Chapter 1

Lipid Peroxidation: Chemical Mechanism, Biological Implications and Analytical Determination

Marisa Repetto, Jimena Semprine and Alberto Boveris

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/45943

1. Introduction

Currently, lipid peroxidation is considered as the main molecular mechanisms involved in the oxidative damage to cell structures and in the toxicity process that lead to cell death. First, lipid peroxidation was studied for food scientists as a mechanism for the damage to alimentary oils and fats, nevertheless other researchers considered that lipid peroxidation was the consequence of toxic metabolites (e.g. CCl_4) that produced highly reactive species, disruption of the intracellular membranes and cellular damage (Dianzani & Barrera, 2008).

Lipid peroxidation is a complex process known to occur in both plants and animals. It involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds in unsaturated lipids and the eventual destruction of membrane lipids, with the production of a variety of breakdown products, including alcohols, ketones, alkanes, aldehydes and ethers (Dianzani & Barrera, 2008).

In pathological situations the reactive oxygen and nitrogen species are generated at higher than normal rates, and as a consequence, lipid peroxidation occurs with α-tocopherol deficiency. In addition to containing high concentrations of polyunsaturated fatty acids and transition metals, biological membranes of cells and organelles are constantly being subjected to various types of damage (Chance et al., 1979; Halliwell & Gutteridge, 1984). The mechanism of biological damage and the toxicity of these reactive species on biological systems are currently explained by the sequential stages of reversible oxidative stress and irreversible oxidative damage. Oxidative stress is understood as an imbalance situation with increased oxidants or decreased antioxidants (Sies, 1991a; Boveris et al., 2008). The concept implies the recognition of the physiological production of oxidants (oxidizing free-radicals and related species) and the existence of operative antioxidant defenses. The imbalance

concept recognizes the physiological effectiveness of the antioxidant defenses in maintaining both oxidative stress and cellular damage at a minimum level in physiological conditions (Boveris et al., 2008).

Lipid peroxidation is a chain reaction initiated by the hydrogen abstraction or addition of an oxygen radical, resulting in the oxidative damage of polyunsaturated fatty acids (PUFA). Since polyunsaturated fatty acids are more sensitive than saturated ones, it is obvious that the activated methylene (RH) bridge represents a critical target site. The presence of a double bond adjacent to a methylene group makes the methylene C-H bond weaker and therefore the hydrogen in more susceptible to abstraction. This leaves an unpaired electron on the carbon, forming a carbon-centered radical, which is stabilized by a molecular rearrangement of the double bonds to form a conjugated diene which then combines with oxygen to form a peroxyl radical. The peroxyl radical is itself capable of abstracting a hydrogen atom from another polyunsaturated fatty acid and so of starting a chain reaction (Halliwell & Gutteridge, 1984) (Fig. 1).

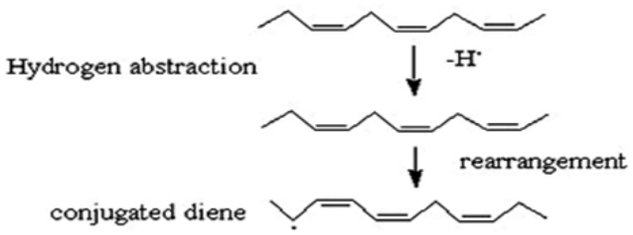

Figure 1. Initiation step of lipid peroxidation process.

Molecular oxygen rapidly adds to the carbon-centered radicals (R·) formed in this process, yielding lipid peroxyl radicals (ROO·). Decomposition of lipid peroxides is catalyzed by transition metal complexes yielding alcoxyl (RO·) or hydroxyl (HO·) radicals. These participate in chain reaction initiation that in turn abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. The formation of peroxyl radicals leads to the production of organic hydroperoxides, which, in turn, can subtract hydrogen from another PUFA. This reaction is termed propagation, implying that one initiating hit can result in the conversion of numerous PUFA to lipid hydroperoxides. In sequence of their appearance, alkyl, peroxyl and alkoxyl radicals are involved. The resulting fatty acid radical is stabilized by rearrangement into a conjugated diene that retains the more stable products including hydroperoxides, alcohols, aldehydes and alkanes. Lipid hydroperoxide (ROOH) is the first, comparatively stable, product of the lipid peroxidation reaction (Halliwell & Gutteridge, 1984) (Fig. 2).

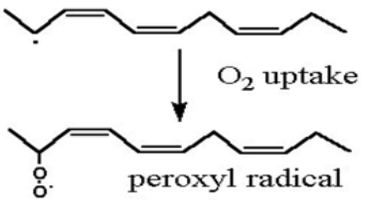

Figure 2. Initial phase of the propagation step of lipid peroxidation process indicating the oxygen uptake.

Reduced iron complexes (Fe^{2+}) react with lipid peroxides (ROOH) to give alkoxy radicals, whereas oxidized iron complexes (Fe^{3+}) react more slowly to produce peroxyl radicals. Both radicals can take part in the propagation of the chain reaction. The end products of these complex metal ion-catalyzed breakdowns of lipid hydroperoxides include the cytotoxic aldehydes and hydrocarbon gases such as ethane.

The free radical chain reaction propagates until two free radicals conjugate each other to terminate the chain. The reaction can also terminate in the presence of a chain-breaking anti-oxidant such as vitamin E (α-tocopherol) (Halliwell & Gutteridge, 1984).

In conditions in which lipid peroxidation is continuously initiated it gives non-radical products destroying two radicals at a time. In the presence of transition metal ions, ROOH can give rise to the generation of radicals capable of re-initiating lipid peroxidation by redox-cycling of these metal ions (Halliwell & Gutteridge, 1984).

Lipid peroxidation causes a decrease in membrane fluidity and in the barrier functions of the membranes. The many products of lipid peroxidation such as hydroperoxides or their aldehyde derivatives inhibit protein synthesis, blood macrophage actions and alter chemotactic signals and enzyme activity (Fridovich & Porter, 1981).

2. Biological implications of lipid peroxidation

The biological production of reactive oxygen species primarily superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) is capable of damaging molecules of biochemical classes including nucleic acids and aminoacids. Exposure of reactive oxygen to proteins produces denaturation, loss of function, cross-linking, aggregation and fragmentation of connective tissues as collagen (Chance et al., 1979). However, the most damaging effect is the induction of lipid peroxidation. The cell membrane which is composed of poly-unsaturated fatty acids is a primary target for reactive oxygen attack leading to cell membrane damage.

The lipid peroxidation of polyunsaturated fatty acids may be enzymatic and non-enzymatic. Enzymatic lipid peroxidation is catalyzed by the lipoxygenases family, a family of lipid peroxidation enzymes that oxygenates free and esterified PUFA generating as a consequence, peroxy radicals. Non enzymatic lipid peroxidation and formation of lipid-peroxides are initiated by the presence of molecular oxygen and is facilitated by Fe^{2+} ions (Repetto et al., 2010a).

Oxidative breakdown of biological phospholipids occurs in most cellular membranes including mitochondria, microsomes, peroxisomes and plasma membrane. The toxicity of lipid peroxidation products in mammals generally involves neurotoxicity, hepatotoxicity and nephrotoxicity (Boveris et al., 2008). The principal mechanism involves detoxification process in liver. Toxicity from lipid peroxidation affect the liver lipid metabolism where cytochrome P-450s is an efficient catalyst in the oxidative transformation of lipid derived aldehydes to carboxylic acids adding a new facet to the biological activity of lipid oxidation metabolites. Cytochrome P-450-mediated metabolism operates in parallel with other metabolic transformations of aldehydes; hence, the P450s could serve as reserve or

compensatory mechanisms when other high capacity pathways of aldehyde elimination are compromised due to disease or toxicity. Finally, 4-hydroxynonenal (HNE), unsaturated aldehydes, such as acrolein, trans-2-hexenal, and crotonaldehyde, are also food constituents or environmental pollutants, P-450s may be significant in favoring lipid peroxidation that has significant downstream effects and possibly play a major role in cell signaling pathways. Oxidized lipids appear to have a signaling function in pathological situations, are pro-inflammatory agonists and contribute to neuronal death under conditions in which membrane lipid peroxidation occurs. For example, mitochondrial lipid cardiolipin makes up to 18% of the total phospholipids and 90% of the fatty acyl chains are unsaturated. Oxidation of cardiolipin may be one of the critical factors initiating apoptosis by liberating cytochrome c from the mitochondrial inner membrane and facilitating permeabilization of the outer membrane. The release of cytochrome c activates a proteolytic cascade that culminates in apoptotic cell death (Navarro & Boveris, 2009).

Previous results indicate that lipid peroxidation has a role in the pathogenesis of several pathologies as neurodegenerative (Dominguez et al., 2008; Famulari et al., 1996; Fiszman et al., 2003), inflammatory (Farooqui & Farooqui, 2011), infectious (Repetto et al., 1996), gastric (Repetto et al., 2003) and nutritional diseases (Repetto et al., 2010b).

Oxidative damage in liver is associated with hepatic lipid metabolism, and may be affecting the absorption and transport mechanisms of α-tocopherol in this organ. In the liver, the morphological damage is previous to the lipid peroxidation and the consumption of endogenous antioxidants. In kidney and heart, indeed, lipid peroxidation and oxidative damage preceded necrosis (Repetto et al., 2010b).

Lipid peroxidation is a chain reaction process characterized by repetitive hydrogen abstraction by $HO^{\cdot}$ and $RO^{\cdot}$, and addition of O_2 to alkyl radicals ($R^{\cdot}$) resulting in the generation of $ROO^{\cdot}$ and in the oxidative destruction of polyunsaturated fatty acids, in which the methylene group (=RH-) is the main target (Halliwell & Gutteridge, 1984).

The association between increased phospholipid oxidation, free-radical mediated reactions and pathological states was early recognized (Cadenas, 1989; Verstraeten et al., 1997; Liu et al., 2003). The contribution by Sies of the concept of oxidative stress followed (Sies, 1991a,1991b) with the implication that increased free-radical mediated reactions, basically by $HO^{\cdot}$ and $RO^{\cdot}$, would produce phospholipid, protein, lipid, DNA, RNA or carbohydrate oxidation, whatever is close (Halliwell & Gutteridge, 1984). The increased oxidation of the cell biochemical constituents is associated with ultra structural changes in mitochondrial morphology with mitochondrial swelling and increased matrix volume (Boveris et al., 2008). In human liver, the morphological changes can affect the organ structure and function as it is the case for the bile canaliculi that are damaged in liver transplanted patients; a fact that is interpreted as consequence of the oxidative damage that is associated to ischemia-reperfusion (Cutrin et al., 1996). Interestingly, there are reports in rat liver experimental models, of increased peroxidation secondary to increased mitochondrial production of $O_2^{\cdot-}$ and H_2O_2 (Fridovich, 1978; Navarro &Boveris, 2007; Navarro et al., 2009).

