydrogen peroxide and alcohol-derived free radicals and a decrease in both enzymatic and non-enzymatic antioxidant systems[16-18]. However, it has been suggested, by some clinical and epidemiologic studies,that moderate alcohol consumption is associated with a decrease in the incidence of myocardial infarction[19,20], ischemic stroke[21] and an increase in the survival rate after both these events[22].

Detection of chemiluminescence (CLS) emission was proposed as a method to visualize liver OFR generation in real time using specific CLS enhancers for anion superoxide and hydrogen peroxide[23]. This method has been used to evaluate the massive OFR production in the liver during the reoxygeneration phase that follows a period of prolonged anoxia[24,25].

There has long been speculation that liver toxicity secondary to chronic EtOH consumption is partly due to ROS generated in the oxidative pathways of alcohol metabolism[26-28]. The generation of acetaldehyde, mediated by the cytosolic alcohol dehydrogenase, may initiate lipid peroxidation, correlated with the presence of lipoperoxides, conjugated dienes, MDA[29] and a decrease in GSH tissue concentration[30]. Although a marked depletion of this antioxidant defens can be found in rats given RtOH for a long time, this event *per se* does not lead to a liver injury[31]. In fact, simultaneous production of superoxide, hydrogen peroxyde and hydroxyl radical, including xanthine oxidase, microsomal monoxygenase enzymes and the mitochondrial respiratory chain, may be required to generate alcohol-related cytotoxicity[32,33].

Addolorato et al[34] wanted to investigate effects of different doses of ethanol on liver oxidative injury. Ethanol-containing diets were administered to rats (24, 30, 36, 40% for groups A, B, C, D, respectively). After 4 weeks, livers were exposed to ischemia-reperfusion. Chemiluminescence was recorded; total lipids, adenosine triphosphate, malondialdehyde, reduced glutathione and lactic dehydrogenase were measured. In all groups, ischemia resulted in the disappearance of $O_2^{\bullet -}$, a decrease in glutathione and adenosine triphosphate, and stable malondialde-

hyde values. During the reperfusion phase, $O_2^{\bullet -}$ production, malondialdehyde and lactic dehydrogenase increased, reaching significantly higher values in groups C and D and significantly lower values in group B. The effect of ethanol on ischemia-reperfusion injury seems to be a dose-related response, with an additional toxic effect only at high doses of ethanol.

Steatosis of the liver is mediated by a multiple factors such as an increase in dietary fat intake, release of FFA from adipose tissue, insufficient hepatic lipid secretion and development of insulin resistance[35]. FFA oxidation, CYP2E1 induction, leukocyte infiltration and activation of NADPH oxidase and mitochondrial dysfunction involving electron transfer inhibition in the respiratory chain increase the production of ROS such as singlet oxygen, superoxide anion, hydrogen peroxide and hydroxyl radical.

In order to reflect the true state of oxidative stress in the liver, it is more ideal to measure lipid peroxidation markers and anti-oxidant components in hepatic tissue. Nevertheless, it is impossible to perform multiple tests on very limited amounts of biopsy specimen that obtained in needle biopsy. Additionally, liver biopsy carries a significant morbidity and even mortality risk. It is not an ethical approach to perform liver biopsy in healthy controls to provide a comparison with nonalcoholic steatohepatitis (NASH) subjects.

Steatohepatitis is a disease that can be progressive, cause fibrosis and cirrhosis and can ultimately lead to liver failure and hepatocellular carcinoma in a minority of patients[36-39]. The pathogenesis of NASH remains unclear and the factors, which cause the progression from bland steatosis to steatohepatitis, often termed the "second hit", remain poorly understood[40-42]. Oxidative stress, an increase in oxidants and/or a decrease in antioxidant capacity, is one of the potential biochemical mechanisms involved in the pathogenesis of NASH[43,44]. Oxidative stress is a cardinal feature of alcoholic steatohepatitis[45]. Histological similarity between NASH and alcohol induced liver disease suggests some shared pathogenic features such as oxidative stress and cytokine-mediated injury[46,47]. Additionally, evidence of oxidative stress have been found in human livers showing steatosis or NASH[48], and in experimental models of NASH[49]. The efficacy of several anti-oxidant agents on hepatic steatosis, inflammation and fibrosis in subjects with NASH has been investigated in several small, open-label studies[50-52]. In all of these studies, those anti-oxidant agents exerted beneficial effects in improving necroinflammatory activity or fibrosis or both. These results are also suggestive for the role of oxidative stress in the pathogenesis of NASH.

Various techniques have been developed for the measurement of total anti-oxidant status. Moreover, there is not yet an accepted "gold standard" reference method[53], and decisions concerning standardization, and the terms and units used for the measurement of total anti-oxidant response (TAR)[54]. This implies that this topic needs to be studied further[55].

In a few studies, in NASH subjects, anti-oxidant and oxidant capacities have been investigated[56-58]. Koruk et al[56] reported an increase in oxidative stress in NASH subjects. However, in their study, plasma anti-oxidant capacities observed to be comparable in NASH subjects and controls. They suggested that impaired anti-oxidant defense mechanisms in responding to increased oxidative stress might be an important factor in the pathogenesis of NASH. In a study of Fierbinteanu-Braticevici et al[57] serum index of oxidative stress have been suggested as an independent risk factor for fibrosis in the course of NASH. In a Videla et al study[58] it has been showed that nonalcoholic fatty liver disease (NAFLD) patients with steatosis exhibit a substantial pro-oxidant condition in the liver at early stages of steatosis. This pro-oxidant condition was observed to occur concomitantly with a significant decrease in hepatic SOD activity, changes involving an overall derangement in the anti-oxidant status of the liver, with the consequent diminution in the anti-oxidant capacity of plasma. They also observed that further exacerbation in oxidative stress was associated with CYP2E1 induction in patients with steatohepatitis.

Gastric Mucosa

Helicobacter pylori is an important agent in the pathogenesis of active chronic gastritis, peptic ulcer and low-grade gastric MALT lymphoma and in gastric carcinogenesis[59]. Generation of cytotoxins, urease, ammonia, T-cell mediated damage and a mainly humoral reaction are among the events which involve mucosal integrity following *H. pylori* infection[60]. Moreover, reactive oxygen metabolites (ROMs) have been found to play a relevant role in gastroduodenal inflammatory

damage[61]. Particularly, the acute mucosal inflammatory infiltrate (e.g. polymorphonuclear cells, PMNs), which characterize *H. pylori*-related chronic active gastritis, could be an important source of free radicals[62], as both *in vivo* an *in vitro* studies have reported a positive relation between *H. pylori* infection and ROMs generation[63,64]. Infact, the host immune response to the infection may involve generation of great amounts of ROMs to kill microrganisms[65]: when neutrophils are activated, ROMs concentration is very high[66]. However, *H. pylori* seems to be resistant to the antimicrobial action of ROMs and such resistance has been attributed to bacterial antioxidant species generation. Consequently, the excessive ROMs generation by mucosal inflammatory cells may damage other gastric cellular components and induce structural and functional changes in proteins, carbohydrates and DNA, resulting in the derangement of gastric mucosal cell biology[67]. More virulent *H. pylori* strains, bearing the cytotoxic associated gene A (CagA), have been shown to induce a more severe local inflammatory reaction[68], resulting in more serious degrees of gastric inflammation and enhanced mucosal amounts of cytokines and acute phase reactants[69,70]. *In vitro* studies also demonstrated that CagA-positive strains induce an increased oxidative burst in PMNs with higher ROMs generation.

