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Review Article

Oxidative Stress in Liver Diseases

Nagy S. El-Rigal, *Manal A. Hamed, Maha Z. Rizk

Therapeutic Chemistry Department, National Research Centre, Dokki, Cairo, Egypt.

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ABSTRACT

Oxidative stress corresponds to an imbalance between the rate of oxidant production and that of their degradation. The main biological targets of free radicals are proteins, lipids and DNA. Protein oxidation leads to loss of function or premature degradation in proteasomes. Lipid peroxidation, occurring mostly in the plasma membrane or the stratum corneum- the most superficial layer of the epidermis- alters the physical properties of cell membranes or the epidermis, and thereby their biological function. Oxidation of DNA can lead to gene mutation, and thus abnormal protein synthesis, alteration in gene expression, apoptosis and cell death. An oxidative stress is often associated to all kinds of diseases, although it is not always easy to determine whether it is a cause or a consequence of the observed condition. Liver fibrosis, hepatocellular carcinoma, alcoholism and non alcoholic fatty liver were the main diseases that will be discussed in this work.

Keywords: Oxidative stress, liver, hepatocellular carcinoma, alcoholism

INTRODUCTION

Except for anaerobic microorganisms, all living organisms require molecular oxygen as an electron acceptor for efficient production of energy. However, oxygen is a strong oxidant, and it is impossible to avoid secondary oxidations not involved in physiological metabolism. These random oxidations would have deleterious consequences if they were not neutralized by an efficient antioxidant system[1].

From this theoretical evidence, biophysicists introduced the concept of 'oxidative stress' to describe a situation occurring in aerobes when the undesired secondary oxidations induced by oxygen and its derivatives are not efficiently neutralized, and lead to abnormal metabolism, loss of physiological function, disease, and potentially death[2].

For many years, biophysicists and theoretical chemists have communicated about oxidative stress and its biological consequences using an abstruse language that did not really appeal to most biologists and physicians. For this reason, the latter did not pay much attention to the growing number of publications focused on this subject, and eventually imagined oxidative stress more as a theoretical model unusable for applied biology and medicine than a biological reality that could have interesting applications and help finding the search for new treatments for human diseases[3].

Oxidative stress definition: Oxidative stress corresponds to an imbalance between the rate of oxidant production and that of their degradation[2]. Aerobic organisms such as vertebrates and man in particular produce their energy from the oxidation of organic substrates by molecular oxygen. The complete four-electron reduction of molecular oxygen occurs within mitochondria and produces water, at the end of the respiratory chain. Sometimes molecular oxygen is partly reduced instead of the proteins of the respiratory chain, and superoxide and various reactive oxidant intermediates are produced, leading to secondary oxidations[4]. Besides these physiological oxidations and their unavoidable secondary reactions, many substances contained in food are either oxidants by themselves or oxidant precursors[5,6].

Other oxidants come from the environment: (*i*) air pollutants and oxidant contained in tobacco and fire smoke react with molecules of the skin surface, and some of them penetrate into the skin and reach the circulation[7,8]; (*ii*) UV radiation from the sun are absorbed by cutaneous chromophores and induce the formation of a variety of oxidants[9-10].

Finally, although the organism adapts to any unstable situation by preventing undesirable reactions and repairing damaged molecules and tissues, the very few undesirable reactions that escape the prevention and repair systems accumulate little by little, and will invariably be deleterious after a long period of time[12,13], thus confirming the free radical theory of ageing developed by Denham Harman[14]. All these conditions lead to the production of various oxidants, and if the organism fails to neutralise them, these oxidants accumulate and react with a variety of biomolecules, creating an undesirable situation known as oxidative stress[2] (Fig.1).

Molecules mediate oxidative stress: Two classes of molecules mediate most of the reactions leading to oxidative stress: free radicals and reactive oxygen species (ROS) (Fig. 2). Free radicals are molecules possessing at

*Author for correspondence: E- mail: manal_hamed@yahoo.com

least one unpaired electron; for this reason, they are unstable and promote electron transfers, i.e., oxidations and reductions. Lipid radicals are formed by hydrogen abstraction, a process elicited by UV irradiation or the hydroxyl radical (•OH)[15,16]. The latter, as well as other reactive radicals, belong to the class of ROS. These unstable intermediates derive from molecular oxygen (•O2• or ^{3}O2), a biradical. In the case of molecular oxygen, the triplet (biradical) state is more stable than the singlet one (non radical, with a double bond); thus singlet oxygen (^{1}O2) is a ROS. It can be produced by several biochemical oxidations involving peroxidases and lipoxygenases, by reactions between various ROS or in the presence of light, oxygen and a photosensitizer such as porphyrins, as it is the case in congenital erythropoietic porphyria[1,17,18].