3. Chemical mechanisms for lipid peroxidation process

The spectrum of oxygen reactive species that are considered responsible for biological oxygen toxicity include the intermediates of the partial reduction of oxygen, superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and other reactive species as hydroxyl radicals ($HO^{\cdot}$), peroxyl radical ($ROO^{\cdot}$), nitric oxide (NO), peroxinitrite (ONOO-) and singlet oxygen ($^1O_2$).

The biological effects of excess levels of the spectrum of these species are quite similar, and that is the reason they are collectively called reactive oxygen species (ROS). The main free-radical mediated chain reactions in biological systems are summarized in Fig. 3. The Beckman-Radi-Freeman pathway and the Cadenas-Poderoso shunt have been added to the original consecutive reactions of the Fenton/Haber-Weiss pathway and lipid peroxidation process to incorporate NO and $ONOO^{\cdot}$ to the biochemical free-radical mediated chain reaction (Moncada et al., 1991; Boveris et al., 2008) (Fig. 3).

In the last years the denominations "reactive oxygen species" (ROS) and "reactive nitrogen species" (RNS) had became very popular. The ROS denomination involves the three chemical species of the Fenton/Haber-Weiss pathway ($O_2^{\cdot-}$, H_2O_2 and $HO^{\cdot}$), the products of the partial reduction of oxygen. Similarly, the RNS denomination is loosely referring to the three chemical species of the Beckman-Radi-Freeman pathway (NO, $ONOO^{\cdot}$, and NO_2) (Moncada et al., 1991). The reference as a whole to either group, ROS and RNS, is usually made to explain or to refer to their biological activity, what reflects the fact that each group, ROS and RNS, are auto-propagated in biological systems from their promoters, $O_2^{\cdot-}$ and NO. Nevertheless, the advantage and facility in referring to the biological effects implies the ignorance of the biochemistry of the process.

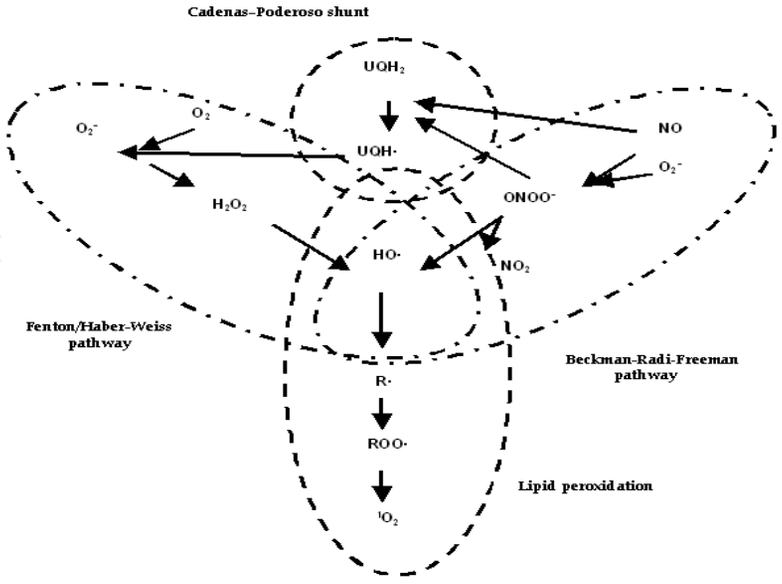

Figure 3. The free-radical mediated chain reaction in biochemistry. $O_2^{\cdot-}$, superoxide radical; H_2O_2, hydrogen peroxide, $HO^{\cdot}$, hydroxyl radical; NO, nitric oxide; $ONOO^{\cdot}$, peroxinitrite; ·NO2, nitrogen dioxide; UQH2, ubiquinol; $UQH^{\cdot}$, ubisemiquinone; $R^{\cdot}$, alkyl radical; $ROO^{\cdot}$, peroxyl radical; $^1O_2$, singlet oxygen.

The individual steps of the free-radical mediated chain reaction of biological systems (Fig. 3) are in majority non-enzymatic second order reactions with fast reaction rates, about 10^7 M^{-1} s^{-1}. The exceptions are the enzymatic dismutation of O_2^- (10^{10} M^{-1} s^{-1}, catalyzed by the antioxidant enzyme superoxide dismutase, SOD), the first order reaction of decomposition of $ONOO^-$, and the relatively lower rate (10^5 M^{-1} s^{-1}) of the homolysis of H_2O_2 catalyzed by Fe^{2+} (Boveris et al., 2008).

Concerning the molecular mechanisms that produces lipid peroxidation in biological systems previous, it is accepted that lipid peroxidation may be a consequence of a) intermediates of the partial reduction of oxygen (homolysis of H_2O_2 and $HO^{\cdot}$ generation), b) direct autoxidation of lipids, c) intermediates of the nitric oxide metabolism, and d) modifications of lipid membrane surface structure (Fridovich & Porter, 1981; Boveris et al., 2008; Navarro & Boveris, 2009; Repetto et al., 2010a;).

The lipid peroxidation process is induced for the pro-oxidant effect of transition metals. A vast evidence supports the occurrence of reactions of metal ions with H_2O_2, and hydroperoxides in the cytosol and in biological membranes. The latter ones are the main target of oxidative damage. In other words, by one mechanism, transition metals produce lipid peroxidation by stimulation of the oxidative capacity of H_2O_2 by promoting free-radical mediated processes (Fridovich, 1978; Moncada et al., 1991; Verstraeten et al., 1997; Repetto et al., 2010a; Repetto & Boveris, 2012), and by another mechanism, they bind to negatively charged phospholipids which alters the physical properties of the bilayer and favors the initiation and propagation reactions of lipid peroxidation (Repetto et al., 2010a; Repetto & Boveris, 2012).

Lipid peroxidation is a chain reaction initiated by hydrogen abstraction or by addition of an oxygen radical, resulting in the oxidative damage of polyunsaturated fatty acids (PUFA). Since polyunsaturated fatty acids are more sensitive than saturated ones, it is obvious that the activated methylene (RH) bridge represents a critical target site. This initiation is usually performed by a radical of sufficient reactivity (Eq.1):

$$R_1H + R^{\cdot} \rightarrow R_1^{\cdot} + RH \quad (1)$$

Molecular oxygen rapidly adds to the carbon-centred radical ($R^{\cdot}$) formed in this process, yielding the lipid peroxyl radical (ROO) (Eq. 2):

$$R^{\cdot} + O_2 \rightarrow ROO \quad (2)$$

The formation of peroxyl radicals leads to the production of organic hydroperoxides, which, in turn, can abstract hydrogen from another PUFA, analogous to reaction 1:

$$R_1H + ROO^{\cdot} \rightarrow R_1^{\cdot} + ROOH \quad (3)$$

This reaction is termed propagation, implying that one initiating hit results in the conversion of numerous PUFA to lipid hydroperoxides.

In the sequence of their appearance, alkyl, peroxyl, and alkoxyl radicals are generated in the free radical chain reaction.

The alkyl radical is stabilized by rearrangement into a conjugated diene that is a relatively stable product.

Lipid hydroperoxide (ROOH) is the first stable product of the lipid peroxidation reaction. Under conditions where lipid peroxidation is continuously initiated, radical anhilation or termination occurs with the destroying of two radicals at once:

$$ROO\cdot + ROO\cdot \rightarrow ROH + RO\cdot + {}^1O_2 \quad (4)$$

In the presence of transition metal ions, ROOH gives raise to the generation of radicals capable of (re-)initiating the lipid peroxidation by redox-cycling of the metal ions (Repetto et al., 2010a; Repetto & Boveris, 2012):

$$ROOH + Me^{n+} \rightarrow RO\cdot + Me^{(n-1)+} \quad (5)$$

$$ROOH + Me^{(n-1)+} \rightarrow ROO\cdot + Me^{n+} \quad (6)$$

3.1. Autoxidation of lipids: Non-enzymatic lipid peroxidation

Non-enzymatic lipid peroxidation is a free radical driven chain reaction in which one free radical induces the oxidation of lipids, mainly phospholipids containing polyunsaturated fatty acids. Autoxidation of lipids in biological systems is a direct process that occurs by homolysis of endogenous hydroperoxides by scission of ROOH and production of RO· and ROO·.

The polyunsaturated fatty acids such as linoleic and arachidonic acids, which are present as phosphoglyceride esters in lipid membranes, are particularly susceptible to autoxidation. Moreover, autoxidation in biological systems has been associated with such important pathological events as damage to cellular membranes in the process of aging and the action of certain toxic substance. The autoxidation of most organic substrates in homogeneous solution is a spontaneous free-radical chain process at oxygen partial pressures above 100 torr (Repetto et al., 2010a).

Lipid hydroperoxides, in presence or absence of catalytic metal ions, produce a large variety of products including short and long chain aldehydes and phospholipids and cholesterol ester aldehydes, which provide an equivalent hydrogen abstraction from an unsaturated fatty acid and formation of free radical. The secondary products can be used to assess the degree of lipid peroxidation in a system (Sies, 1991a) (Eq. 7 to 9).

Eq. 7 requires some comments. As written is thermodinamically non spontaneous since it involves the breaking of a C-H bond (435 kJ/mol). However, polyunsaturated fatty acids in solutions are readily autooxidized, likely catalized by transition metal ions. The R· radicals reaction with O_2 yielding ROO·.

$$RH \rightarrow R\cdot + H\cdot \quad (7)$$

$$R\cdot + O_2 \rightarrow ROO\cdot \quad (8)$$

$$ROO\cdot + RH \rightarrow ROOH + R\cdot \quad (9)$$

Transition metal ions Fe^{2+} and Cu^{+} stimulate lipid peroxidation by the reductive cleavage of endogenous lipid hydroperoxides (ROOH) of membrane phospholipids to the corresponding alkoxyl ($RO\cdot$) and peroxyl ($ROO\cdot$) radicals in a process that is known as ROOH-dependent lipid peroxidation (Eqs. 10 and 11):

$$Fe^{2+} + ROOH \rightarrow RO\cdot + OH^{-} + Fe^{3+} \quad (10)$$

$$Fe^{3+} + ROOH \rightarrow RO_2\cdot + H^{+} + Fe^{2+} \quad (11)$$

The mechanisms of these two reactions appear to involve the formation of Fe(II)-Fe(III) or Fe(II)-O_2-Fe(III) complexes with maximal rates of $HO\cdot$ radical formation at a ratio Fe(II)/Fe(III) of 1 (Repetto et al., 2010a; Repetto & Boveris, 2012).