Danese et al[71] wanted to assess ROMs generation in mucosal biopsy specimens of patients with *H. pylori* CagA-positive- and *H. pylori* CagA-negative-related gastritis and to verify whether ROMs generation and *H. pylori* CagA status are related to differences in gastric mucosal macroscopic injury and in microscopic inflammatory infiltration. Patients undergoing gastroscopy were enrolled. *H. pylori* infection was assessed by histology and 13C urea breath test. CagA status was assessed through serology. ROMs were assayed in gastric biopsies by luminol-enhanced chemiluminescence (CLS). Gastric mucosal inflammation was histologically graded and neutrophils were individually counted. Macroscopical damage was scored according to a modified Lanza score. 40 out of 60 patients evaluated were *H. pylori* (HP) positive. Of the 40 infected patients, 24 were CagA-positive. CLS emission was significantly higher in HP-CagA-positive patients than in HP-CagA-negatives and uninfected. ROMs production showed a significant correlation to neutrophil infiltrate in all groups. Gastric mucosa of patients infected by HP-CagA-positive strains is characterized by a higher generation of ROMs and by greater neutrophil counts than that observed in HP-CagA-negative subjects. Since ROMs production is associated with DNA oxidative damage, a long-term stimulation by these strains might be relevant in the pathogenesis of gastric malignancies. Assessment of CagA status might be useful to discriminate patients in which H. pylori eradication is advisable.

Modzelewski[72] examined 92 patients with gastro-esophageal reflux disease (GERD) confirmed by 24-hour pH-metry, upper gastrointestinal tract endoscopy, and X-ray barium scan. All patients were divided into 2 groups: first consisted of patients (51) with no signs of esophagitis and the second one (41) included individuals with gastro-oesophageal reflux complicated with inflammation. Inflammation of the mucosa was confirmed by pathological examination. In these patients SOD (superoxide dismutase) and glutathione peroxidase (GP) activities were measured in serum and mucosal homogenates. Tests were performed before and 8 weeks after treatment. Control group consisted of patients scheduled for elective inguinal hernia repair procedure. Serum SOD activity in oesophagitis patients was 1173.7 U/gHb before the treatment, and 1401.7 U/gHb afterwards. These differences were statistically significant. Also differences in SOD serum activity in oesophagitis and non-oesophagitis patient groups were significant. Serum GP activity in oesophagitis patients was 11.17 U/gHb before and 25.12 U/gHb after treatment. The differences showed statistical significance. Measured activities did not reach the control values too. Serum SOD and GP activity was lower than those measured in tissue homogenates from corresponding patient groups.

Hernia

Polat et al[73] wanted to investigate markers of oxidative stress–malondialdehyde (as TBARS), protein carbonyls and protein sulfhydryls–in patients undergoing Lichtenstein tension-free hernioplasty (LH) or laparoscopic preperitoneal hernia (LPPH) repair. Seventeen patients with unilateral inguinal hernia and no complications or recurrence were included in this study. Ten were randomized to undergo LH and seven to LPPH repair. Heparinized blood samples were

taken to measure the levels of oxidative stress markers in the patients undergoing hernia repair. Levels of malondialdehyde, protein carbonyls and protein sulfhydryls were measured preoperatively and at 6 and 24 hours postoperatively in all patients. Both types of hernia repair caused a significant increase in the oxidative stress response and a decrease in antioxidant activity. Plasma levels of malondialdehyde and carbonyls (indicators of oxidant activity) were significantly higher in the LH than in the LPPH repair group ($p < .05$) and plasma sulfhydryl levels (indicators of antioxidant activity) were significantly lower in the LH than in the LPPH group ($p < .05$). In both groups, significant differences were also found between the preoperative levels and the postoperative levels 6 and 24 hours ($p < .05$). These data demonstrate that both LH and LPPH repair cause a significant increase in markers of oxidative stress; however, the oxidative stress response associated with LH is greater than that associated with LPPH repair.

Acquired Immune Deficiency Syndrome (AIDS)

AIDS is the terminal phase of Human Immunodeficiency Virus (HIV) infection when it runs its natural course. OS plays a relevant pathogenetic role in HIV infections. HIV-infected patients are in oxidative imbalance early in the disease: serum and tissue anti-oxidants levels are low and peroxidation products elevated. High plasma levels of MDA, reduced plasma GSH, decreased GSHPx (glutathione peroxidase) and SODs activities are normally found. HIV infection results also in considerably reduced vitamin E and C concentrations and very low plasma Zn and Se levels[74]. Particularly, Se deficiency is related to the occurrence, virulence and disease progression of some virus infections including HIV progression to AIDS.

Cumulative effects of OS and antioxidant depletion increase AIDS-linked anorexia that is accentuated by alcohol abuse, as well as other xenobiotics and periodic infections. Given the prevalence of hepatitis B and C in alcoholics and drug users and their propensity to acquire HIV infections, it is obvious that these disorders combinations further deplete the host and liver of anti-oxidants and accentuate redox imbalance. Because immune cells normally requiring a higher anti-oxidant concentration than other cells to retain redox balance, anti-oxidant depletion indicates a decrease in immune function and, on the contrary, an optimal anti-oxidant capacity preserves integrity and function of the immune system.

HIV infection represents another example where the progression of HIV infection to AIDS seems to be influenced by nutritional factors, particularly by anti-oxidants and vitamin B12. Insufficient intake, malabsorption diarrhea, impaired storage, altered metabolism and increased usage can all contribute to the nutritional deficiencies observed in HIV infected individuals. It has beeen suggested that redox imbalance to the oxidant state favours disease progression with increased viral replication, immune dysfunction and carcinogenesis whereas the anti-oxidant state inhibits viral replication and potentiates latency and healing.

Rheumatoid Arthritis

Rheumatoid arthritis (RA), a chronic inflammation polyarthritis that destroys synovial joints, is characterized by the presence of activated T lymphocytes, neutrophils, macrophages and synoviocytes in the synovial and periarticular tissues. Here, proliferation, inflammation, cellular and humoral immune responses result in the release of metallo-protease enzymes and other mediators that damage connective tissue and cartilage. The progressive deterioration and loss of articular cartilage leading to an irreversible impairment of joint motion are the final pathogenic events common to osteoarthritis and rheumatoid arthritis. In both arthritis the loss of integrity of the cartilage extracellular matrix depends on an imbalance between anabolic and catabolic pathways.

Then ROS and RNS can directly or indirectly be responsible of articular constituents damage and lead to the clinical expression of the inflammatory arthritis. Several factors are involved in the development of oxidative stress in the joints of RA patients; firstly, ROS generation from locally activated leukocytes, but even increased pressure in the synovial cavity[75], reduced capillary density, vascular changes and an increased metabolic rate of synovial tissue. ROS production could be facilitated also by a repetitive ischemia reperfusion injury in the joint. Addition-

ally, tissue injury releases iron and copper ions, as well as heme proteins, which are catalytic for free radical reactions. Electron transport chain are also disrupted in the mitochondria and endoplasmic reticulum, leading to electrons leakage to give superoxide. Increased production of ROS in RA patients has been suggested by enhanced levels of ascorbic acid in serum and synovial fluid and increased breath pentane excretion. Moreover, thioredoxin concentrations, a marker of OS, are significantly higher in synovial fluid from RA patients with other forms of arthritis[76]. Peripheral blood lymphocyte DNA from RA patients contains significantly increased levels of the promutagenic 8-oxohydrodeoxyguanosine (8-oxodG), a product of oxidative damage to DNA, pointing to the genotoxic effects of oxidative stress. The NO (nitric oxide) production is also up-regulated in rheumatoid synovial tissue. HOCl, $ONOO^-$ and O_2^- react with ascorbate, which is essential for cartilage function, leading to low levels of ascorbate in synovial fluid.

In RA, phagocytosis of immune complexes by neutrophils may be self perpetuated by the ROS release from these phagocytes in the diseases joint. ROS, infact, may alter the antigenic behaviour of immunoglobulin G, producing a protein aggregates that can further activate phagocytic cells to release more free radicals. Free radicals are then believed to play a role in the exacerbation and chronicity of the inflammatory reaction.

All this knowledge might serve to apply a rational selection of anti-oxidants for possible therapeutic purposes, enforcing combination therapy of the inflammatory joint disease.