Other ROS derive from the secondary reduction of ^{3}O2 in the inner membrane of mitochondria (mainly from ubiquinol oxidation), where oxygen receives one electron in the place of a metalloprotein of the respiratory chain[4,19]. The superoxide radical anion thus formed (•O2−) can then dismutate into oxygen (^{3}O2) and hydrogen peroxide (H2O2), either spontaneously at acidic pH or in a reaction catalysed by superoxide dismutase (SOD)[1].

Superoxide and hydrogen peroxide can react with transition metals such as iron or copper to form the strong oxidant hydroxyl radical (•OH)[1,20,21].

Superoxide and hydrogen peroxide are also enzymatically formed by NADPH oxidase in activated neutrophils or macrophages during inflammatory processes [21,22].

Generally speaking, all endogenous oxidases-enzymes that transfer two electrons from a substrate (AH2) to molecular oxygen- produce superoxide and hydrogen peroxide[1].

Myeloperoxidase, an enzyme present in neutrophils, but not in macrophages, can produce hypochlorous acid (HOCl) by oxidizing chloride ions with hydrogen peroxide[1,24].

Hypochlorous acid (or its conjugated base hypochlorite, depending of the pH), is a powerful oxidant towards various biomolecules, especially amino groups; furthermore, in acidic pH it readily decomposes to liberate the highly toxic chlorine gas Cl_2[1,25].

As mentioned above, hydrogen peroxide can react with hypochlorite to form singlet oxygen. Under physiological conditions, 1–2% of the consumed oxygen is converted to ROS. Nitric oxide (•NO), an intercellular messenger, is produced from oxygen by various nitric oxide synthases[1,26]. This free radical, by reacting with superoxide- another radical- produces the strong oxidant peroxynitrite (ONOO−)[1,27,28].

The main biological targets of free radicals and ROS are proteins, lipids and DNA. Protein oxidation leads to loss of function or premature degradation in proteasomes. Lipid peroxidation, occurring mostly in the plasma membrane or the stratum corneum- the most superficial layer of the epidermis- alters the physical properties of cell membranes or the epidermis, and thereby their biological function. Oxidation of DNA can lead to gene mutation, and thus abnormal protein synthesis, alteration in gene expression, apoptosis and cell death[29,31].

Endogenous defenses against oxidative stress: Strict aerobic organisms cannot live in the presence of oxygen, because they have insufficient defenses against the multiple secondary reactions induced by oxygen[3]. All other organisms which can or must live in the presence of oxygen possess an efficient battery of antioxidant defenses able to trap reactive intermediates before they have time to oxidize biomolecules or reduce those which have been oxidized (Fig. 1)[32-34].

Due to the great variety of reactive intermediates that must be neutralized, as well as the variety of oxidized biomolecules which must be reduced, there are a lot of different antioxidants; moreover, there is a need for antioxidants for both hydrophilic (cytosol, extracellular fluids) and lipophilic (membranes, lipids) phases[1,32,37].

The most efficient antioxidants are enzymes that catalyze the reduction of ROS: superoxide dismutase (SOD) catalyzes the dismutation of superoxide (•O2−) into hydrogen peroxide (H2O2) and oxygen (3O2), catalase catalyses hydrogen peroxide (H2O2) dismutation into water (H2O) and oxygen (^{3}O2), glutathione peroxidases (GPX) reduce both hydrogen (H2O2) and organic (R-OOH) hydroperoxides. The oxidized glutathione cofactor (GS–SG) is then reduced (2 GSH) by glutathione reductase (GSR). Various GPX isoforms exist, which are specific for hydrophilic or lipophilic phases[3].