Cu^{2+} and Cu^{+} are known for their capacity to decompose organic hydroperoxides (ROOH) to form $RO\cdot$ and $ROO\cdot$ (Eqs. 12 and 13) (Sies, 1991a; Repetto et al., 2010a; Repetto & Boveris, 2012).

$$Cu^{+} + ROOH \rightarrow RO\cdot + OH^{-} + Cu^{2+} \quad (12)$$

$$Cu^{2+} + ROOH \rightarrow RO_2\cdot + H^{+} + Cu^{+} \quad (13)$$

3.2. Lipid peroxidation generated for intermediates of the partial reduction of oxygen

The physiological generation of the products of the partial reduction of oxygen, O_2^{-} and H_2O_2, constitute the biological basis of the process of lipid peroxidation in mammalian aerobic cells. From a molecular point of view hydroxyl radical ($HO\cdot$) generation, formed from H_2O_2 and Fe^{2+} by the Fenton reaction, has been considered for a long time as the likely rate-limiting step for physiological lipid peroxidation (Verstraeten et al., 1997; Repetto & Boveris, 2012). The Fenton reaction and Fenton-like reactions (Eq. 14) are frequently used to explain the toxic effects of redox-active metals (Eq. 5), where $M^{(n)+}$ is usually a transition metal ion:

$$Fe^{2+} + H_2O_2 \rightarrow [Fe(II)H_2O_2] \rightarrow Fe^{3+} + HO^{-} + HO \quad (14)$$

Trace (nM) levels of cellular and circulating active transition metal ions seem enough for the catalysis of a slow Fenton reaction *in vivo*, at the physiological levels of H_2O_2 (0.1-1.0 μM) (Chance et al., 1979).

Reactive oxygen species mainly include $O_2^{\cdot-}$ and H_2O_2, which are physiologically generated as by-products of mitochondrial electron transfer. The formation of $O_2^{\cdot-}$ is originated from the auto-oxidation of the ubisemiquinone of complexes I and III and the production of H_2O_2 occurs by intramitochondrial Mn-SOD catalysis (Navarro & Boveris, 2004; Navarro et al., 2007, 2010). When the electron transfer process is blocked at complexes I and III, electrons pass directly to O_2 producing $O_2^{\cdot-}$. The reactive oxygen and nitrogen species, although kept

in low steady-state concentrations by antioxidant systems, are able to react and damage biomolecules (Fig. 3). Mitochondria are considered the main intracellular source of oxidizing reactive oxygen species (Navarro Boveris, 2004; Navarro et al., 2005, 2009, 2010).

At low level of H_2O_2, Fe^{2+} induces lipid peroxide decomposition, generating peroxyl and alkoxyl radicals and favoring lipid peroxidation. These results indicate that the onset of the Fe^{3+} stimulatory effect on Fe^{2+}-dependent lipid peroxidation is due to reactive oxygen species production via Fe^{2+} oxidation with endogenous ROOH (Repetto & Boveris, 2012).

The Cu^{+} ion is considered an effective catalyst for the Fenton reaction (Eq. 15) [3].

$$Cu^{+} + H_2O_2 \rightarrow [Cu(I)\text{-}H_2O_2] \rightarrow Cu^{2+} + HO^{-} + HO^{\cdot} \quad (15)$$

The process of lipid peroxidation has been recognized as a free radical-mediated and physiologically occurring with the supporting evidence of in situ organ chemiluminescence (Boveris et al., 1980). The main initiation reaction is understood to be mediated by $HO^{\cdot}$ or by a ferryl intermediate, both with the equivalent potential for hydrogen abstraction from an unsaturated fatty acid, with formation of an alkyl radical ($R^{\cdot}$) (Repetto & Boveris, 2012) (Eq. 16):

$$HO^{\cdot} + RH \rightarrow H_2O + R^{\cdot} \quad (16)$$

One effect of the reaction of hydroxyl radicals, their formation catalyzed by iron ions, with lipids is to make those lipids insoluble or fibrotic that can be considered causative of membrane disruption and oxidative damage associated in different pathologies.

3.3. Lipid peroxidation generated from intermediates of the nitric oxide metabolism

An area of interest that has currently increased over the past decades is the study of nitric oxide (NO) since the demonstration, in 1987, of its formation by the enzyme NO synthase in vascular endothelial cells. This NO radical accounts for the properties of the called endothelial derived relaxing factor, is the endogenous stimulator of the soluble guanylate cyclase and is a potent vasodilator *in vitro* (Moncada et al., 1991). Unsaturated fatty acids are susceptible to nitration reactions. The nitric oxide (NO)-derived species are diffusible across membranes, their concentration in the hydrophobic core of membranes and lipoproteins lead to react fast with fatty acids and lipid peroxyl radicals ($ROO^{\cdot}$) during the lipid oxidadation process generating oxidized and nitrated products of free lipids (arachidonic acid, arachidonate oleate, linoleate) and esterified (cholesteryl linoleate). Lipid nitration process includes *in vivo* different molecular mechanisms: a) NO autooxidation to nitrite, which has oxidant and nitrating properties, b) electrophilic addition of NO relates species to unsaturated fatty acids, c) radical reactions between $ROO^{\cdot}$ and NO, d) peroxynitrite ($ONOO^{\cdot}$) derived free radicals mediate oxidation, nitrosation and nitration reactions. These species are considered currently as mediators of adaptative inflammatory responses.

NO is an endogenous mediator of many physiological functions through stimulation of the guanylate cyclase enzyme including the regulation of vascular relaxing, post-traslational

protein changes, gene expression and inflammatory cell function (Moncada et al., 1991). Free and esterified fatty acids as arachidonic and linoleic acids are important components of lipoproteins and membranes that may be oxidized for different compounds. The NO and NO-derived radicals react with fatty acids generating oxidized and nitrated species as nitroalkenes and consequently, nitroalcohols. At low oxygen concentrations the most important biological NO derivatives is $ONOO^{\cdot}$. The nitroalkylation process occurs *in vitro* and in vivo, is involved in redox processes and cell signaling through the reversible covalent bound and post-traslational modifications responsible for structure, function and subcellular distribution of proteins (Valdez et al., 2011) and regulating the pro-inflammatory effect of oxidant exposure (Nair et al., 2007).

A novel mechanism for hydroxyl radical production, which is not dependent on the presence of transition metals, has recently been proposed. This involves the production of peroxynitrite (Beckman et al., 1990, 1994; Rachmilewitz et al., 1993) which has proinflammatory effects *in vitro* (Moncada et al., 1991), from the reaction of NO with $O_2^{\cdot-}$ (Eqs. 17 to 20):

$$NO + O_2^{\cdot-} \rightarrow ONOO^- \quad (17)$$

$$ONOO^- + H^+ \rightarrow ONOOH \quad (18)$$

$$ONOOH \rightarrow HO^{\cdot} + NO_2 \quad (19)$$

$$2\,H^+ + O_2^{\cdot-} + O_2^{\cdot-} \rightarrow H_2O_2 + O_2^{\cdot-} \quad (20)$$

In pathological situations, macrophages and neutrophils, recruited to a site of injury, are activated to produce NO as part of the inflammatory response. Furthermore, SOD activity rapidly scavenges $O_2^{\cdot-}$ and also prolongs the vaso-relaxant effects of NO (Murphy & Sies, 1991; Hogg et al., 1992; Rachmilewitz et al., 1993).

3.4. Modifications of lipid membrane structure

The presence of cholesterol in cell surface membranes influences their susceptibility to peroxidation, probably both by intercepting some of the radicals present and by affecting the internal structure of the membrane by interaction of its large hydrophobic ring structure with fatty acid-side-chains. As lipid peroxidation precedes in any membrane, several of the products produced have a detergent-like activity, specially released fatty acids or phospholipids with one of their fatty-acid side-chains removed. This will contribute to increased membrane disruption and further peroxidation.

The onset of lipid peroxidation within biological membranes is associated with changes in their physicochemical properties and with alteration of biological function of lipids and proteins. Polyunsaturated fatty acids and their metabolites play physiological roles: energy provision, membrane structure, fluidity, flexibility and selective permeability of cellular membranes, and cell signaling and regulation of gene expression (Catala, 2006). The hydroxyl radical generated as a consequence of the Fenton reaction, oxidizes the cellular components of biological membranes (Fig. 4).

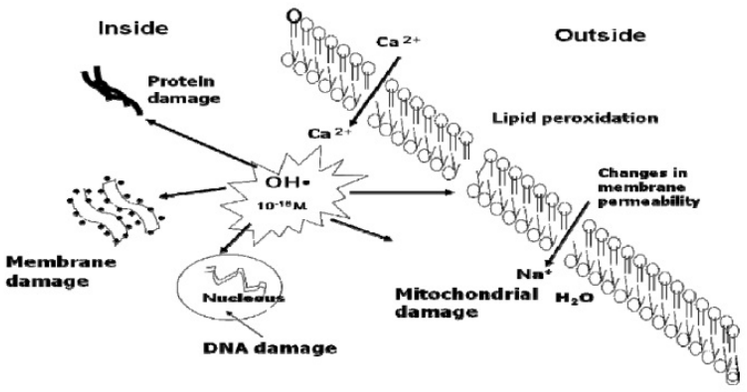

Figure 4. Lipid, DNA and protein oxidative damage from reactive hydroxyl radical.

The binding of positively charged species to a membrane (to the negatively-charged head-groups of phospholipids) can alter the susceptibility of the membrane to oxidative damage. This can be seen as either an enhancement or an inhibition of the rate of lipid peroxidation. Several metal ions such as Ca^{2+}, Co^{2+}, Cd^{2+}, Al^{3+}, Hg^{2+} and Pb^{2+} alter the rate of peroxidation in liposomes, erythrocytes and microsomal membranes, often stimulating the peroxidation induced by iron ions.