A male patient with rheumatoid arthritis (RA) developed acute stroke and was treated with the free radical scavenger, edaravone. Polyarthralgia improved with a reduction in serum C-reactive protein concentration soon after the start of edaravone administration. The disease activity score 28 (DAS28) also decreased. Edaravone appears to be effective for the control of RA. The usefulness of this potentially novel therapeutic agent should be tested in a well designed randomized controlled trial[77].

Hearing Loss

Sensorineural hearing loss following acute bacterial meningitis could be caused by hydroxyl radicals generated by the inflammatory response. Obstruction of cerebrospinal fluid circulation through the tela choroidae of the choroid plexuses, with subsequent rupture of the tela choroidae, would expose the auditory nerve to selective radical damage. Acute administration of lipophilic antioxidants might provide the auditory nerve with increased protection[78].

Hearing loss is the most frequent long-term complication of pneumococcal meningitis, affecting up to 40% of survivors. Unfortunately, adjuvant therapy with dexamethasone has failed to satisfactorily reduce its incidence. Therefore, Klein et al[79] evaluated the use of antioxidants for the adjunctive therapy of meningitis-associated deafness. Eighteen hours after intracisternal injection of 7.5 × 10(5) colony-forming units of *Streptococcus pneumoniae*, rats were treated systemically either with ceftriaxone and the antioxidants and peroxynitrite scavengers Mn(III)tetrakis(4-benzoic acid)-porphyrin (MnTBAP) or N-acetyl-L-cysteine (NAC) or placebo (1 ml phosphate-buffered saline) for 4 days. Hearing was assessed by auditory brainstem response audiometry. Adjunctive antioxidant therapy significantly reduced the long-term hearing loss (14 days after infection) for square wave impulses (mean hearing loss ± SD: ceftriaxone and placebo, 45 ± 26 dB; ceftriaxone and MnTBAP, 9 ± 23 dB; ceftriaxone and NAC, 19 ± 30 dB) as well as 1 kHz (ceftriaxone and placebo, 28 ± 19 dB; ceftriaxone and MnTBAP, 10 ± 16 dB; ceftriaxone and NAC, 10 ± 17 dB), and 10 kHz tone bursts (ceftriaxone and placebo, 62 ± 27 dB; ceftriaxone and MnTBAP, 16 ± 13 dB; ceftriaxone and NAC, 25 ± 26 dB). Furthermore, both antioxidants attenuated the morphological correlates of meningogenic hearing loss, namely, long-term blood-labyrinth barrier disruption, spiral ganglion neuronal loss and fibrous obliteration of the perilymphatic spaces. Adjuvant antioxidant therapy is highly otoprotective in meningitis and therefore is a promising future treatment option.

ROS have been implicated in hearing loss associated with aging and noise exposure. SODs form a first line of defense against damage mediated by the superoxide anion, the most common ROS. Absence of Cu/Zn SOD (SOD1) has been shown to potentiate hearing loss related to noise exposure and age. Conversely, overexpression of SOD1 may be hypothesized to afford a protection from age- and noise-related hearing loss. This hypothesis may be tested by Coling et al[80] using a transgenic mouse model carrying the hu-

man SOD1 gene. Contrary to expectations, here, we report that no protection against age-related hearing loss was observed in mice up to 7 months of age or from noise-induced hearing loss when 8 week old mice were exposed to broadband noise (4-45 kHz, 110 dB for 1 h). Mitochondrial DNA deletion, an index of aging, was elevated in the acoustic nerve of transgenic mice compared to nontransgenic littermates. The results indicate the complexity of oxidative metabolism in the cochlea is greater than previously hypothesized.

Assuming that superoxide anion radicals (O_2^-) may play a role in damage to the inner ear, Joachims et al[81] investigated the possible benefit of vitamin E as an antioxidant in the treatment of idiopathic sudden hearing loss. A total of 66 patients, aged 15 to 70 years, with diagnoses of idiopathic sudden hearing loss of less than 7 days' duration during 1998 to 2001, were included in the study. All were treated with bed rest, steroids, magnesium and carbogen inhalation. The study group received vitamin E in addition. The recovery rate, calculated as hearing gain divided by the difference in hearing level between the affected and unaffected ear, was better than 75% in 41 of 66 (62.12%) patients. This rate was achieved in 26 (78.78%) patients in the study group treated with vitamin E, compared with 15 (45.45%) patients in the control group. Patients treated with the addition of vitamin E achieved better recovery than did the control patients. Further studies should be directed toward a better understanding of the role of antioxidants in idiopathic sudden hearing loss.

Reactive oxygen species play a major role in drug-, noise-, and age-dependent hearing loss, but the source of ROS in the inner ear remains largely unknown. Banfi et al[82] demonstrate that NADPH oxidase (NOX) 3, a member of the NOX/dual domain oxidase family of NADPH oxidases, is highly expressed in specific portions of the inner ear. As assessed by real-time PCR, NOX3 mRNA expression in the inner ear is at least 50-fold higher than in any other tissues where its expression has been observed (e.g. fetal kidney, brain, skull). Microdissection and in situ hybridization studies demonstrated that NOX3 is localized to the vestibular and cochlear sensory epithelia and to the spiral ganglions. Transfection of human embryonic kidney 293 cells with NOX3 revealed that it generates low levels of ROS on its own but produces high levels of ROS upon co-expression with cytoplasmic NOX subunits. NOX3-dependent superoxide production required a stimulus in the absence of subunits and upon co-expression with phagocyte NADPH oxidase subunits p47(phox) and p67(phox), but it was stimulus-independent upon co-expression with colon NADPH oxidase subunits NOX organizer 1 and NOX activator 1. Pre-incubation of NOX3-transfected human embryonic kidney 293 cells with the ototoxic drug cisplatin markedly enhanced superoxide production, in both the presence and the absence of subunits. These results suggest that NOX3 is a relevant source of ROS generation in the cochlear and vestibular systems and that NOX3-dependent ROS generation might contribute to hearing loss and balance problems in response to ototoxic drugs.

Noise induced hearing loss (NIHL) is a complex disease caused by an interaction between genetic and environmental factors. Damage in the cochlea as a result of noise exposure appears to be mediated by ROS. To investigate whether genetic variation in the human protective antioxidant system is associated with high or low susceptibility to NIHL, genetic polymorphisms derived from genes involved in the oxidative stress response were analysed in the 10% most susceptible and 10% most resistant extremes of 1200 Swedish noise-exposed workers. The genetic polymorphisms included 2 deletion polymorphisms for the GSTM1 and GSTT1 gene, and 14 SNPs derived from the CAT (catalase), SOD, GPX, GSR and GSTP1 genes. No significant differences were found between susceptible and resistant groups, providing no support for a major role of genetic variation of antioxidant enzymes in the susceptibility to NIHL[83].

Critically ill Surgical Patients

Nathens et al[84] wanted to determine the effectiveness of early, routine antioxidant supplementation using alpha-tocopherol and ascorbic acid in reducing the rate of pulmonary morbidity and organ dysfunction in critically ill surgical patients. Oxidative stress has been associated with the development of the Adult Respiratory Dystress Syndrome (ARDS) and organ failure through direct tissue injury and activation of genes integral to the inflammatory response. In addition, depletion of endogenous antioxidants has been associated with an increased risk of nosocomial infections. The authors postulated

that antioxidant supplementation in critically ill surgical patients may reduce the incidence of ARDS, pneumonia and organ dysfunction. This randomized, prospective study was conducted to compare outcomes in patients receiving antioxidant supplementation (alpha-tocopherol and ascorbate) versus those receiving standard care. The primary endpoint for analysis was pulmonary morbidity (a composite measure of ARDS and nosocomial pneumonia). Secondary endpoints included the development of multiple organ failure, duration of mechanical ventilation, length of ICU (intensive care unit) stay and mortality. Five hundred ninety-five patients were enrolled and analyzed, 91% of whom were victims of trauma. The relative risk of pulmonary morbidity was 0.81 (95% confidence interval 0.60-1.1) in patients receiving antioxidant supplementation. Multiple organ failure was significantly less likely to occur in patients receiving antioxidants than in patients receiving standard care, with a relative risk of 0.43 (95% confidence interval 0.19-0.96). Patients randomized to antioxidant supplementation also had a shorter duration of mechanical ventilation and length of ICU stay. The early administration of antioxidant supplementation using alpha-tocopherol and ascorbic acid reduces the incidence of organ failure and shortens ICU length of stay in this cohort of critically ill surgical patients.