Metallothioneins are small proteins with several cysteine residues which bind transition metal ions: this can both detoxify metals and avoid them catalzsing the Haber–Wei ß and Fenton reactions that lead to the production of the hydroxyl radical (•OH). The tripeptide glutathione and the selenoprotein thioredoxin are efficient in reducing disulphur bridges (R–S–S–R`) into thiols (R–SH + R`-SH), as well as reducing thiyl radicals (R–S•); the whole system involving thioredoxin, thioredoxin peroxidase and thioredoxin reductase is known as peroxiredoxin. Besides being an antioxidant by itself, glutathione is also the cofactor for GPX and GSR[3].

Ubiquinol (coenzyme Q, QH2), plays an essential role in the mitochondrial electron-transport chain in creating a proton gradient between both sides of inner mitochondrial membrane. Due to its two-step oxidation via a radical intermediate. Ubiquinol is both a promoter of superoxide formation and an efficient free radical scavenger, especially in lipid phase[3].

Lipoic acid, a component of pyruvate dehydrogenase complex, and its reduced form dihydrolipoic acid, are both metal chelators and ROS scavengers; moreover, dihydrolipoic acid recycles vitamins C and E[38]. Thus, besides their central role in energy metabolism, lipoic and dihydrolipoic acids prevent oxidative stress at various stages[39,40].

L-Ascorbic acid (vitamin C) is a water-soluble low molecular weight antioxidant required for collagen synthesis, iron absorption and maintenance of the redox status of cells[41]. It recycles vitamin E, the predominant membrane antioxidant, as well as many other oxidized biomolecules, and scavenges free radicals[42].