In the lipid peroxidation of the brain phosphatidylcholine-phosphatidylserine (PC-PS) liposomes (Repetto et al., 2010a) hydrogen abstraction occurred at the allilic carbons 9 and 10 of the oleic acid chain. Secondary initiation reactions are provided by hydrogen abstraction by RO· and ROO· (Eqs. 21 to 23) at the mentioned tertiary carbons:

$$RO\cdot + RH \rightarrow ROH + R \quad (21)$$

$$RO_2\cdot + RH \rightarrow ROOH + R\cdot \quad (22)$$

$$R\cdot + O_2 \rightarrow RO_2\cdot \quad (23)$$

The R· and ROO· radicals (Eqs.21-23) are central to the free radical-mediated process of lipid peroxidation. The addition reaction of R· with O_2 to yield ROO· (Eq. 23) yield a product that is able to abstract hydrogen atoms and to regenerate R· for a new cycle of the free-radical chain-reaction [38]. The whole process, by repetition of reaction 23, consumes O_2 and produces malondialdehyde (O=HC-CH_2-CH=O), 4-hydroxynonenal and other dialdehydes as secondary and end products of lipid peroxidation. The process produces TBARS at an approximate ratio of 0.12 TBARS/O_2 and normally utilized as measurement of the rate and extent of lipid peroxidation (Junqueira et al., 2004).

There are two consequences of lipid peroxidation: structural damage to membranes and generation of secondary products. Membrane damage derives from the production of broken fatty acyl chains, lipid-lipid or lipid-protein cross-links, and endocyclization reactions to produce isoprostanes and neuroprostanes (Catala, 2006). This effect is severe for biological systems, produce damage of membrane function, enzymatic inactivation and toxic effects on cellular division and function.

4. Role of transition metal on lipid peroxidation process

Studies in the past two decades have shown that redox active metals undergo redox cycling reactions and possess the ability to produce reactive radicals such as superoxide anion radical and nitric oxide in biological systems. Disruption of metal ion homeostasis leads to oxidative stress, a state with increased formation of reactive oxygen species that overwhelms antioxidant protection and subsequently induces DNA damage, lipid peroxidation, protein modification and other effects, all symptomatic for numerous diseases, involving cancer, cardiovascular disease, diabetes, atherosclerosis, neurological disorders, and chronic inflammation.

The mechanism of lipid peroxidation in biological systems caused by free radicals has been the focus of scientific interest for many years (Chance et al., 1976; Fridovich & Porter, 1981; Fraga et al., 1988; Gonzalez-Flecha et al., 1991a, 1991b; Famulari et al., 1996; Fiszman et al., 2003; Junqueira et al., 2004; Catala, 2006; Boveris et al., 2008; Dianzani & Barrera, 2008; Dominguez et al., 2008; Repetto, 2008; Repetto et al., 2010b). Currently, it is known that the $OH^{\cdot}$ radical, is formed mainly by the Haber-Weiss reaction, and it is responsible for the biological damage (Repetto et al., 2010a; Repetto et al., 2010b) (Eq.24):

$$O_2^{-} + H_2O_2 \rightarrow O_2 + HO^{\cdot} + HO^{-} \quad (24)$$

However, this reaction would not proceed significantly *in vivo* because the rate constant for the reaction is lower than that of the dismutation reaction. Nevertheless, a modification of the Haber-Weiss reaction, the Fenton reaction and Fenton-like reactions, utilizes the redox cycling ability of iron to increase the rate of reaction, is more feasible *in vivo* (Chance et al., 1979; Boveris et al., 1980; Gonzalez Flecha et al., 1991b), and is frequently used to explain the toxic effects of redox-active metals where $M^{(n)+}$ is usually a transition metal ion.

As a transition metal that can exist in several valences and that can bind up to six ligands, iron is an important component of industrial catalysts in the chemical industry especially for redox reactions (Repetto et al., 2010a; Repetto & Boveris, 2012).

There are several reports on the role of transition metals in lipid peroxidation process associated with cellular toxicities, because once they enter our physiological systems, these metals play a role in oxidative adverse effects. Some transition metals including iron, chromium, lead, and cadmium generate lipid peroxidation *in vitro* e *in vivo*: fatty acids, cod liver oil, biological membranes, tissues and organs, suggesting that metals contribute to the oxidative effects of lipid peroxidation observed in various diseases (Repetto et al., 2010a; Repetto & Boveris, 2012).

The Fenton reaction occurs *in vivo* at a very low rate, and hence cannot account for any substantial production of $OH^{\cdot}$ radicals in biology. On the other hand, when catalysed by transition metal ions, $OH^{\cdot}$ radicals can be formed through reactions 25 and 26:

$$M^{(n)+} + O_2^{-} \rightarrow M^{(n-1)+} + O_2 \quad (25)$$

$$M^{(n-1)+} + H_2O_2 \rightarrow M^{(n)+} + HO^{\cdot} + HO^{-} \quad (26)$$

The concentration of intracellular redox active transition metals is either low or negligible: free Fe^{2+} is 0.2-0.5 μM and the pool of free Cu^{2+} is about a single ion per cell. However, trace (nM) levels of cellular and circulating active transition metal ions seem enough for the catalysis of a slow Fenton reaction *in vivo* at the physiological levels of hydrogen peroxide (H_2O_2, 0.1-1.0 μM) (Repetto et al., 2010a; Repetto & Boveris, 2012).

It is well known that iron serves as a catalyst for the formation of the highly reactive hydroxyl radical via Fenton reaction. In addition to ferrous ion, many metal ions including Cu (I), Cr (II), and Co (II) were found to have the oxidative features of the Fenton reagent. Therefore, the mixtures of these metal compounds with H_2O_2 were named ''Fenton like reagents". In actual in vivo systems, once organic peroxides (ROOH) are formed by the action of ROS, heat, and/or photo-irradiation, ROOH can be substituted for $HO^{\cdot}$, where ROOH reacts with metal ions to form alkoxyl radicals. Subsequently, a chain reaction of lipid peroxidation occurs.

The mechanisms for metal transition ions promoted lipid peroxidation are H_2O_2 decomposition and direct homolysis of endogenous hydroperoxides. The Fe^{2+}-H_2O_2-mediated lipid peroxidation takes place by a pseudo-second order process, and the Cu^{2+}-mediated process by a pseudo-first order reaction. Co^{2+} and Ni^{2+} alone, do not induce lipid peroxidation. Nevertheless, when they are combined with Fe^{2+}, Fe^{2+}-H_2O_2-mediated lipid peroxidation is stimulated in the presence of Ni^{2+} and is inhibited in the presence of Co^{2+} (Fig. 5) (Repetto et al., 2010a).

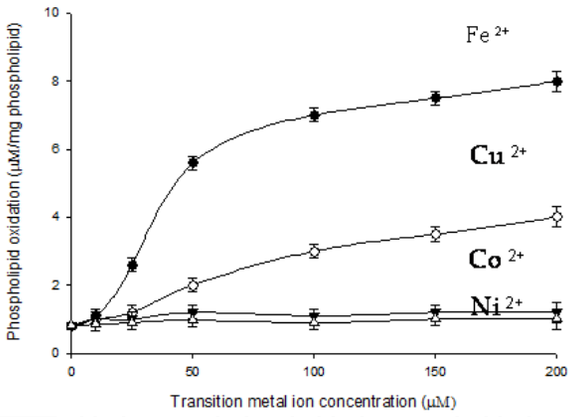

Figure 5. Phospholipid oxidation at different concentrations of transition metals.

There are many factors influencing lipid peroxidation products formation from lipids catalyzed by various metals. For example, the quantitative measurement of the reaction of Fe (II) and H_2O_2 has shown that a stoichiometric amount of hydroxyl radical is spin-trapped when ion concentration was less than 1 μM, suggesting that the strength of the Fenton system depended on the metal concentration. Since Fenton reported that a mixture of hydrogen peroxide and ferrous salts was an effective oxidant of a large variety of organic substrates in 1894 this reagent (the Fenton's reagent) has been used to investigate many subjects related to *in vitro* oxidation of organic substrates including lipids (Repetto et al., 2010a; Repetto & Boveris, 2012).

In the *in vitro* model of phosphatidylcholine/phosphatidyserine (60:40) liposomes and hydrogen peroxide (H_2O_2), Fe and Cu promote lipid peroxidation, interpreted as the consequence of the homolytic scission of H_2O_2 and of endogenous hydroperoxides (ROOH) and of the generation of hydroxyl ($HO^{\bullet}$) and alcoxyl ($RO^{\bullet}$) radicals (Cadenas, 1989) depending strictly on the participation of Fe and Cu as redox-reactive metals . However, Co^{2+} and Ni^{2+} alone, do not induce lipid peroxidation. Nevertheless, when they are combined with Fe^{2+}, Fe^{2+}-H_2O_2-mediated lipid peroxidation is stimulated in the presence of Ni^{2+} and inhibited in the presence of Co^{2+} (Repetto et al., 2010a; Repetto & Boveris, 2012).

Cr(III) occurs in nature and is an essential trace element utilized in the regulation of blood glucose levels. Cr(III) reacts with superoxide, subsequently Cr(II) yields hydroxyl radical via Fenton-like reaction with H_2O_2 to initiate lipid peroxidation.

Cadmium intoxication was shown to increase lipid peroxidation in rat liver, kidney and heart. However, the mechanisms of cadmium toxicity are not fully understood. Cadmium indirectly affects the generation of various radicals including superoxide and hydroxyl radical. The generation of hydrogen peroxide by cadmium ion may become a source of radicals in the Fenton system (Jomova & Valko, 2011).

5. Toxic effects of secondary products of lipid peroxidation

Many aldehydes are produced during the peroxidative decomposition of unsaturated fatty acids. Compared with free radicals, aldehydes are highly stable and diffuse out from the cell and attack targets far from the site of their production. About 32 aldehydes were identified as products of lipid peroxidation: a) saturated aldehydes (propanal, butanal, hexanal, octanal, being the decanal the most important); b) 2,3-trans-unsaturated-aldehydes (hexenal, octenal, nonenal, decenal and undecenal); c) a series of 4-hydroxylated,2,3-trans-unsaturated aldehydes: 4-hydroxyundecenal, being 4-hydroxinonenal (HNE) the most important quantitatively. Malonyldialdehyde (MDA) was considered for a long time as the most important lipid peroxidation metabolite. However, MDA is practically no toxic.

Recent studies have demonstrated that the most effective product of lipid peroxidation causing cellular damage is HNE. HNE produces different effects: acts as an intracellular signal able to modulate gene expression, cell proliferation, differentiation and apoptosis. The hydroxyl-group close to a carbonyl group present in HNE chemical structure is related to its high reactivity with different targets (thiol and amine groups). HNE is easily diffusible specie, but its biological effect depends on the molecule target and behavior as a signal to produce the damage.