Oxidative stress is a consequence of critical illness and may have an impact on survival. Mishra et al[85] studied markers of oxidative damage and antioxidant (AO) protection and compared them with clinical scores and outcome. Blood sampling and clinical scoring was carried out on 60 consecutively admitted intensive therapy unit (ITU) patients within 24 h of admission and then every three days of ITU stay. The patients included 30 surgical and 30 medical patients, of whom 46 survived their stay in ITU. Clinical scoring was by Acute Physiology and Chronic Health Evaluation (APACHE) II score, multiple organ dysfunction (MOD) score and sepsis rating. Oxidative damage was assessed by measurement of plasma malondialdehyde (MDA) and F2 isoprostanes (F2 IsoPs). AO protection was assessed by measurement of plasma total AO status, AO gap, ascorbic acid and the enzymes glutathione peroxidase and superoxide dismutase. Both clinical markers, APACHE II and MOD, and oxidative damage markers MDA and F2 IsoPs were significantly higher in non-survivors (NS) than in survivors (S) at the time of admission. Median (interquartile ranges) were (APACHE II), 14[12–17] (S), 20.5[16.7–22.2] (NS), $p < 0.0001$; (MOD), 3.0[2.0–5.0] (S), 8.0[4.7–9.2] (NS), $P < 0.0005$; (MDA, mumol/L), 0.22[0.19–0.27] (S), 0.25[0.20–0.34] (NS), $p = 0.04$ and (F2 IsoPs, pg/mL), 9.7[6.0–9.9] (S), 11.0[9.0–12.0] (NS), $p = 0.01$. Oxidative damage markers reduced (improved) in the survivors but increased in the non-survivors. There was little difference between the groups in AO protection markers. There was a significant positive correlation between MOD and markers of oxidative damage at the time of admission ($r = 0.40$, $p = 0.003$, F2 IsoPs; $r = 0.28$, $p = 0.035$, MDA) and between the oxidative damage markers themselves ($r = 0.32$, $p = 0.017$). Increased oxidative stress is associated with poor outcome in critically ill patients and may be a prognostic indicator. Oxidative damage markers are more useful than AO protection markers in predicting outcome.

Obstetric, Gynecologic and Androgenic Conditions

Pregnancy and Preeclampsia

Oxidative processes exert a fundamental regulatory function during pregnancy. It depends on the influence of oxygen, nitric oxide, ROS and RNS metabolic pathways upon the vascular changes in the maternal organism, as well as on the regulation of uterine and cervical tone throughout gestation and delivery. These functions are strictly linked with the mediators of the inflammatory pathway. At the beginning of the pregnancy, when a certain grade of inflammatory change is necessary to the trophoblast invasion of maternal tissue, the activation of the process by nitric oxide and RNS is welcome. Indeed, these products modulate the metalloproteinases, which are responsible for the remodelling of uterine extracellular matrix. At this stage estrogens are involved as well in the regulation of the delicate balance of pro-oxidant and anti-oxidant effects. Furthermore, ROS and RNS appear to play a relevant role both in normal and pathologic embryogenesis. During advanced pregnancy, a derangement of the oxidative balance can lead to the improper activation of inflammatory changes, thus triggering premature labour as well as other complications, such as foetal growth restriction and preeclampsia[86]. Thus, pregnancy is a condition showing increased sensitivity to OS:

- Pregnancy is characterized by dinamic changes in multiple body systems resulting in a high-energy demand and increased basal oxygen consumption. This process is related to the biological production of free radicals;
- From early pregnancy the human placenta influences maternal homeostasis: placenta has abundant mitochondrial mass (one of the major biological sourec of ROS), it is also highly vascular and is exposed to high maternal oxygen partial pressure. These characteristics explain partially the production of free radicals. NO is also locally generated by the placenta and contributes to potential OS in presence of transition metals[87]. The placenta is also rich in macrophages favoring the local generation of ROS.
- The high estrogen level might favor an augmentation of ROS;
- Natural antioxidant system, such as SOD enzyme, ascorbic acid and thiols, were found to be lower during pregnancy suggesting an oxidative enviroment;
- Lipid peroxidation products increase in normal pregnant women, reaching their maximal levels in the second trimester;
- Over enthusiastic and uncontrolled iron supplementation may further support an OS condition[88].

Preeclampsia is a potentially dangerous complication of the second half of pregnancy, labor or early period after delivery, characterized by hypertension, edema, proteinuria and other systemic disturbances. In clinical practice, preeclampsia is defined by its clinical manifestations and is often discovered late in its course: it's one of the most common reasons for induced preterm delivery. Since preeclampsia cannot be prevented, an objective screening test to predict the onset of preeclampsia would be clinically valuable to identify women who require closer clinical monitoring during pregnancy and also to aid in evaluating new preventive therapies before the onset of clinical symptoms or signs. The cause of preeclampsia remains largely unknown but OS plays a key role. Then a marker of oxidative stress could potentially provide a screening test[89]. Moreover, an antioxidant intervention may be effective in the prevention of the disease.

Growing evidence suggests that placental OS could be involved in the etiopathogenesis of preeclampsia. Reduced perfusion as a result of abnormal placentation leads to ischemia reperfusion injury to the placenta enhancing placental OS. It has been proposed as a promoter of lipid peroxidation and the endothelial cell dysfunction that is commonly seen in this condition. Preeclampsia is characterized by increased lipid peroxidation and diminished antioxidant capacity. Lipid peroxidation and leukocyte activation may play a relevant role in endothelial cell dysfunction[90].

Endometriosis

Endometriosis is a puzzling disease with obscure pathogenesis. It is defined by the presence of endometrial tissue outside of the uterus. Severe pelvic pain is often associated with endometriosis and this pain can be diminished with therapies that suppress oestrogen production. It has been showed an association between endometriosis and the presence of pro-inflammatory cytokines into peritoneal fluid[91]. Most of these active molecules have been implicated as inducers of attachment, proliferation and neovascularization of endometrial cells. Endometriosis is associated with a general inflammatory response in the peritoneal cavity and OS has been proposed as a potential factor involved in the pathophysiology of the disease. Endothelial nitric oxide synthase, the enzyme that produce NO, is also overexpressed in endometriosis and adenomyosis. The endometrium shows altered enzymes expression such as SOD and GSHPx involved in defence against OS. Also vitamin E levels are significantly lower in the peritoneal fluid of women with endometriosis. Sometimes an apparent discrepancy between results may be observed because of the only persistent markers of OS, such as enzymes or stable by-products of oxidative reactions, can still be detected when endometriosis is diagnosed. Another possible explanation is that OS occurs only locally, for example at the site of bleeding and does not result in an increase in total fluid concentrations[92].

Erythrocytes, apoptotic endometrial tissue and cell debris transplanted into the peritoneal cavity by menstrual reflux and macrophages have been implicated as potential inducers of OS. Erythrocytes are likely to release pro-oxidant and pro-inflammatory factors, such as hemoglobin and its highly toxic by-products heme and iron, into the peritoneal enviroment. Iron and heme are essential to living cells, but, unless they are appropri-

ately chelated, free iron and, to a lesser extent, heme play an important role in the generation of deleterious ROS. However, erythrocytes are found in the peritoneal cavity of 90% of menstruating women. Thus, why do some patients develop macroscopically visible peritoneal endometriotic lesions but others do not? One hypothesis is that in some patients, peritoneal protective mechanisms are swamped by menstrual reflux, either because of the abundance of the reflux or because of defective scavenging systems[93]. Additionally, senescent erythrocytes and apoptotic endometrial cells may be responsible for recruitment and activation of mononuclear phagocytes in the peritoneal cavity.