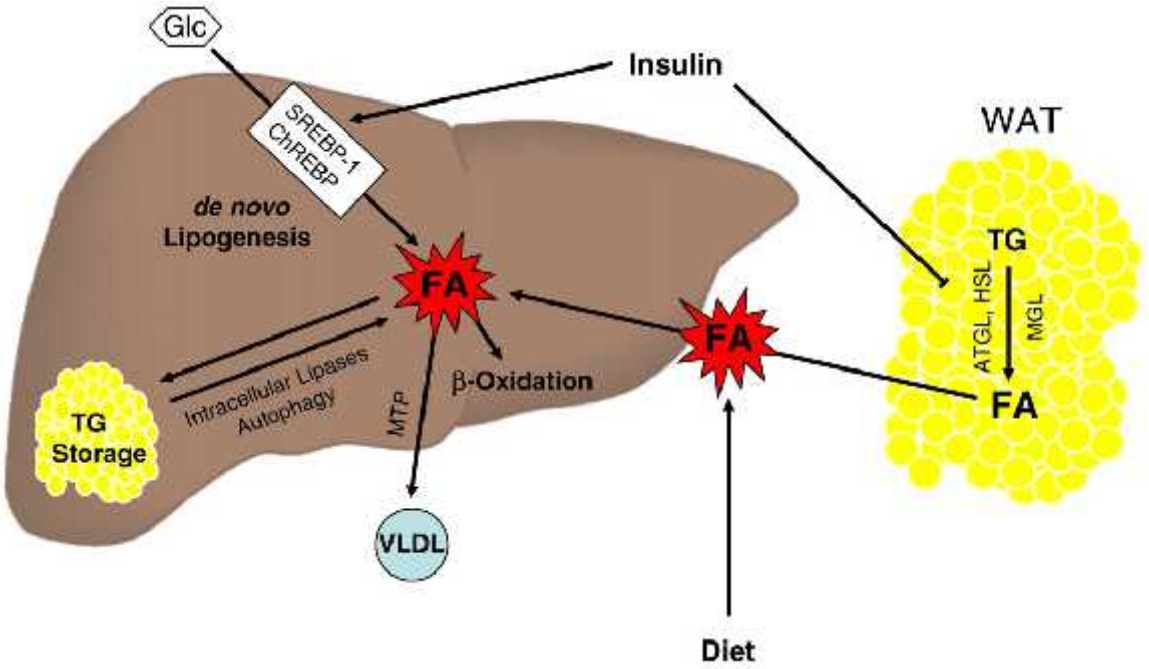

Fig. 5. Pathogenesis of NAFLD. Three major sources for hepatic fatty acids (FA) include white adipose tissue (WAT), hepatic de novo lipogenesis and diet. In WAT insulin blocks lipolysis via inhibition of hormone-sensitive lipase (HSL). In insulin-resistant states, esterification of FA is inhibited and lipolytic activity is increased, which results in enhanced FA flux to the liver. The second important source of FA comes from hepatic de novo lipogenesis. Hyperinsulinemia induces SREBP-1c expression, leading to transcriptional activation of lipogenic genes. Simultaneously, hyperglycemia (Glc) activates ChREBP, which additionally transcriptionally activates lipogenic genes. The coordinated actions of SREBP-1c and ChREBP activate the enzymatic machinery necessary for the conversion of excess glucose to FA, which are preferentially esterified to triglycerides (TG). FA can be released from TGs storages by intracellular lipases or via autophagy. Beside esterification and storage as TG, hepatic FA can either be oxidized in mitochondria to form ATP or incorporated into VLDL particles via microsomal triglyceride transfer protein (MTP) for export. ATGL, adipose triglyceride lipase; MGL monoglyceride lipase.

Carotenoids (terpenoid molecules synthesized by plants, among which vitamin A precursors such as β-carotene) are free radical scavengers, and most importantly, singlet oxygen quenchers. This is particularly important for the retina, where singlet oxygen is produced following interactions between visible light, oxygen (triplet) and various photosensitizers[43-46].

Tocopherols (vitamin E) are the main antioxidants in the lipophilic phase; once oxidized, they become radicals, then they are converted to their functional reduced state by ascorbic acid[32-34,42,47].

Selenium is a trace element present in the food as selenites, selenates or selenomethionine, which are precursors for selenocysteine (Fig. 1). The latter is directly incorporated into glutathione peroxidases and thioredoxins from the UGA codon.

Oxidative stress involved in many pathogens: An oxidative stress is often associated to all kinds of diseases, although it is not always easy to determine whether it is a cause or a consequence of the observed condition[48].

Carcinogenicity: The increased incidence of cancer over the last 50–60 years may be largely attributed to two factors: the ageing of the population and the diffusion of carcinogenic agents, present not only in the occupational, but also in the general environment[48]. There are studies supporting evidence that lifespan exposure to carcinogenic agents, beginning during developmental life, produces an overall increase in carcinogenic processes[48].

Metals carcinogenicity: It has long been known that some metals are toxic and carcinogenic. Epidemiological studies have indicated that people exposed to high levels of arsenic (As) are prone to develop skin, bladder, liver, and lung cancers[49]. Chromium (Cr), widely used in industry and an environmental pollutant, is another metal that has been shown to be a human carcinogen, and can cause asthma attacks in people who are allergic to chromium. Other metal ions that are known to be human carcinogens include nickel (Ni), beryllium (Be), and cadmium (Cd). From ongoing investigations on the underlying mechanisms of metal-induced carcinogenesis, accumulated evidence suggests that oxidative stress, resulting from the imbalance of cellular free radical generation and antioxidative defense, plays an important role in metal induced cellular response and carcinogenesis[50]. Reactive oxygen and nitrogen species such as superoxide anion ($O2^{-}$), hydrogen peroxide (H2O2), hydroxyl radical (SOH), and nitric oxide (NO) are known to be important in mutagenesis and carcinogenesis. Studies have shown that free radicals are generated in cells exposed to many metals such as arsenic and chromium. Reactive oxygen species (ROS) and

reactive nitrogen species (RNS) can be directly involved in oxidative damage to lipids, proteins, and DNA. Additionally, metals can alter cellular signaling pathways through generation of reactive and transient intermediate metal compounds. Various studies have proven that ROS are involved in the activation of redox-sensitive transcriptionfactors. ROS may act as ‘‘fast-acting third messenger molecules’’ in inducing the activation of redox-sensitive transcription factors such as NF-nB and AP-1[51].

Metals induced apoptosis: As mentioned above, toxic metals are capable of disturbing the natural oxidation/reduction balance in cells through various mechanisms stemming from their own complex redox reactions with endogenous oxidants and effects on cellular antioxidant systems. The resulting oxidative stress causes lipid peroxidation, protein damage, and DNA damage, which may contribute to metals' toxicity and carcinogenesis. The resulting oxidative stress may also affect the levels and functions of redox-sensitive signaling molecules, such as AP-1, NF-ќB, and p53, derange the cell signaling and gene expression systems, and/or induce apoptosis. This, in turn, may produce a variety of toxic effects, including carcinogenesis[52].