Oxidative stress is a well known mechanism of cellular injury that occurs with increased lipoperoxidation of cell phospholipids and that has been implicated in various cell dysfunctions (Sies, 1991a,b; Catala, 2006). Aldehydes exhibit high reactivity with bio-molecules, such as proteins, DNA and phospholipids generating intra and intermolecular adducts.

The physiological concentrations of these products are low; however, higher concentrations correspond to pathological situations. Therefore, DNA damage caused by lipid peroxidation

end products could provide promising markers for risk prediction and targets for preventive measures. DNA-reactive aldehydes can damage DNA either by reacting directly with DNA bases or by generating more reactive bifunctional intermediates, which form exocyclic DNA adducts. Of these, HNE and MDA, acrolein, and crotonaldehyde have been shown to modify DNA bases, yielding promutagenic lesions and to contribute to the mutagenic and carcinogenic effects associated with oxidative stress-induced lipid peroxidation and HNE and MDA implicated carcinogenesis.

The end-products of lipid peroxidation (HNE and MDA) cause protein damage by addition reactions with lysine amino groups, cysteine sulfhydryl groups, and histidine imidazole groups (Esterbauer et al., 1991; Esterbauer, 1996). Modifications of protein by aldehyde products of lipid peroxidation contribute to neurodegenerative disorders, activation of kinases (Uchida et al., 1999; Uchida, 2003) and inhibition of the nuclear transcription factor (Camandola et al., 2000).

6. Lipid peroxidation of subcellular fragments

6.1. Microsomes

Microsomes isolated from liver have been shown to catalyze an NADPH-dependent peroxidation of endogenous unsaturated fatty acids in the presence of ferric ions and metal chelators, such as ADP or pyrophosphates. Microsomal membranes are particularly susceptible to lipid peroxidation owing to the presence of high concentrations of polyunsaturated fatty acids (Poyer & McCay, 1971). The mechanism involved in the initiation of peroxidation in the NADPH-dependent microsomal system do not appear to involve neither superoxide nor hydrogen peroxide, since neither superoxide dismutase nor catalase cause inhibition of peroxidation. Nevertheless, reduced iron plays an important role in both the initiation and propagation of NADPH-dependent microsomal lipid peroxidation (Shires, 1975).

Microsomal membrane lipids, particularly the polyunsaturated fatty acids, undergo degradation during NADPH-dependent lipid peroxidation. The degradation of membrane lipids during lipid peroxidation has been observed to result in the production of singlet oxygen, which is detected as chemiluminescence (Boveris et al., 1980).

Nonenzymatic peroxidation of microsomal membranes also occurs and is probably mediated in part by endogenous hemoproteins and transition metals. High concentrations of transition metals (50 μM) promote auto-oxidation of phospholipids (Repetto et al., 2010a).

6.2. Mitochondria

It is currently accepted that mitochondrial complex I is particularly sensitive to inactivation by oxygen free radicals and reactive nitrogen species. This special characteristic is frequently referred as complex I syndrome, with the symptoms of reduced mitochondrial respiration with malate-glutamate and ADP and of reduced complex I activity. This complex I syndrome has been observed in aging (Navarro et al., 2005; Navarro & Boveris, 2004, 2008),

in ischemia-reperfusion (Gonzalez-Flecha et al., 1993), in Parkinson's disease, and in other neurodegenerative diseases (Schapira et al., 1990a, 1990b; Sayre et al., 1999; Carreras et al., 2004; Schapira, 2008; Navarro et al., 2009), and in this study, with the addition of the increased rates of production of $O_2^{\cdot -}$ and H_2O_2 by complex I mediated reactions, reactions with the free radicals intermediates of the lipid peroxidation process (mainly ROO·), and amine-aldehyde adduction reactions. It is now understood that the three processes above mentioned alter the native non-covalent polypeptide interactions of complex I and promote synergistically protein damage and inactivation by shifting the noncovalent bonding to covalent cross linking (Navarro et al., 2005). Complex I oxidative protein damage has also been considered the result of protein modification by reaction with malonaldehyde and 4-HO-nonenal (Sayre et al., 1999). It was hypothesized that protein damage in the subunits of complexes I and IV follows to free radical-mediated cross-linking and inactivation. The subunits that are normally held together by noncovalent forces are shifted to covalent cross-linking after reaction with the hydroperoxyl radicals (ROO·) and the stable aldehydes produced during the lipid peroxidation process.

The hypothesis that cumulative free radical-mediated protein damage is the chemical basis of respiratory complexes I and IV inactivation (Berlett & Stadtman, 1997) offers the experimental approach of the chronic use of vitamin E, as an antioxidant for the lipid phase of the inner mitochondrial membrane and for the prevention of the mitochondrial /damage associated with aging. The adduction reactions of malonaldehyde and 4-HO-nonenal with protein evolve to stable advanced lipid peroxidation products (Sayre et al., 1999) and protein carbonyls (Nair et al., 2007; Navarro et al., 2008). The molecular mechanism involved in the inactivation of complex I is likely accounted for by $ROO^{\cdot}$ and $ONOO^{-}$. Upon aging, frontal cortex and hippocampal mitochondria show a decreased rate of respiration, especially marked with NAD-dependent substrates, and decreased enzymatic activities of complexes I and IV associated with an increase in the content of oxidation products (TBARS and protein carbonyls) (Navarro et al., 2008) (Fig. 6).

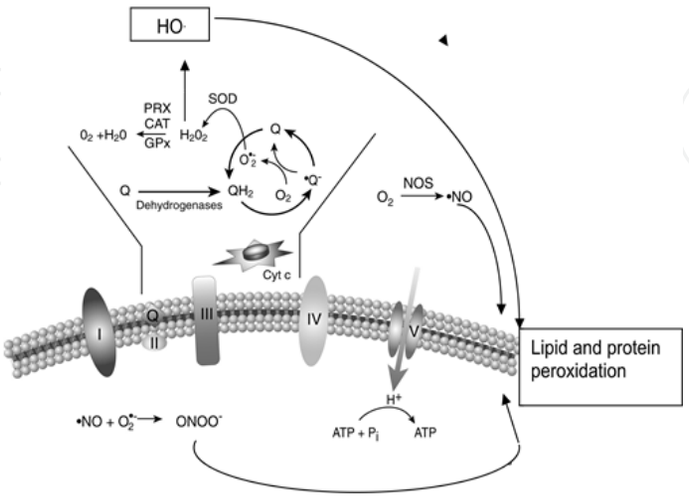

Figure 6. Lipid peroxidation and protein peroxidation by secondary products of lipid peroxidation in mitochondria.

7. Lipid peroxidation and human pathologies

The organism must confront and control the balance of both pro-oxidants and antioxidants continuously. The balance between these is tightly regulated and extremely important for maintaining vital cellular and biochemical functions. This balance often referred to as the redox potential, is specific for each organelle and biological site, and any interference of the balance in any direction might be deleterious for the cell and organism. Changing the balance towards an increase in the pro-oxidant over the capacity of the antioxidant is defined as oxidative stress and might lead to oxidative damage. Changing the balance towards an increase in the reducing power, or the antioxidant, might also cause damage and can be defined as reductive stress.

Oxidative stress and damage have been implicated in numerous disease processes, including inflammation, degenerative diseases, and tumor formation and involved in physiological phenomena, such as aging and embryonic development. The dual nature of these species with their beneficial and deleterious characteristics implies the complexities of their effects at a biological site.

Lipid peroxidation has been pointed out as a key chemical event in the oxidative stress associated with several inborn and acquired pathologies. Disruption of organelle and cell membranes together with calcium homeostasis alterations are the main supramolecular events linked to lipid peroxidation. However, it is not clear if lipid peroxidation process is a cause, triggering step of the clinical manifestations of the disease, or a consequence of toxic effects of lipid peroxidation products.

In pathological situations the reactive oxygen species are generated and as a consequence lipid peroxidation occurs with α-tocopherol deficiency. In addition to containing high concentrations of polyunsaturated fatty acids and transitional metals, red blood cells are constantly being subjected to various types of oxidative stress. Red blood cells however are protected by a variety of antioxidant systems which are capable of preventing most of the adverse effects under normal conditions. Among the antioxidant systems in the red cells, α-tocopherol possesses an important and unique role. α-tocopherol may protect the red cells from oxidative damage via a free radical scavenging mechanism and as a structural component of the cell membrane (Chitra & Shyamaladevi, 2011).

Levels of Met-Hb· are regarded as an index of intracellular damage to the red cell and it is increased when α-tocopherol is consumed and the rate of lipid peroxidation is increased. Scavenging of free radicals by α-tocopherol is the first and the most critical step in defending against oxidative damage to the red cells. When α-tocopherol is adequate, GSH and ascorbic acid may complement the antioxidant functions of α-tocopherol by providing reducing equivalents necessary for its recycling/regeneration.

On the other hand, when α-tocopherol is absent, GSH and ascorbic acid release transitional metals from the bound forms and/or maintain metal ions in a catalytic state. Free radical generation catalysed by transition metal ions in turn initiates oxidative damage to cell

membranes. Membrane damage can lead to release of heme compounds from erythrocytes. The heme compounds released may further promote oxidative damage especially when reducing compounds are present (Boveris et al., 2008).

8. Lipid peroxidation and aging

Aging is a process directly related to systemic oxidative stress. Two components of the oxidative stress situation have been recognized in human aging: a decrease in availability of nutritional molecular antioxidants and an accumulation of products derived from the oxidation of biological structures. Oxidation of biomolecules is related to susceptibility to diseases, such as cancer and heart disease, as well as associated with the process of aging (Navarro et al., 2005; Navarro & Boveris, 2007, 2008).

The products derived from lipid peroxidation, measured in plasma by Junqueira et al., (2004) as fluorescent products, were higher in elderly than younger human subjects and even higher in disabled octogenarians and nonagenarians. This increase in lipid peroxidation products was directly correlated with age, and was associated with decreases in vitamin E and C.

9. Analytical determination of lipid peroxidation

Since the acceptation of the oxidative stress concept, scientists and physicians have been searching for a simple assay or a small group of determination that would result useful for the assessment of oxidative stress and lipid peroxidation in clinical situations. The determinations of marker metabolites are usually performed in blood, red blood cells or plasma. The markers for systemic oxidative stress are normally present in healthy humans and the assays for systemic oxidative stress are comparative, which makes necessary to have reference values from normal individuals.