Most reports discuss the potential consequences of increased OS in endometriosis relative to decreased fertility. OS is thought to be one of the factors responsible for impaired fertility in patients with endometriosis. The peritoneal fluid of patients with endometriosis is toxic to sperm[94] and this effect may be mediated by ROS. ROS and NO may also impair ovulation[95]. The effects of OS in fertilization, pre-implantation embryo development and implantation are infact well documented.

Hormonal Treatments

Oral contraceptive pills are combined formulations of a progestin and a synthetic oestrogen. In addition to hormonal activity, both natural and synthetic estrogenic molecules possess an antioxidant activities that is associated with the phenolic group present in their structure. On the other hand, in the presence of redox-active metal ions, estrogens are established oxidants. This mechanism might be due to oestrogen's pro-inflammatory effect, infact hormone therapy use resulted to be correlated with increased amount of C-reactive protein, a marker of inflammation[96]. Then, estrogens and their metabolites have both pro-oxidant and anti-oxidant properties depending on the availability of metal ions and/or their dose and formulation.

Oral contraceptive use modifies slightly but significantly the oxidative status in women and in animals by decreasing in plasma and blood cells the antioxidant defences (vitamins and enzymes). The changes in the oxidative status are related to an increase in plasma lipid peroxides apparently responsible for the hyperaggregability and possibly the imbalance in clotting factors associated with the oral contraceptive-induced prethrombotic state. These effects appear to be increased by a high intake of polyunsaturated fat and counteracted by supplements of vitamin E. Additionally, smoking, diabetes and, to some extent, dyslipidemia are related to an increased lipid peroxides levels and concomitant changes in the antioxidant defences that can be additive to those induced by oral contraception. Thus, free radicals and lipid peroxidation could be the underlying mechanism in the predisposition to thrombosis induced by most risk factors in oral contraceptive users[97].

Thyroid Disorders

Mano et al[98] to clarify whether the changes of free radicals and its scavengers are induced by thyroid disorders, measured levels of free radical scavengers and checked O_2 radical generating systems in the human thyroid gland. Thyroid specimens from patients with Graves' disease, follicular adenoma, and papillary and follicular carcinomas contained significantly higher concentrations of xanthine oxidase (XOD) and GSH-PX, compared to those in the normal thyroid tissue. Catalase concentration was significantly lower in thyroid specimens from patients with Graves' disease and significantly lower in thyroid specimens from patients with follicular adenoma, compared to those in the normal thyroid tissue. Cu/Zn SOD concentration was significantly lower in the specimens from follicular adenoma and papillary carcinoma and Mn SOD concentration was significantly higher in the specimens from papillary carcinoma than those in the normal thyroid tissue. The lipid peroxide concentration, expressed as MDA concentration, was significantly higher in the specimens from papillary carcinoma than those in the normal thyroid tissue. These findings suggest that the levels of free radicals are increased and are scavenged and catalyzed in the thyroid of Graves' disease, whereas free radicals and lipid peroxide are not completely scavenged in papillary carcinoma tissues, suggesting that these substances affect some role in cell function of thyroid tumors.

Menopausal Disturbances

Climacteric is defined as the critical transitional phase of the reproductive life in women from the fertile to unfertile status. It is characterised

by an initial instability of endocrine ovarian function and subsequently the drop of ovarian hormones production and definitive loss of endometrial cycling. This phase of female life is followed by established menopause, which is frequently associated with severe pathologic states, linked to both the increase in age and the long-lasting absence of endogenous hormones.

Lack of estrogens in menopause leads to a redox status imbalance and a change in the lipid profile. The symptoms accompanying the menopause (e.g. hot flushes) suggest that when they are manifest there is increased metabolic activity[99] which together with their repetitive character could cause a redox status imbalance toward oxidative processes. ROS react with many biologic substrates, especially polyunsatured fatty acids leading to lipid peroxidation, with increased lipid peroxides in plasma and to membrane destruction. Other components of the biologic response to the presence of reactive oxygen intermediates is the decrease in anti-oxidants, including reduced sulphydryl groups.

Studies have documented that estrogens are potent anti-oxidants and decrease LDL oxidation *in vitro* and *in vivo*[100,101]. This concept was raised by studies which employed pharmacological concentrations, but it has been appreciated more recently that 17β-estradiol is active even at physiological concentrations[102]. The antioxidative properties of estradiol have been more definitely appreciated in the case of vascular biology and of lipid metabolism[103,104], but it is conceivable that they are exerted also in other districts. For instance animal evidences demonstrate that estrogen deprivation after ovariectomy induces an alteration in redox state that may be responsible for the decreased release of NO and therefore the decrease in systemic vascular conductance[105,106].

The chemical structure of estrogens enables them to act as free-radical scavengers preventing oxidative damage. The key structural element of estrogen molecule required for antioxidant effect is the presence of a phenolic ring in the A position. Under this aspect estrogens can be ascribed to the class of the Polycyclic Phenolic Compounds (PPCs), molecules able to intercalate into the cellular and mitochondrial membranes where they interrupt lipid peroxidation, a free radical-induced chain reaction that undermines membrane integrity or alternatively to integrate into circulating lipoproteins to exert in the microenvironment the antioxidative action, either directly or through poorly defined lipophilic derivatives[107].

Some of the therapies commonly used in menopause present as additional benefit antioxidant activity. The therapeutic agents that demonstrate anti-oxidant activity are all characterised by the presence of a phenolic ring, a key structural element required for cell protection against OS and can be classified as PPCs. It must be kept in mind that the antioxidant activity of PPCs is greatly amplified when cellular content in glutathione is high and predominantly in the reduced state. Glutathione is a small molecule composed of 3 amino acids that operate in cells with some enzymes, such as glutathione peroxidase and glutathione reductase, to scavenge reactive free radicals, in addition to be itself susceptible of oxidation-reduction by a thiol-disulfide exchange.

All estrogen molecules (native or synthetic) have phenol ring in A position of the cyclopentaneperhydrophenantrene structure and for this reason they can be classified as PPCs. The main scientific evidences of the anti oxidant activity of the estrogens came from neurological, cardiovascular and metabolic fields.

Neurological Evidences

Postmenopausal estrogen withdrawal constitutes an important factor for an increase to a susceptibility of CNS to free radicals in the woman. It is well-known that AD is a "female disease" and that the incidence increases progressively during the postmenopausal years.

The evidence is now specifically compelling that estrogens are neuroprotective for AD for the OS *via* several mechanisms[108]:

- estrogen protects PC12 cells expressing mutant presenilin from apoptosis induced by amyloid or serum withdrawal, preventing and reducing free radical generation and preserving cellular ATP pools;
- estrogens protect cortical neurons from hydroxyl radical and amyloid toxicity by moderating intraneuronal free Ca^{2+} and preserving the function of membrane proteins as Na-K-ATPase and glucose transporters;
- estrogens protect neuroblastoma cells from the mitochondrial toxin 3-nitropropionic acid by preserving Dym, thereby moderating both ATP depletion and ROS production;
- estrogen protects spinal cord cultures from mice expressing the human mutant superoxide dismutase correlated to familial SLA against excitotoxicity by preserving mitochondrial function and reducing radical generation;

- estrogen induces activity of constitutive NOS with a rapid increase of NO synthesis: NOS is widespread in the CNS and NO acts as neurotransmitter/neuromodulator. Besides its relevance in relation to CNS degenerative disorders[109], modulation of NO generation by estrogens might not reveal as an important and widespread mechanism also for the rapid non-genomic actions of the hormones.