Both AP-1 and NF-ќB are considered stress response transcription factors that govern the expression of a variety of pro-inflammatory and cytotoxic genes[53]. p53 gene is an important tumor-suppressor gene whose protein product plays an important role in cell cycle control, apoptosis, and control of DNA repair. NF-nB is involved in a wide variety of biological responses (Fig. 3). In particular, it is implicated in inflammatory reactions, growth control, apoptosis, and initiation or acceleration of carcinogenesis, and is the first eukaryotic transcription factor shown to respond directly to oxidative stress in certain types of cells. NF- ќB also mediates cell transformation in an epidermal model[54], suggesting that ROS activation of NF- ќB is a factor in metal-induced carcinogenesis.

Hepatocellular carcinoma: Hepatocellular carcinoma (HCC) is one of the most frequent tumor types worldwide. It is the fifth most common cancer and the third leading cause of cancer death[55]. There are multiple etiological agents that are associated with the development of HCC, the most frequent being chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, and long-term exposure to the mycotoxin, aflatoxin B1[56]. HCC is also a common complication of alcoholic cirrhosis, although ethanol appears to not be directly carcinogenic[57].

ROS are potential carcinogens because of their roles in mutagenesis, tumor promotion, and progression[58]. If not regulated properly, the excess ROS can damage lipids, protein or DNA, inhibiting normal function[59]. ROS alterations in different signaling pathways may modulate gene expression, cell adhesion, cell metabolism, cell cycle and cell death. These events may induce oxidative DNA damage, which in turn increases chromosomal aberrations associated with cell transformation[60]. ROS may also activate cellular signal pathways, such as those mediated by mitogen-activated protein kinase (MAPK), nuclear factor-κB (NF-κB), phosphatidylinositol 3-kinase (PI3K), p53, β-catenin/Wnt and associated with angiogenesis[61-63]. Importantly, HBx stimulates the activities of MAPK, NF- κB, PI3K, and β-catenin (as well as other pathways) that are thought to contribute importantly to the development of HCC. Perhaps this is why carriers with chronic liver disease (CLD) develop a high incidence of HCC, while asymptomatic carriers do not.

Liver fibrosis and stellate cell activation: Several *in vitro* and *in vivo* observations suggest that oxidative stress and associated damage could represent a common link between different forms of chronic liver injury and hepatic fibrosis. For example, oxidative stress contributing to lipid peroxidation is one of the critical factors involved in the genesis and the progression of nonalcoholic steatohepatitis and liver cancer[64,65]. Viral infection or alcohol abuse greatly increased the highly variable miscoding etheno-modified DNA like epsilondA [1, N(6)-etheno-2'- deoxyadenosine] levels by triggering lipid peroxidation. Patients with chronic hepatitis, liver cirrhosis, and HCC due to HBV infection had more than 20 times higher urinary epsilondA levels[66] compared to uninfected individuals with no liver disease.

Among the mechanisms involved in mediating the process of liver fibrosis, an important role is played by ROS[67]. During the progression of liver injury, hepatic stellate cells (HSCs) become activated, which produce extracellular matrix such as collagen I[68]. Collagen I gene regulation has revealed a complex process involving ROS as a key mediator[69-71]. ROS-sensitive cytokines contribute to HSC activation during inflammation through paracrine signals released from immune cells[72]. The activated HSCs become responsive to platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β. PDGF facilitates the progression of hepatic fibrosis in human CLD. It increased the accumulation of hydrogen peroxide in HSCs. Specifically PDGF-induced increases in collagen deposition and liver fibrosis is markedly reduced by treatment with the anti-oxidant drug Mn-TBAP[73,74]. TGF-β increases ROS production and decreases the concentration of glutathione (GSH)[75].

In this context, it is important to note that HBx trans-activation activity is stimulated by ROS. Given that HBx is also associated with the development of HCC in both human carriers and in transgenic mice, and that HCC is associated with chronic inflammation[75].