At present, the plasma levels of oxidation products derived from free-radical mediated reactions and of antioxidants are used as indicators of systemic oxidative stress in humans and experimental animals. The more utilized determination of an oxidation product is MDA, which is determined with low specificity but with great efficiency by the simple and useful assay of TBARS with measurements made by spectrophotometry or spectrofluorometry. The normal plasma levels of TBARS are 2-3 μM (Junqueira et al., 2004).

Oxidative damage is characterized by increases in the levels of the oxidation products of macromolecules, such as thiobarbituric acid reactive substances (TBARS), and protein carbonyls. Many of these products can be found in biological fluids, as well as addition-derivatives of these reactive end-products. As a result of lipid peroxidation a great variety of aldehydes can be produced, including hexanal, malondialdehyde (MDA) and 4-hydroxynonenal (Catala, 2006).

Oxidation of an endogenous antioxidant reflects an oxidative stress that is evaluated by measuring the decrease in the total level of the antioxidant or the increase in the oxidative

form. The only way not to be influenced by nutritional status is to measure the ratio between oxidized and reduced antioxidants present in blood. The published literature provides compelling evidence that a) MDA represents a side product of enzymatic PUFA-oxygenation and a secondary end product of no enzymatic (autoxidative) fatty peroxide formation and decomposition and b) sensitive analytical methods exist for the unambiguous isolation and direct quantification of MDA. Conceptually, these two facts indicate that MDA is an excellent index of lipid peroxidation. However, this conclusion is limited in practice by several important consideration: a) MDA yield as a result of lipid peroxidation varies with the nature of the PUFA peroxidised (specially its degree of instauration) and the peroxidation stimulus, b) only certain lipid oxidation products decompose yield MDA, c) MDA is only one of several end product of fatty peroxide formation and decomposition, d) the peroxidation environment influences both the formation of lipid-derived precursors and their decomposition to MDA, e) MDA itself is a reactive substance which can be oxidative and metabolically degraded, f) oxidative injury to no lipid biomolecules has the potential to generate MDA. With biological materials, it appears prudent to consider the TBARS test more than an empirical indicator of the potential occurrence of peroxidative lipid damage and not as a measure of lipid peroxidation (Repetto, 2008). The thiobarbituric acid test (TBARS) has been employed to a uniquely great degree over the last five decades to detect and quantify lipid peroxidation in a variety of chemical as well as biological material. Two underlying assumptions are implicit from the widespread use of the TBARS test to assess lipid peroxidation: a) an operative and quantitative relationship exists between lipid peroxidation and MDA, b) product formation during the TBARS test is diagnostic of the presence and amount of fatty peroxides.

Lipid peroxidation proceeds by a free-radical mediated chain reaction that includes initiation, propagation and termination reactions. The chain reaction is initiated by the abstraction of a hydrogen atom from a methylene group of an unsaturated fatty acid. Propagation is cycled through rounds of lipid peroxyl radical abstraction of the bis-methylene hydrogen atoms of a polyunsaturated fatty acyl chain to generate new radicals, after O_2 addition, resulting in the conversion of alkyl radical in hydroperoxyl radical. Termination involves the reaction of two hydroperoxyl radicals to form non-radical products. This reaction is particularly interesting since it is accompanied, although at low yield, by emission of light or chemiluminiscence. Some lipid peroxidation products are light-emitting species and their luminescence is used as an internal marker of oxidative stress (Chance et al., 1979; Boveris et al., 1980, Gonzalez-Flecha et al., 1991b; Sies, 1991a; Repetto, 2008). The measurement of light emission derived from $^1O_2$ and excited triplet carbonyl compounds, which are the most important chemiluminiscent species in the lipid peroxidation of biological systems, is directly related to the rate of lipid peroxidation and allows an indirect assay of the content of lipophilic antioxidants in the sample (Gonzalez-Flecha et al., 1991a). Lipophilic antioxidants react with lipid peroxyl radicals and lower antioxidant content is associated with higher chemiluminescence (Repetto, 2008).

The low-level chemiluminescence which accompanies the peroxidation of polyunsaturated fatty acids has been used as a tool in kinetic and mechanistic studies of biological samples to estimate the extent of the reactions and even to indicate tissue damage promoted by oxidants. Triplet carbonyls and singlet oxygen formed in the annihilation of intermediate peroxyl radicals (ROO·) have been identified as the chemiluminescence emitters.

Chemiluminescence is a very interesting way to evaluate an oxidative stress and lipid peroxidation in biological samples and living systems. The emission of light has been observed during stress in different experimental models. Chemiluminescence is very sensitive and thus can be applied to measure free radical production in human tissues.

Chemiluminescent systems may be classified in two classes based on the origin of the emitting molecule. In the first class, the emitter is a product of the chemical reaction (direct chemiluminescence). In the second class, there is energy transfer between an electronically excited product molecule and a second substance which then becomes the emitter (sensitized chemiluminescence) (Boveris et al., 1980; Gonzalez-Flecha et al., 1991b; Repetto, 2008).

The chemical mechanism responsible for spontaneous organ light emission is provided by the Russell's reaction in which two secondary or tertiary peroxyl radicals (ROO•) yield $^1O_2$ and excited carbonyl groups (=CO*) as products. In turn, two $^1O_2$, through dimol emission, lead to photoemission at 640 and 670 nm, whereas =CO* yields photons at the 460-470 nm band (Boveris et al., 1980). The main sources of the chemiluminescence detected in the direct and sensitized chemiluminescence is the dimol emission of $^1O_2$ (reaction 27) and the photon emission from excited carbonyl groups (reaction 28) (Boveris et al., 1980).

$$2\ {}^1O_2 \rightarrow 2\ O_2 + h\upsilon\ (634\text{-}703\ nm) \quad (27)$$

$$RO^* \rightarrow RO + h\upsilon\ (380\text{-}460\ nm) \quad (28)$$

These reactions are accompanied by chemiluminescence whose intensity may serve as an indirect measure of peroxide free radical and α-tocopherol concentration in the sample.

Lipid peroxidation has been recognized as free radical-mediated and physiologically occurring (Navarro & Boveris, 2004, Navarro et al., 2010; Repetto & Boveris, 2012) with the supporting evidence of *in situ* organ chemiluminescence (Repetto, 2008). Spontaneous chemiluminescence of *in situ* organs directly reports the intracellular formation of singlet oxygen ($^1O_2$) (Boveris et al., 1980) and represents an issue of direct chemiluminescence. The generation of $^1O_2$ implies the collision of two peroxyl radicals (ROO·) with formation of excited species, $^1O_2$ itself and excited carbonyls, followed by photoemission. Light emission from *in situ* organs is a physiological phenomenon that provides a determination of the steady state concentration of singlet oxygen and indirectly of the rate of oxidative free radical reactions (Boveris et al., 1980). *In situ* liver chemiluminescence has been recognized as a reliable indicator of oxidative stress and damage in rat liver upon hydroperoxide infusion (Gonzalez-Flecha et al., 1991b), ischemia-reperfusion (Gonzalez-Flecha et al., 1993),

and chronic and acute alcohol intoxication (Videla et al., 1983). The increases in photoemission observed were parallel to increased contents of indicators of lipid peroxidation (malonaldehyde and 4-HO-nonenal) but with a higher experimental/control ratio in organ chemiluminescence (Boveris et al., 1980).

Tert-butyl hydroperoxide initiated chemiluminescence is an example of sensitized chemiluminescence, and it has been used to enhance the chemiluminescence accompanying lipid peroxidation and the α-tocopherol content of tissues. This method has been successfully utilized to detect the existence of oxidative damage associated to experimental or pathological situations in tissue homogenates, subcellular fractions, and in human heart, liver and muscle biopsies (Gonzalez-Flecha et al., 1991b).

Tissue homogenates or blood samples are subjected to *in vitro* oxidative damage by supplementation with tert-butyl hydroperoxide. It reacts with hemoproteins and Fe^{2+} producing peroxyl and alcoxyl free radicals, which enter to the propagation phase of the lipid peroxidation radical chain reaction. The termination steps of the chain reaction generate compounds in an excited state: singlet oxygen and carbonyl groups. This assay is useful to evaluate the integral level of the non-enzymatic antioxidant defenses of a tissue (Gonzalez-Flecha et al., 1991a, 1993).

The increase of tert-butyl hydroperoxide-initiated chemiluminescence is indicative that α-tocopherol is the antioxidant consumed in erythrocytes and suggest that reactive oxygen species and lipid peroxidation catalyzed by reduced transition metals may be responsible for the onset of oxidative damage and the occurrence of systemic oxidative stress in patients suffering oxidative damage associated to neurological pathologies as Parkinson (Famulari et al., 1996, Dominguez et al., 2008), Alzheimer disease (Famulari et al., 1996; Repetto et al., 1999; Dominguez et al., 2008; Serra et al., 2009), and vascular dementia (Famulari et al., 1996, Dominguez et al., 2008; Serra et al., 2009); immunological diseases as HIV infection and AIDS (Repetto et al., 1996), hyperthyroidism and hypothyroidism (Abalovich et al., 2003). These methods were used to evaluate lipid peroxidation and oxidative damage in experimental models of oxidative stress in rats (Repetto et al., 2003, 2010; Ossani et al., 2007; Repetto & Ossani, 2008; Repetto & Boveris, 2010).

A common question of the researchers in the field is which the method of choice is. The answer is: none of them, and all of them. Each assay measures something different. Diene conjugation tells one about the early stages of peroxidation, as a direct measurement of lipid peroxides. In the absence of metal ions to decompose lipid peroxides there will be little formation of hydrocarbon gases, carbonyl compounds, or their fluorescent complexes, which does not necessarily mean therefore that nothing is happening. Even if peroxides do not decompose, the TBARS test can still detect them because of decomposition of peroxides. Changes in the mechanism of peroxide decomposition might alter the amount generated without any change in the overall rate of lipid peroxidation. Whatever method is chosen, one should think clearly what is being measured and how it relates to the overall lipid peroxidation process. Whatever possible, two or more different assay methods should be used.

10. Conclusion

Lipid peroxidation is a physiological process that takes place in all aerobic cells. Unsaturated fatty acids which are structural part of cell membranes are subjected to lipid peroxidation by a non enzymatic and free-radical mediated reaction chain. The molecular mechanisms of the lipid peroxidation process are known and it can be estimated that about 1 % of the total oxygen uptake of cells, organs and bodies in taken up by the reactions of lipid peroxidation. The initiation reactions are provided by the transition-metal catalyzed hemolytic scission of H_2O_2 and ROOH. In turn, H_2O_2 is mainly generated from the mitochondrial dismutation of superoxide radical ($O_2^{\cdot-}$). The products and by-products of lipid peroxidation are cytotoxic and lead in successive steps to oxidative stress, oxidative damage and apoptosis. In a long series of physiological and pathophysiological processes, including aging and neurodegenerative diseases, the rates of mitochondrial $O_2^{\cdot-}$ and H_2O_2 are increased with a parallel increase in the rate of the lipid peroxidation process. It is expected that supplementation with adequate antioxidants, as for instance, α-tocopherol, will keep sensitive cells and organs in healthy conditions and increase lifespan.