Cardiovascular Evidences

Another protective mechanism of the estrogens involving lipoproteins is the ability of these hormones to reduce the oxidation of LDL. Oxidised LDL play a relevant role in the development of atherosclerosis. The oxidation of LDL isolated from postmenopausal women is inhibited differentially by some estrogens, such as estriol and 17β-estradiol[110-112]. Not only estradiol or equine estrogens, but also estriol seems to have an antioxidant action on LDL at least *in vitro*, although its effect is less potent than estradiol[113].

Metabolic Evidences

Fatty acids can play a pathogenic relevant role in metabolic syndrome favouring insulin resistance throughout involvement of ROS and/or RNS[114,115]. These radicals could induce macromolecular damage either directly or, indirectly, by activation of several stress-sensitive pathways (such as NF-κB, p38MAPK, JNK/SAPK)[116-118]. Infact, infusion of FFA causes increased oxidative stress as judged by increased malondialdehyde concentrations and a decline in the plasma reduced/oxidised glutathione ratio[119]. *In vitro* evidences also indicate that elevated FFA levels have many side effects on mitochondrial function, including the uncoupling of oxidative phosphorylation[120]. Estrogen withdrawal, consequent to bilateral ovariectomy, is accompained by elevated high blood concentration of FFA, while subsequent administration of transdermal estrogens restore the physiological levels[121]. For this reasons, Pansini et al[122] can prospect that the high incidence of "metabolic syndrome", that often complicates the physiological consequences of menopause, can be at least in part due to the increase of FFA concentrations and the consequent oxidative stress.

Raloxifene

Raloxifene has a cardioprotective effect comes from the demonstration that it reduces blood pressure concentrations and improves endothelial dysfunction in male spontaneous hypertensive rats. These effects are probably mediated by an increase in release of NO from vessel wall and by the reduction of the vascular generation of ROS. This is induced by a direct effect of the raloxifene on the membrane-bound NAD(P)H oxidase, which is shown to represent a major source of free radicals in the vascular wall[123,124]. The evidence[125] that the effect of raloxifene on cellular ROS generation is completely blocked by the estrogen receptor antagonist ICI 182,780 indicates that the anti-oxidant effect of raloxifene is estrogen receptor-mediated.

In conclusion, the antioxidant properties of raloxifene on the vessel are demonstrated by the reduction of free radical release, the induction of the eNOS/NO system, the improvement of endothelial dysfunction. All these effects induce a decrease of blood pressure at least in hypertensive rats.

Plant Antioxidants

In the last years, a lot of studies has been published on the relevance of a consistence intake of phytoestrogenic antioxidating agents (either in the form of traditional foods or of dietary supplementation) on human fitness and well-being. The major nutritive elements taken in account are represented by plant isoflavones, which have proven effective also in the frame of menopausal complications, particularly in relation to vasomotor complaints[126] but probably also for skeletal and genitourinary disturbances. Soy, as well as many other plants (e.g. red clover) contain high amounts of isoflavones daidsein and genistein, mostly as their glycon derivatives, daidzin and genistin. These molecules are powerful antioxidants (because of their phenolic rings), but they show also mild estrogenic activity, with comparatively higher affinity[127] for ER (estrogen receptor)β rather ERα, since they are relatively similar to estrogens for solubility and space-fillin organization, despite definitely different chemical structure. Experimental studies have confirmed this general scheme, demonstrating that administration of soy phytoestrogens to ovariectomised monkeys protects against estrogen deprivation effects, without stimulation to both uterus and breast[128], consistently with a selective stimulation of ERβ.

Plant phytoestrogens can accumulate beneficial antioxidant activity with possible *in vivo* estrogenic (or antiestrogenic) effects, depending on the individual hormonal state. In other terms, if they are administered to a woman in the fertile

state they are likely estrogen antagonists (binding to ERs, without being able to fully transactivate them and antagonising the action of endogenous hormones), but can act as estrogens when administered to postmenopausal women, replacing the actions of the declining endogenous hormones[129]. Additionally, it is likely that phytoestrogens can modulate interconversions among the several forms of circulating estrogens (even in the postmenopause), modulating the activity of endogenous interconverting hormones.

Tikkanen et al[130], who proved that supplementation of normal diet with soy food bars containing about 20 mg of isoflavones for periods as short as 2 weeks, could significantly delay oxidation of LDL after *in vitro* challenge with copper salts in human volunteers. In a similar way, 4 months supplementation of diet of premenopausal women with 90 mg of isoflavones significantly delayed oxidability of circulating LDL during *ex vivo* copper treatment[131].

The effects of moderate physical activity were studied in 19 postmenopausal and 25 premenopausal women on the enzymatic (superoxide dismutase and catalase) and non-enzymatic (vitamins A and E) antioxidant systems and on the processes of lipid peroxidation (malondialdehyde), and to see whether there is any variation in oxidative stress indicators between the postmenopausal and non-menopausal women. The subjects were also studied anthropometrically. At the end of a training period, there was a decrease in body fat percentage and in various skin folds. Neither group presented a rise in lipid peroxidation levels, although there was a significant ($p < 0.05$) rise in superoxide dismutase activity in the postmenopausal women[132].

Leal et al[133] wanted to assess the association of hot flushes during postmenopause with oxidative stress and to determine whether HRT affects the plasma redox status of postmenopausal women. These researchers conducted a prospective clinical study of 49 postmenopausal women who have (n = 29) or do not have (n = 20) hot flushes. Twelve of the postmenopausal women with hot flushes and six without were treated with HRT (estradiol patches and medroxyprogesterone acetate) for 4 months. Plasma level of estradiol, total antioxidant status, reduced sulfhydryl groups, lipoperoxides, total cholesterol, and triglycerides were measured at 4-month intervals in both groups, before and after treatment. Postmenopausal women who have hot flushes, had lower total basal antioxidant status in plasma (.9 ± .01 compared with 1.14 ± .01 mmol/L), lower concentration of reduced sulfhydryl groups (145 ± 4 compared with 200 ± 3 micromol/L), and higher concentration of lipoperoxides (2.88 ± .04 compared with 2.61 ± .04 micromol/L) than women without hot flushes. After HRT, total antioxidant status and reduced sulfhydryl groups increased while lipoperoxides decreased similarly in both groups. Hormone replacement therapy decreased the frequency of hot flushes per day from 11.2 ± 0.8 to 1.4 ± 0.3. Hot flushes in postmenopausal women were associated with the oxidative process. Hormone replacement therapy decreases oxidative stress and the number of episodes of hot flushes. Because oxidative stress is associated with a high risk for cardiovascular diseases, HRT might protect women with hot flushes. Vitamin E traditionally has been used to treat hot flushes[134,135] but Barton et al[136] found that, in breast cancer survivors who cannot receive HRT, the clinical magnitude of vitamin E for decreasing hot flushes was marginal, suggesting that the oxidative process is not the cause of hot flushes. However, because vitamin E has a marked antioxidant effect[137] it might not be very effective in decreasing hot flushes episodes, but might counteract the deleterious effects of oxidative stress associated with hot flushes.

These findings are in agreement with the theory that estrogens can act as antioxidants. Particularly, oestradiol upregulates the expression of genes encoding for mitochondrial antioxidant enzymes such as Mn-SOD and GSHPx[138].

Kumaraguruparan et al[139] wanted to evaluate the extent of lipid peroxidation and the antioxidant levels in breast cancer patients in relation to different clinical stages and menopausal status. The risk factors associated with breast cancer may exert their effects via generation of ROSs, which are recognized to induce oxidative DNA damage and neoplastic transformation. Of late, ROS are being increasingly implicated in breast cancer development[140-142]. In previous study, these researchers demonstrated the involvement of ROS in breast cancer patients[143,144]. Fifty newly diagnosed women with adenocarcinoma of the breast were divided into different groups based on clinical staging and menopausal status. The extent of lipid peroxidation as reflected by the formation of TBARS, LOOH, and conjugated dienes (CD) as well as the status of the antioxidants SOD, CAT, GSH and GPx in tumor tissues and adjacent normal tissues were estimated in these patients. Enhanced lipid peroxidation ac-

companied by significant elevation in enzymatic and nonenzymatic antioxidants was observed in breast tumor tissues compared to the corresponding uninvolved adjacent tissues irrespective of clinical stage and menopausal status of the patients. The magnitude of change in tissue oxidant-antioxidant status was, however, more pronounced in stage III and in premenopausal patients compared to stage I and II and postmenopausal patients, respectively. A correlation between tissue redox status and tumor progression suggests that upregulation of antioxidants enables tumor cells to counter oxidative stress, thereby conferring a selective advantage for growth compared to corresponding normal cells.