Scar formation and accumulation: Hepatic ECM synthesis is typically associated with activated HSC. In addition, with the progression of the fibrogenic response, qualitative and quantitative changes in ECM may also occur, as HSC not only synthesize new ECM proteins but also produce metalloproteinases leading to the disruption of the normal physiological matrix[76-80]. As far as HSC-dependent ECM synthesis, detailed analysis of the different mediators and their sources has depicted a main role of mesenchymal cells in the expression of TGFβ1 and the other fibrogenic cytokines[81]. In the early steps of excessive fibrogenesis, mediators like PDGF and TGFβ1

are only produced by Kupffer cells, sinusoidal endothelial cells, and hepatocytes, so that a paracrine loop with HSC as a potential target is likely to occur[82]. If the fibrotic process is sustained, HSC may synthesize such mediators in rather large amounts maintaining and amplifying their own fibrogenic activity through an autocrine loop[83].

An increasing volume of literature suggests the involvement of H2O2 in the induction of promoter under TGFβ treatment[83,84]. Thus, the idea that both the expression and the activity of selected profibrogenic cytokines is significantly modulated by ROS-dependent reactions, is gaining relevance[83,84]. Lipid peroxidation-derived reactions are also involved in the profibrogenic response and acetaldehyde has also been demonstrated to have profibrogenic actions[85,86]. ROS (H2O2) and lipid peroxidation products [hydroxy- 2,3-nonenal (4-HNE), 4-hydroxyhexenal (4-HHE), malondialdehyde (MDA)] can easily diffuse through the plasma membrane[87,88]; therefore it is likely that dif- fusible ROS and LPO-derived products and not only cytokines and growth factors may also impact on the HSC *in vivo* to induce a fibrogenic response[89].

Moreover, supporting evidence is provided by work from Svegliati et al.[90] suggesting increased proliferation and elevated collagen type I synthesis in rat HSC cultured in conditioned medium derived from hepatocytes undergoing stimulated lipid peroxidation, and the work of Nieto et al.[89] showing that HepG2 cells overexpressing CYP2E1 release ROS-derived from CYP2E1 metabolism which impact on the HSC triggering HSC activation, proliferation, and a profibrogenic response as assessed by α-smooth muscle actin and collagen I expression as well as the rate of incorporation of *methyl*[3H]-thymidine into the DNA of HSC[91].

The signaling from CYP2E1-derived ROS triggering activation of HSC may play a role in alcohol-induced liver injury and fibrosis[69,70]. Similarly recent studies using primary Kupffer cells in co-culture with HSC have demonstrated a profibrogenic mechanism whereby Kupffer cell-derived H2O2 may up-regulate *COL1A1* promotor and *COL1A2* transactivation and simultaneously prevent collagen I protein degradation via al IL6-dependent mechanism[89].

Alcoholism: Alcohol-induced oxidative stress is linked to the metabolism of ethanol. Three metabolic pathways of ethanol have so far been described in the human body, which involve the following enzymes: alcohol dehydrogenase, microsomal ethanol oxidation system (MEOS) and catalase. Each of these pathways could produce free radicals that affect the antioxidant system (Fig.4).

The classical pathway of ethanol metabolism, which is catalyzed by alcohol dehydrogenase to form acetaldehyde results in the formation of free radicals. Concomitant changes are seen in the NADH levels and NADH/NAD+ redox ratios[92]. When consumed in moderate amounts, the major part of the ethanol is metabolized by alcohol dehydrogenaze in liver. In this reaction a hydride ion is transferred from ethanol to NAD^+ [93]. The microsomal electron transport system also participates in ethanol oxidation via catalysis by the cytochrome P450 isoenzymes[94]. The enzymes in this family include the 2E1, 1A2 and 3A4 isoforms[95], which vary in their capacity to oxidize ethanol. The cytochrome P450 2E1 isoform is induced by chronic ethanol consumption. Hence this mechanism becomes more important quantitatively in the alcohol abuser.

In addition to this reaction in the mitochondria, the 2E1 isoenzyme may also be a significant catalyst for formation of ROS in the alcohol consumer, as it has been demonstrated to generate higher amounts of H2O2[96]. Its elevation in the livers of ethanol consumers has also been linked to increased generation of hydroxyl radicals[97].