Author details

Marisa Repetto, Jimena Semprine and Alberto Boveris
University of Buenos Aires, School of Pharmacy and Biochemistry,
General and Inorganic Chemistry,
Institute of Biochemistry and Molecular Medicine (IBIMOL-UBA-CONICET), Argentina

Acknowledgement

We thank to Dr. Jorge Serra for helping in the revision of this version.

11. References

Abalovich, M.; Llesuy, S.; Gutierrez, S. & Repetto, M. (2003) Peripheral markers of oxidative stress in Graves´ disease. The effects of methimazole and 131 Iodine treatments. *Clinical Endocrinology*. Vol. 59, pp. 321-327, ISSN: 1365-2265

Beckman, J.; Beckman, T.; Chen, J.; Marshall, P. & Freeman, B. (1990) Apparent hydroxyl radical production from peroxynitrite. Implications for endothelial injury from nitric oxide oxide and superoxide. *Proceeding of the National Academy of Sciences of the United States.* Vol. 87, pp. 1620-1624, ISSN: 0027-8424

Beckman, J.; Chen, J.; Ischiropulos, H. & Crow, J. (1994) Oxidative chemistry of peroxynitrite. *Methods in Enzymology.* Vol. 233, pp. 229-240, ISSN: 0076-6879

Berlett, B.S. & Stadtman, E.R. (1997) Protein oxidation in aging, disease, and oxidative stress. *The Journal of Biological Chemistry.* Vol. 272, pp. 20313–20316, ISSN: 0021-9258

Boveris, A.; Cadenas, E.; Reiter, R.; Filipkowski, M.; Nakase, Y. & Chance, B. (1980) Organ chemiluminescence: noninvasive assay for oxidative radical reactions *Proceeding of the National Academy of Sciences of the United States.* Vol. 177, pp. 347-351, ISSN: 0027-8424

Boveris, A.; Fraga, C.; Varsavsky, A. & Koch, O. (1983) Increased chemiluminescence and superoxide production in the liver of chronically ethanol-treated rats. *Archives of Biochemistry and Biophysics.* Vol. 227, pp. 534-541, ISSN: 0003-9861

Boveris, A. & Navarro, A. (2008) Brain mitochondrial dysfunction in aging. *Life,* Vol. 60, No.5, pp. 308-314, ISSN: 1521-6543

Boveris, A.; Repetto, M.G.; Bustamante, J.; Boveris, A.D. & Valdez, L.B. (2008). The concept of oxidative stress in pathology. In: Álvarez, S.; Evelson, P. (ed.), *Free Radical Pathophysiology*, pp. 1-17, Transworld Research Network: Kerala, India, ISBN: 978-81-7895-311-3

Cadenas, E. (1989) Biochemistry of oxygen toxicity. *Annual Review of Biochemistry.* Vol. 58, pp. 79-110, ISSN:0066-4154

Camandola, S.; Poli, G. & Mattson, M. (2000) The lipid peroxidation product 4-hydroxy-2,3-nonenal inhibits constitutive and inducible activity of nuclear factor-bin neurons. *Molecular Brain Research.* Vol. 85, pp. 53–60, ISSN: 0021-9258

Carreras, M.C.; Franco, M.C.; Peralta, J.G. & Poderoso, J.J. (2004) Nitric oxide, complex I, and the modulation of mitochondrial reactive species in biology and disease. *Molecular Aspects of Medicine.* Vol. 25, pp. 125–139, ISSN: 0098-2997

Catala, A. (2006) An overview of lipid peroxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay. *The International Journal of Biochemistry and Cell Biology.* Vol. 38, pp. 1482-1495, ISSN: 1357-2725

Chance, B.; Sies, H. & Boveris, A. (1979) Hydroperoxide metabolism in mammalian organs. *Physiological Reviews*. Vol. 59, pp. 527-605, ISSN:®0031-9333

Cutrin, JC.; Cantino, D.; Biasi, F.; Chiarpotto, E.; Salizzoni, M.; Andorno, E.; Massano, G.; Lanfranco, G.; Rizetto, M.; Boveris, A. & Poli, G. (1996) Reperfusion damage to the bile canaliculi in transplanted human liver. *Hepatology.* Vol. 24, pp. 1053-1057, ISSN: 1527-3350

Dianzani, M. & Barrera, G. (2008) Pathology and physiology of lipid peroxidation and its carbonyl products. In: Álvarez, S.; Evelson, P. (ed.), *Free Radical Pathophysiology*, pp. 19-38, Transworld Research Network: Kerala, India, ISBN: 978-81-7895-311-3

Domínguez, R.O.; Marschoff, E.R.; Guareschi, E.M.; Repetto, M.G.; Famulari, A.L.; Pagano, M.A. & Serra, J.A. (2008). Insulin, glucose and glycated haemoglobin in Alzheimer's and vascular dementia with and without superimposed Type II diabetes mellitus condition. *Journal of Neural Transmission,* Vol. 115, pp. 77-84, ISSN: 0300-9564.

Esterbauer, H.; Schaur, J. & Zollner, H. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. *Free Radical in Biology & Medicine.* Vol. 11, pp. 81–128, ISSN: 0891-5849

Esterbauer, H. (1996) Estimation of peroxidative damage. A critical review. *Pathologie Biologie.* Vol. 44, pp. 25–28, ISSN: 0031-3009

Famulari, A.; Marschoff, E.; Llesuy, S.; Kohan, S.; Serra, J.; Domínguez, R.; Repetto, M.G.; Reides, C. & Lustig, E.S. de (1996). Antioxidant enzymatic blood profiles associated with risk factors in Alzheimer's and vascular diseases. A predictive assay to differentiate demented subjects and controls. *Journal of the Neurological Sciences*, Vol. 141, pp. 69-78, ISSN: 0022-510X

Farooqui, T. & Farooqui, A. (2011) Lipid-mediated oxidative stress and inflammation in the pathogenesis of Parkinson´s disease. *Parkinson´s disease*. DOI: 10.4061/2011/247467

Fiszman, M.; D´Eigidio, M.; Ricart, K.; Repetto, M.G.; Llesuy, S.; Borodinsky, L.; Trigo, R.; Riedstra, S.; Costa, P.; Saizar, R.; Villa, A. & Sica, R. (2003). Evidences of oxidative stress in Familial Amyloidotic Polyneuropathy Type 1. *Archives of Neurology*, Vol. 60, pp. 593-597, ISSN 0003-9942

Fraga, C.; Leibovitz, B. & Tappel, A. (1988). Lipid peroxidation measured as thiobarbituric acid-reactive substances in tissue slices: characterization and comparison with homogenates and microsomes. *Free Radicals in Biology and Medicine*, Vol. 4, pp. 155-161, ISSN: 0891-5849

Fridovich, I. (1978) Superoxide radicals, superoxide dismutases and the aerobic lifestyle. *Photochemistry and Photobiology*. Vol. 28, pp. 733-741, ISSN: 1010-6030

Fridovich, S. & Porter, N. (1981) Oxidation of arachidonic acid in micelles by superoxide and hydrogen peroxide. *The Journal of Biological Chemistry.* Vol. 256, pp. 260-265, ISSN: 0021-9258

Gatto, E.; Carreras, M.C.; Pargament, G.; Reides, C.; Repetto, M.G.; Llesuy, S.; Fernández Pardal, M. & Poderoso, J. (1996). Neutrophil function nitric oxide and blood oxidative stress in Parkinson's disease. *Movement Disorders*, Vol. 11, pp. 261-267, ISSN: 0885-3185

Gatto, E.; Carreras, C.; Pargament, G.; Riobó, N.; Reides, C.; Repetto, M.; Fernández Pardal, N.; Llesuy, S. & Poderoso, J. (1997). Neutrophyl function nitric oxide and blood oxidative stress in Parkinson´s Disease. *Focus Parkinson´s Disease,* Vol. 9, pp. 12-14

Gonzalez Flecha, B., Repetto, M.; Evelson, P. & Boveris, A. (1991a) Inhibition of microsomal lipid peroxidation by α -tocopherol and α -tocopherol acetate. *Xenobiotica*. 21: 1013–1022, ISSN: 0049-8254

González Flecha, B.; Llesuy, S. & Boveris, A. (1991b). Hydroperoxide-initiated chemiluminescence: assay for oxidative stress in biopsies of heart, liver and muscle. Free Radicals in Biology and Medicine, Vol. 10, pp. 93-100, ISSN: 0891-5849

Gonzalez-Flecha, B.; Cutrin, J.C. & Boveris, A. (1993) Time course and mechanism of oxidative stress and tissue damage in rat liver subjected to in vivo ischemia-reperfusion. *Journal of Clinical Investigation.* Vol. 91, pp. 456–464, ISSN:◎0021-9738

Halliwell, B. & Gutteridge, J.M.C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, Vol. 218, pp. 1-14, ISSN: 0264-6021

Hogg, N.; Darley-Usmar, V.; Wilson, M. & Moncada, S. (1992) Production of hydroxyl radicals from the simultaneous generation of superoxide and nitric oxide. *Biochemical Journal.* Vol. 281, pp. 419-424, ISSN: 0264-6021

Jomova, K. & Valko, M. (2011) Advances in metal-induced oxidative stress and human disease. *Toxicology.* Vol. 283, pp. 65-87, ISSN: 0300-483X.