ROS are a double-edged sword – they serve as key signal molecules in physiological processes but also have a role in pathological processes involving the female reproductive tract. ROS affect multiple physiological processes from oocyte maturation to fertilization, embryo development and pregnancy. It has been suggested that OS modulates the age-related decline in fertility. It plays a role during pregnancy and normal parturition and in initiation of preterm labor. Most ovarian cancers appear in the surface epithelium, and repetitive ovulation has been thought to be a causative factor. Ovulation-induced oxidative base damage and damage to DNA of the ovarian epithelium can be prevented by antioxidants. There is growing literature on the effects of OS in female reproduction with involvement in the pathophysiology of preeclampsia, hydatidiform mole, free radical-induced birth defects and other situations such as abortions. Numerous studies have shown that OS plays a role in the pathophysiology of infertility and assisted fertility. There is some evidence of its role in endometriosis, tubal and peritoneal factor infertility and unexplained infertility. Trials investigating combination intervention strategy of vitamin E and vitamin C supplementation in preventing preeclampsia are highlighted. Antioxidants are powerful and there are few trials investigating antioxidant supplementation in female reproduction. Pregnant women with HIV infection, selenium deficiency or micronutrient deficiencies like vitamin C and A, were found to have side clinical outcomes in large prospective studies[145,146]. There is increasing argument for increasing the selenium intake in these patients. There is emerging enthusiasm in the use of antioxidants, natural or synthetic. Small molecules that mimic antioxidant enzymes are the new tools being developed in the antioxidant armamentarium[147]. These are cell membrane permeable unlike the natural superoxide dismutase. Anti-oxidants targeting cellular organelles like mitochondria are also being investigated. Gene polymorphisms of the glutathione S-transferase family and myeloperoxides and their association with endometriosis, is an area of recent interest, which is promising[148]. However, before clinicians recommend antioxidants, randomized controlled trials with sufficient power are necessary to prove the efficacy of antioxidant supplementation in disorders of female reproduction. Serial measurement of oxidative stress biomarkers in longitudinal studies may help delineate the etiology of some of the disorders in female reproduction such as preeclampsia[149].

Miquel et al[150] supports the hypothesis that oxygen stress contributes to menopause and that some of its physiopathological effects may be prevented and/or treated improving the antioxidant defense of menopausic and postmenopausic women. Accordingly, a selection of micronutrients may be useful as a dietary supplement for protection against the decline of physiological functions caused by age-related oxygen stress. Nutritional supplements should also include the key antioxidant vitamins C and E, as well as beta-carotene and the mineral micronutrients found in the oxygen radical-detoxifying enzymes glutathione peroxidase and superoxide dismutase. The integration of the diet with vitamin C and E would be in agreement with the declaration of Upston et al[151] that the balance between vitamin E and the other anti-oxidants may be essential for antioxidant protection *in vivo*. Thus, the joint administration of vitamin C and E must increase the protective action against ROS both in the aqueous phase of the organism and in the lipid phase of the mitochondrial membranes, who are enriched in polyunsaturated fatty acids quite susceptible to oxidation[152-154]. According to Vatassery et al[155], these two antioxidant micronutrients (that also interact with the other key physiological antioxidant glutathione) may be very efficient mitochondrial protectors when there is an excessive formation of superoxide and nitric oxide in normal or pathological conditions. De la Fuente et al[156] showed that the immune function of elderly women is improved by ingestion of a dietary supplement of vitamin C and E.

The inclusion of vitamin B_6 in micronutrient supplements is due to the relevant contribution in

the maintenance of an adequate GSH/GSSG ratio. This is very important for health preservation in women of menopausic or postmenopausic age, since according to the results of Vina et al[157], Miquel and Weber[158], Harding et al[159] and Chen et al[160], normal aging and even more aging related to health loss are linked to a progressive oxidation of glutathione and other thiolic molecules.

Moreover, other antioxidants such as lipoic acid and the precursors of glutathione thioproline (TP) and l-2-oxothiazolidine-4-carboxylic acid (OTC), as well as the soy isoflavones and the "coantioxidants" of an hydroalcoholic extract of Curcuma longa may help to prevent antioxidant deficiency with resulting protection of mitochondria against premature oxidative damage with loss of ATP synthesis and especialized cellular functions. α-lipoic acid (ALA) is a very hydrophobic normal component of mitochondria with a heterocyclic ring containing an –S-S- group. A lot of studies in several model systems have shown that ALA is a powerful neutralizer of ROS such as the $OH^{\cdot}$ and singlet oxygen[161]. From a practical viewpoint of health preservation in menopausic and postmenopausic women it is worth-mentioning that ALA has a preventive action against hypertension and insuline resistance[162].

TP is a cyclic thiolic molecule that acts as an antioxidant and free radical scavenger[163]. As regards the probable uselfuness of TP administration in menopause, it should be realized that the immune system (essential for health in menopause) is very susceptible to both the age-related injurious effects of oxygen stress[164] and the redox preserving and anti-aging effects of thiol supplementation[165]. Interestingly, in a model of premature mouse aging linked to high anxiety levels (that interfere with performance in a behavioral test), the favorable immuno-modulating action of diet supplementation with TP is more evident in the prematurely aged mice than in those showing normal aging[166,167].

Therefore, the administration under medical advice of synergistic combinations of some of the above mentioned antioxidants in the diet as well as topically (for skin protection) may have favorable effects on the health and quality of life of women, especially of those who cannot be treated with HR, suffer high levels of oxygen stress and do not consume a healthy diet that includes five daily rations of fresh fruit and vegetables.

Male Infertility

The correlation between varicocele and male infertility is still obscure. Several factors seems to be involved in sperm modifications: backflow of noxious substances from the kidney or adrenal glands, testicular temperature increased, tissue hypoxia induced by venous stasis, hypothalamic or pituitary dysfunctions[168]. Oxidative damage because of the testicular venous backflow may represent one of the causes of gonad injury and seems to precede the histological alteration[169]. Therefore, determining the values of spermatic or intratesticular nitric oxide could be useful in evaluating this oxidative stress. The NO act also on the peritubular lamina propria regulating its permeability and NO controls the activity of muscle cells and pericytes of testicular vessels[170]. Moreover, NO has been reported to be relevant in the control of male reproductive function and fertility, Leydig cell function, myofibroblast contraction and therefore tubular peristalsis[171,172]. Experimental studies have shown that NO at a high concentrations can be harmful for both testicular and sperm function[173,174]. Some authors have considered the correlation between varicocele and semen NO levels, stating that increased NO generation may interfere sperm production, motility and morphology in patients with varicocele[175-178]. Köksal et al[179] suggested that oxidative status, which reflects a relative balance between ROS produced and ROS scavenged, may not be responsible for the testicular dysfunction correlated with experimentally induced varicocele during adolescence in rats. De Stefani et al[180] study the role of testicular NO in early detection of the damages induced by an experimental varicocele in the Wistar rat. A left varicocele was induced in 10 animals (group A). A control group of 10 rats was performed (group B). Animals were sacrificed 3 months after the operation. Both testicles were harvested, weighed and sectioned in two equal parts: one for the evaluation of the NO level and the other one for histological examination. All the rats in group A showed a conspicuous dilatation of the left spermatic vein. The histopathological analysis was normal in both the groups. Biochemistry showed a relevant statistical difference ($p < 0.001$) in the concentrations of NO among the specimens of the left and right gonads in group A but no difference was found in group B. The increase in NO values and the presence of other oxidant agents represent the first sign of testicular distress and it seems to anticipate

histopathological changes. Based on a great difference exist between human and animal sperm, NO could therefore in the future be taken into consideration together with others parameters for the evaluation of patient who is affected by varicocele. Interestingly should be the possibility of preventing or delaying oxidative stress with the aid of antioxidant molecules. Semercioz et al[169] reported their experience with melatonin, a potent free radical scavenger; Koltuksuz et al[181] described the advantages of the administration of caffeic acid phenetyl ester, an active component of propolis, that exhibits antioxidant capacity.