Peroxisomal activity also contributes to ethanol oxidation in the liver. This mechanism might be more prominent in heavy ethanol consumers where there is an accumulation of fatty acids in the liver[98], due to the increased peroxisomal oxidation of fatty acids. Ethanol oxidation gives rise to acetaldehyde, which is further oxidized by hepatic aldehyde dehydrogenases. The mitochondrial form of aldehyde dehydrogenase plays a prominent role in maintaining a low concentration of acetaldehyde. The acetate is then activated by acetyl CoA synthase to acetyl CoA[93].

As a result of the oxidation of ethanol by alcohol dehydrogenase and subsequent oxidation of acetaldehyde there is a significant increase in the hepatic NADH/ NAD+ ratio. This shift occurs both in the cytoplasm and in the mitochondria, as measured by the lactate/pyruvate and β- hydroxybutyrate/acetoacetate ratios, respectively. The mitochondrial, low Km aldehyde dehydrogenase generates much of the NADH within the mitochondria. The reducing equivalents of the cytoplasmic NADH are transported into the mitochondria, primarily via the malate–aspartate shuttle. This increased NADH is readily reoxidized by the mitochondrial electron transport system, if the tissue is aerobic. Thus, ethanol oxidation increases the availability of NADH in the mitochondria[93].

Nonalcoholic fatty liver disease: Nonalcoholic fatty liver disease (NAFLD) is a rapidly growing entity that is becoming a major cause of chronic liver disease, due to the increasing incidence of obesity and type 2 diabetes in the general population. NAFLD includes simple triacylglycerol (TAG) accumulation in hepatocytes (hepatic steatosis) or steatosis with inflammation, fibrosis, and cirrhosis (non-alcoholic steatohepatitis, NASH), with oxidative stress, insulin resistance, and nutritional factors playing major contributing roles[99] (Fig.5).

Under most circumstances, fatty acids (FA) are the major oxidative fuel in the liver. However, carbohydrate and lipid affluence induce significant changes in hepatic intermediary metabolism. In fact, high glucose and insulin levels stimulate FA synthesis from glucose and inhibit FA β-oxidation, re-directing FA towards the formation of TAG[100]. Considering that the amount of TAG exported as VLDL depends on synthesis of the protein components, FA in excess are likely to be converted to TAG and stored as lipid droplets within hepatocytes, upon consumption of calorie-enriched diets.

Since non-adipose tissues have limited capacity for TAG storage, the lipids in excess that accumulate under conditions of overnutrition determine high intracellular levels of saturated FA, which can
induce cell dysfunction and/or cell death, a phenomenon known as lipotoxicity[101]. Consequently, higher rates of FA oxidation and ROS generation are achieved, which might explain the increase in the oxidative stress-related parameters and antioxidant depletion found in the liver of obese patients with NAFLD[100].

Furthermore, prolonged oxidative stress may favour: (1) liver *n-3* LCPUFA depletion, which may be compounded by dietary imbalance and defective desaturation activity[102]; and (2) insulin resistance, in association with the redox activation of multiple stress-sensitive serine/threonine kinases that alters insulin signaling[103]. The latter phenomenon is a membrane-mediated process that might be also compromised by *n-3* LCPUFA depletion, due to loss of membrane polyunsaturation. Both IR and liver *n-3* LCPUFA depletion can determine hepatic steatosis by different mechanisms, namely, (1) insulin resistancedependent higher peripheral mobilization of FA and glycerol to the liver; and (2) *n-3* LCPUFA depletioninduced changes in the DNA-binding activity of the peroxisome proliferator-activated receptor-α (PPAR-α) as well as of the sterol regulatory element binding protein- 1c (SREBP-1c), determining a metabolic imbalance between FA oxidation and lipogenesis in favour of the latter. This notion is based on the findings that *n-3* LCPUFA are signaling biomolecules regulating hepatic lipid metabolism through (1) down-regulation of the expression of SREBP-1c and its processing, with inhibition of the transcription of lipogenic and glycolytic genes; and (2) up-regulation of the expression of genes encoding enzymes of the oxidation of FA, which act as ligands of PPAR-α[104].

Are reactive oxygen species always deleterious?