Junqueira, V.; Barros, S.; Chan, S.; Rodríguez, L.; Giavarotti, L.; Abud, R. & Deucher, G. (2004) Aging and oxidative stress. *Molecular Aspects of Medicine.* Vol. 25, pp. 5–16, ISSN: 0098-2997

Liu, Q.; Raina, A.K.; Smith, M.A.; Sayre,. LM. & Perry, G. (2003) Hydroxynonenal, toxic carbonyls, and Alzheimer disease. *Molecular Aspects of Medicine.* Vol. 24, pp. 305–313, ISSN: 0098-2997

Moncada, S.; Palmer, R. & Higgs, E. (1991) Nitric oxide: Physiology, patophysiology and pharmacology. *Pharmaceutical Reviews.* Vol. 43, pp. 109-141, ISSN:⊙1918-5561

Murphy, M. & Sies, H. (1991) Reversible conversion of nitroxyl anion to oxide by superoxide dismutase. *Proceeding of the National Academy of Sciences of the United States.* Vol. 88, pp. 10860-10864, ISSN: 0027-8424

Nair, U.; Barstsch, H. & Nair, J. (2007) Lipid peroxidation-induced DNA damage in cancer-prone inflammatory diseases: a review of published adduct types and levels in humans. *Free Radical in Biology & Medicine.* Vol. 43, pp. 1109-1120, ISSN: 0891-5849

Navarro, A. & Boveris, A. (2004). Rat brain and liver mitochondria develop oxidative stress and lose enzymatic activities on aging. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, Vol. 287, pp. 1244-1249, ISSN: 0363-6119

Navarro, A.; Gomez, C.; Sanchez-Pino, MJ.; Gonzalez, H.;, Bandez, MJ.; Boveris, AD.; Boveris, A. (2005) Vitamin E at high doses improves survival, neurological performance, and brain mitochondrial function in aging male mice. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, Vol. 289, pp. 1392–1399, ISSN: 0363-6119

Navarro, A.; Boveris, A. (2007) The mitochondrial energy transduction system and the aging process. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, Vol. 292, pp. 670-686, ISSN: 0363-6119

Navarro, A.; Lopez-Cepero, JM.; Bandez, MJ.; Sanchez-Pino, MJ.; Gomez, C.; Cadenas, E.; Boveris, A. (2008) Hippocampal mitochondrial dysfunction in rat aging. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, Vol. 294, pp. 501-509, ISSN: 0363-6119

Navarro, A. & Boveris, A. (2009). Brain mitochondrial dysfunction and oxidative damage in Parkinson's disease. *Journal of Bioenergetics and Biomembranes,* Vol. 41, pp. 517-521, ISSN: 0145-479X

Navarro, A.; Boveris, A.; Bández, M.J.; Sánchez-Pino, M.J.; Gómez, C.; Muntane, G. & Ferrer, I. (2009). Human brain cortex: mitochondrial oxidative damage and adaptive response in Parkinson's disease and in dementia with Lewy bodies. *Free Radicals in Biology and Medicine*, Vol. 46, pp. 1574-1580, ISSN: 0891-5849

Navarro, A.; Bández, M.; Gómez, C.; Sánchez-Pino, M.; Repetto, M.G. & Boveris, A. (2010). Effects of rotenone and pyridaben on complex I electron transfer and on mitochondrial

nitric oxide synthase functional activity. *Journal of Bioenergetics and Biomembranes*, Vol. 42, pp. 405-412, ISSN: 0145-479X

Ossani, G.; Dalghi, M. & Repetto, M. (2007) Oxidative damage and lipid peroxidation in the kidney of choline-defficient rats. *Frontiers in Bioscience.* Vol. 12, pp. 1174-1183, ISSN:1093-9946

Poyer, J. & McCay, P. (1971) Reduced triphosphopyridine nucleotide oxidase-catalyzed alterations of membrane phospholipids. Dependence on Fe^{3+}. *The Journal of Biological Chemistry.* Vol. 246, pp. 263-269, ISSN: 0021-9258

Rachmilewitz, D.; Stamler, J.; Karmeli, F.; Mollins, M.; Singel, D.; Loscalo, J.; Xavier, R. & Podolsky, D. (1993) Peroxynitrite induced rat colitis-a new model of colonic inflammation. *Gastroenterology.* Vol. 105. pp. 1681-1688, ISSN: 0016-5085

Repetto, M.; Reides, C.; Gomez Carretero, M.; Costa, M.; Griemberg, G., & Llesuy S. (1996) Oxidative Stress in Erythrocytes of HIV infected patients. *Clinica Chimica Acta.* Vol. 255, pp. 107-117, ISSN: 0009-8981

Repetto, M.G.; Reides, C.; Evelson, P.; Kohan, S.; Lustig, E.S. de & Llesuy, S. (1999). Peripheral markers of oxidative stress in probable Alzheimer patients. *European Journal of Clinical Investigation,* Vol. 29, pp. 643-649, ISSN: 0014-2972

Repetto, M.; María, A.; Giordano, O.; Guzmán,J.; Guerreiro, E. & Llesuy, S. (2003) Protective effect of Artemisia douglasiana Besser extracts on ethanol induced oxidative stress in gastric mucosal injury. *Journal of Pharmacy and Pharmacology.* Vol. 55, pp. 551-557, ISSN: 0022-3573

Repetto, M.G. (2008). Clinical use of chemiluminescence assays for the determination of systemic oxidative stress. In: Popov, I.; Lewin, G. (ed.), *Handbook of chemiluminescent methods in oxidative stress assessment*. Transworld Research Network: Kerala, India; pp. 163-194, ISBN: 978-81-7895-334-2

Repetto, M.G. & Ossani, G. (2008) Sequential histopathological and oxidative damage in different organs in choline deficient rats. In: Álvarez, S.; Evelson P. (ed.), *Free Radical Pathophysiology*. Transworld Research Network: Kerala, India; pp. 433-450, ISBN: 978-81-7895-311-3

Repetto, M.G.; Ferrarotti, N.F. & Boveris, A. (2010a) The involvement of transition metal ions on iron- dependent lipid peroxidation. *Archives of Toxicology.* Vol. 84, pp. 255-262, ISSN: 0340-5761

Repetto, M.; Ossani, G.; Monserrat, A. & Boveris, A. (2010b) Oxidative damage: The biochemical mechanism of cellular injury and necrosis in choline deficiency. *Experimental and Molecular Pathology.* Vol. 88, pp. 143-149. ISSN: 0014-4800.

Repetto, M. & Boveris, A. (2010) Bioactivity of sesquiterpenes: novel compounds that protect from alcohol-induced gastric mucosal lesions and oxidative damage. *Mini Reviews in Medicinal Chemistry.* Vol. 10, pp. 615-623. ISSN: 1389-5575

Repetto, M.G. & Boveris A. (2012). Transition metals: bioinorganic and redox reactions in biological systems. In: *Transition metals: uses and characteristics*. Nova Science Publishers Inc (ed.): New York, USA. pp. 349-370., ISBN: 978-1-61761-110-0

Sayre, L.M.; Perry, G. & Smith, M.A. (1999) In situ methods for detection and localization of markers of oxidative stress: application in neurodegenerative disorders. *Methods in Enzymology.* Vol. 309, pp. 133–152, ISSN: 0076-6879

Sayre, L.M.; Sha, W.; Xu, G.; Kaur, K.; Nadkarni, D.; Subbanagounder, G. & Salomon, R.G. (1996) Immunochemical evidence supporting 2-pentylpyrrole formation on proteins exposed to 4-hydroxy-2-nonenal. *Chemical Research in Toxicology.* Vol. 9, pp. 1194–1201, ISSN: 0893-228X

Schapira, A.H. (2008) Mitochondria in the aetiology and pathogenesis of Parkinson's disease. *The Lancet Neurology.* Vol. 7, pp. 97–109, ISSN:⊚1474-4422

Schapira, A.H.; Cooper, J.M.; Dexter, D.; Clark, J.B.; Jenner, P. & Marsden, C.D. (1990) Mitochondrial complex I deficiency in Parkinson's disease. *Journal of Neurochemistry*. Vol. 54, pp. 823–827, ISSN: 0022-3042

Schapira, AH.; Mann, V.M.; Cooper,. JM.; Dexter, D.; Daniel, S.E.; Jenner, P.; Clark, J.B. & Marsden, C.D. (1990) Anatomic and disease specificity of NADH CoQ1 reductase (complex I) deficiency in Parkinson's disease. *Journal of Neurochemistry*. Vol. 55, pp. 2142–2145, ISSN: 0022-3042

Serra, J.A.; Domínguez, R.O.; Marschoff, E.R.; Guareschi E.M.; Famulari, A.L. & Boveris, A. (2009) Systemic oxidative stress associated with the neurological diseases of aging. *Neurochemical Research,* Vol. 34, pp. 2122–2132, ISSN/ISBN: 03643190

Shires, T. (1975) Inhibition by lipoperoxidation of amino acid incorporation by rough microsomal membranes *in vitro* and its partial reversibility. *Archives of Biochemistry and Biobiophys.* Vol. 171, pp. 695-707. ISSN: 0003-9861

Sies, H. (1991a) Oxidative stress: from basic research to clinical application. *American Journal of Medicine.* Vol. 91, pp. 31-38, ISSN: 0002-9343

Sies, H. (1991b). Role of reactive oxygen species in biological processes. *Wiener Klinische Wochenschrift*, Vol. 69, pp. 965–968, ISSN: 1613-7671

Valdez, L.B.; Zaobornij, T.; Bombicino, S.; Iglesias, D.E.; Boveris, A.; Donato, M.; D'Annunzio, V.; Buchholz, B. & Gelpi, R.A. (2011) Complex I syndrome in myocardial stunning and the effect of adenosine. *Free Radical in Biology & Medicine.* Vol. 51, pp. 1203-1212, ISSN: 0891-5849

Verstraeten, S.; Nogueira, L., Schreier, S. & Oteiza, P. (1997) Effect of trivalent metal ions on phase separation and membrane lipid packing: role in lipid peroxidation. *Archives of Biochemistry and Biobiophys.* Vol. 338, pp. 121-127, ISSN: 0003-9861

Videla, L.; Fraga, C.; Koch, O. & Boveris, A. (1983) Chemiluminescence of the in situ rat liver alter acute ethanol intoxication-effect of (+)-cyanidanol-3. *Biochemical Pharmacology.* Vol. 32, pp. 2822-2825,⊚ISSN: 0006-2952

Uchida, K.; Shiraishi, M.; Naito, Y.; Tori, Y.; Nakamura, Y. & Osawa, T. (1999) Activation of stress signaling pathways by the end product of lipid peroxidation, 4-hydroxy-2-nonenal is a potential inducer of intracellular peroxide production. *Journal of Biological Chemistry.* Vol. 274, pp. 2234–2242. ISSN: 0021-9258

Uchida, K. (2003) 4-Hydroxy-2-nonenal: A product and mediator of oxidative stress. *Progress in Lipid Research*. Vol. 42, pp. 318–343. ISSN: 0163-7827

Yin, H.; Xu, L. & Porter, N.A. (2011) Free radical lipid peroxidation: mechanisms and analysis. *Chemical Reviews.* 111: 5944-5972. ISSN: 0009-2665

IntechOpen