Renal Diseases

There is a relevant evidence suggesting that ROS are implicated in the pathogenesis of ischemic, toxic and immunologically-mediated renal injury[182-185]. In experimental renal ischemia, ROS sources include the electron transport chain, oxidant enzymes (xanthine oxidase), phagocytes and auto-oxidation of epinephrine. ROS cause lipid peroxidation of cell and organelle membranes and, hence, disruption of the structural integrity and capacity for cell transport and energy production, especially in the proximal tubule segment. In experimental immune glomerulonephritis, ROS are generated by both infiltrating blood-borne cells (polymorphonuclear leukocytes and monocytes) and resident glomerular cells, mainly mesangial cells. Their formation results in morphologic lesions and in modifications of glomerular permeability to proteins through activation of proteases and reduction of proteoglycan synthesis. Additionally, they promote a reduction in glomerular blood flow and glomerular filtration rate through liberation of vasoconstrictory bioactive lipids (prostaglandins, thromboxane and platelet activating factor) and, possibly, inactivation of relaxing nitric oxide[186].

Ischemia-Reperfusion-Induced Renal Injury

Ischemic acute renal failure is a complex syndrome involving renal vasocostriction, sudden decrease in glomerular filtration rate, tubule obstruction and tubule backleak of glomerular filtrate. ROS have been demonstrated to participate in these lesions in a number of experimental models including whole animal studies employing renal artery occlusion or intrarenal norepinephrine infusion, and in vitro studies with isolated proximal tubule segments or proximal tubule epithelial cells in primary culture subjected to hypoxia and reoxygeneration. In numerous studies, their production has been assessed indirectly by measuring ROS-mediated lipid peroxidation. For example, Greene et al using a model of 60 min renal artery occlusion in rats, have observed that during the period of reperfusion, malondialdehyde accumulates within the kidney and ethane is formed locally before being transported by the blood and excreted in expired gas. Similarly, they have demonstrated that, in vitro, proximal tubule epithelial cells produce superoxide radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) under normoxic conditions[187]. During hypoxia and reoxygeneration, this production is increased and associated with an increase in lipid peroxidation. Evidence supporting a role for ROS in postischemic renal injury has been derived predominantly from studies using a variety of antioxidants. For example, infusion of superoxide dismutase before ischemia and again at the time of reperfusion provides protection against functional (reduction in glomerular filtration rate) and histological (cellular necrosis and tubule obstruction) injury. Such results suggest a role for $O_2^{-\bullet}$.

Numerous reports have also demonstrated the protective effects of catalase and glutathione peroxidase, two enzymes playing a role in the detoxification of H_2O_2. For example, a diet deficient in selenium promotes a reduction of glutathione peroxidase expression and exacerbates structural and functional renal impairment following ischemia[188]. Such results suggest a role for H_2O_2. Thus oxidant injury could be induced by the generation of H_2O_2 and $O_2^{-\bullet}$ and their conversion to $OH^{\cdot}$ through the Haber-Weiss reaction. In accordance with this theory, $OH^{\cdot}$ scavengers (dimethylsulfoxide, dimethylthiourea, reduced glutathione), and iron chelators preventing the production of $OH^{\cdot}$ (desferoxamine), have been shown to protect against renal ischemic injury.

Immune-Mediated Glomerual Injury

In animal models of glomerulonephritis, immune complex formation and component activation within glomerular capillaries are followed by the infiltration of bone marrow-derived inflammatory cells including polymorphonuclear leukocytes and monocytes/macrophages. These cells contribute to induce glomerular injury, i.e. alteration of glomerular haemodynamics and de-

velopment of proteinuria. This could be due, at least in part, to the release of ROS. This evidence has been obtained first from demonstration of ROS formation at the site of injury. Evidence for a role of ROS also derives from studies showing that the infusion of a chemical or cellular source of ROS into the renal artery alone or following myeloperoxidase infusion induces mesangiolysis, endothelial detachment and reversible proteinuria. This is due to a molecular size-selectivity defect of the glomerular capillary wall without apparent ultrastructural anomalies[189]. Finally, studies analyzing the role of H_2O_2 either produced by polymorphonuclear leukocytes upon phorbol myristate acetate challenge, or directly infused into the renal artery have indicated that ROS provoke a fall in glomerular filtration rate and ultrafiltration coefficient and an increase in renal arterial resistance.

Additional evidence for a role of ROS in glomerular pathophysiology has been obtained by demonstrating the protective role of agents removing ROS. With few exceptions, SOD does not provide protection against glomerular functional impairment after local deposition of immune complexes. By contrast, SOD administration reduces glomerular morphological changes and proteinuria in a model of minimal-change disease obtained by a single injection of the aminonucleoside of puromycin.

Several studies have established that polymorphonuclear leukocytes and monocytes/macrophages are possible source of ROS within the glomerulus. Rehan et al[190] have reported that the ROS-dependent proteinuria in the heterologous phase of anti-glomerular basement membrane-induced glomerulonephritis is greatly diminished when animals are neutrophil-depleted. The same authors have shown that, instead, the intravascular activation of neutrophils by the infusion of phorbol miristate acetate or cobra venom factor into the renal artery causes ROS-dependent glomerular injury and proteinuria. However, because blood-borne cells do not contribute to the pathogenesis of proteinuria in the models of membranous nephropathy, glomerular cells themselves represent a second potential source of ROS within the glomerulus. In support of this theory, various studies with glomerular cells in culture have indicated that at least mesangial cells from murine or human origin have the potential to produce ROS. The magnitude of this production is greater than that exhibited by epithelial cells from the proximal tubule, the cortical collecting duct and the papillary collecting duct. Both $O_2^{-\bullet}$ and H_2O_2 are relesased upon interaction of mesangial cells with immune reactants (opsonized particles, immune complexes), complement membrane attack complex and inflammatory mediators (platelet activating factors, interleukin-1, tumor necrosis factor)[191,192]. This generation is limited by cell exposure to dexamethasone and other glucocorticoids with therapeutic effects in experimental glomerulonephritis.

Conclusions

In this short review, we gave un up-to date of the most relevant studies on the role of oxidative stress in clinical pathology and the possible counteracting effects of antioxidant administration. There is now reasonable evidence that ROS play along the human life, a co-factor role in several allegedly diseases, and in the ageing process itself with specific morphopathological traits, through cytokyine-mediated inflammation to necrosis and final fibrosis.

Biochemical investigations to promptly detect the danger of an oxidative imbalance and to restore antioxidant reservoir before triggering the irreversible damage cascade are thus mandatory, and should be performed in the future as routine exams, during standard blood evaluation, and repeated along the treatment plan, to monitor the efficacy of antioxidants compounds administered as primary unique therapy or complementary to some disease – specific drug (like antibiotics is severe infections and sepsis).

The improvement of symptoms as well as prevention of inflammatory damage, degenerative diseases or cancer should be the goals of antioxidant therapy with confirmation, step by step of the serum red-ox balance restoration and inflammation/degeneration markers disappearance.

We cannot exclude, in the future, that an oxidative-status check-up followed by immediate targeted treatment will achieve better social health perspectives, especially in the old age, preventing major illnesses and heavier social costs burden.

The next following paper will be dedicated to understand and properly use each of the many oxidative stress tests, with special emphasis to the simplest and cheapest ones, in order to have a standardized evaluation background, and, finally, a common feed-back based effective protocol of intervention.

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