The reactive oxygen or nitrogen species nitric oxide, besides being an intermediate in the formation of the strong oxidant peroxynitrite, has several physiological functions: in the nervous system, it acts as a neuromodulator and plays a role in synaptic plasticity and long-term memory, whereas in the vascular system, it controls blood pressure, inhibits platelet aggregation and kills certain pathogenic microorganisms[105.106]. Many physiological reactions are oxidoreduction reactions, i.e., electrons are transferred from one molecule to another, giving rise to free radical intermediates; furthermore, many enzyme-catalyzed reactions produce free radicals as intermediates, which allow decreasing the activation energy of the general reaction. When considering a 'useful role' for ROS, the killing mechanism of microorganisms by neutrophils and other phagocytic cells must taken into consideration. Phagocytosis is a complex process, requiring the activation of neutrophils or macrophages[107]. One of its many steps involves the production in the extracellular space of superoxide and hydrogen peroxide. Hydrogen peroxide crosses biological membranes easily, and is toxic to microorganisms either directly by promoting several oxidations, or indirectly by forming a more reactive species such as the hydroxyl radical or hypochlorite. The highly reactive hydroxyl radical is produced from superoxide and/or hydrogen peroxide by Haber–Weiß or Fenton reactions, whereas hypochlorite is produced by myeloperoxidase[108]. Thus the production of several ROS by activated neutrophils or macrophages is a defense mechanism against invading microorganisms.

Since the latter are much more sensitive to ROS than human tissue, there is a 'bactericidal window' for which the ROS concentration is sufficient to kill bacteria without causing damage to host tissue (in normal conditions). Eicosanoids constitute an important family of bioactive lipids, comprising prostaglandins, thromboxanes and leukotrienes. They are derived from arachidonate (C20:4) under the action of prostaglandin H synthase (cyclooxygenase) or lipoxygenases. Eicosanoids locally exert various biological roles such as the mediation of inflammation, the regulation of blood flow, ion transport across membranes, the modulation of synaptic activity or the induction of sleep[109,110].

An interesting example of the involvement of ROS in signal transduction for a physiological process is the capacitation of spermatozoa by superoxide. ROS have beneficial or detrimental effects on sperm functions depending on the nature and the concentration of the ROS involved, as well as the moment and the location of exposure. Sperm capacitation and the acrosome reaction are complex processes required for ovule fertilization; they are regulated by signal transduction mechanisms involving G proteins, calcium ions and ROS[111.112]. In particular, low concentrations of superoxide trigger this phenomenon, whereas excessive generation of hydrogen peroxide in semen could be a cause for infertility. Conversely, removal of superoxide by superoxide dismutase prevents sperm hyperactivation and capacitation induced by various biological fluids[111].

Besides the participation of ROS in physiological and defense processes, ROS are used as therapeutic agents too. The best example is probably photodynamic therapy (PDT). It requires a photo sensitizer, visible light, and molecular oxygen to selectively kill cells. When localized in the target tissue, the photo sensitizer is activated by light to produce oxygen intermediates (e.g., singlet oxygen) that destroy target tissue cells. The easy access of skin to visible light and molecular oxygen has led dermatologists to apply PDT to cutaneous disorders. In particular, PDT was shown to be successful in treating actinic keratoses (precancerous skin lesions), basal cell carcinoma, and Bowen's disease. The most popular photo sensitizer is *δ*-aminolevulinic acid, the endogenous precursor of porphyrins, which does not make patients susceptible to phototoxicity for extended periods. Thus besides serious conditions such as non-melanoma skin cancers and pre-malignant lesions, PDT is also used for the treatment of acne and as an adjuvant to photorejuvenation procedures[113.114].

CONCLUSIONS

ROS play a determinable role in frequent pathologies especially liver disorders. Adequate knowledge of the

biochemical mechanisms involving ROS should allow developing new strategies to prevent and care these diseases. Due to the unavoidable presence of ROS and free radicals in all aerobic organisms, the latter possess efficient antioxidant systems that prevent an excessive concentration of these molecules in biological tissues in normal conditions. However, various genetic or environmental conditions sometimes lead to an imbalance between the production and the decomposition of these reactive intermediates that lead to deleterious consequences.

Conversely, an adequate level of certain ROS can have a physiological role, as for instance the catalysis of many biochemical reactions or the defense against invading pathogens.

Conflict of interest

Nagy S. El-Rigal, Manal A. Hamed, and Maha Z. Rizk declare that they have no conflict of interest.

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