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Review

Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions

Angel Catalá*,[1]

Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA-CCT La Plata-CONICET), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CC 16, Sucursal 4, 1900 La Plata, Argentina

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ABSTRACT

Polyunsaturated fatty acids (PUFAs) and their metabolites have a variety of physiological roles including: energy provision, membrane structure, cell signaling and regulation of gene expression. Lipids containing polyunsaturated fatty acids are susceptible to free radical-initiated oxidation and can participate in chain reactions that increase damage to biomolecules. Lipid peroxidation, which leads to lipid hydroperoxide formation often, occurs in response to oxidative stress. Hydroperoxides are usually reduced to their corresponding alcohols by glutathione peroxidases. However, these enzymes are decreased in certain diseases resulting in a temporary increase of lipid hydroperoxides that favors their degradation into several compounds, including hydroxy-alkenals. The best known of these are: 4-hydroxy-2-nonenal (4-HNE) and 4-hydroxy-2-hexenal (4-HHE), which derive from lipid peroxidation of *n*-6 and *n*-3 fatty acids, respectively. Compared to free radicals, these aldehydes are relatively stable and can diffuse within or even escape from the cell and attack targets far from the site of the original event. These aldehydes exhibit great reactivity with biomolecules, such as proteins, DNA, and phospholipids, generating a variety of intra and intermolecular covalent adducts. At the membrane level, proteins and amino lipids can be covalently modified by lipid peroxidation products (hydoxy-alkenals). These aldehydes can also act as bioactive molecules in physiological and/or pathological conditions. In addition this review is intended to provide an appropriate synopsis of identified effects of hydroxy-alkenals and oxidized phospholipids on cell signaling, from their intracellular production, to their action as intracellular messenger, up to their influence on transcription factors and gene expression.

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Contents

Abbreviations: ANT, adenine nucleotide translocase; C18:1 *n*-9, oleic acid; C18:2 *n*-6, linoleic acid; C18:3 *n*-3, α-linolenic acid; C20:4 *n*-6, arachidonic acid; C20:5 *n*-3, eicosapentaenoic acid; C22:5 *n*-3, docosapentaenoic acid; C22:6 *n*-3, docosahexaenoic acid; HHE, 4-hydroxy-2-hexenal; HNE, 4-hydroxy-2-nonenal; L•, lipid radical; LH, lipid molecules; LO•, lipid alkoxyl radical; LOO•, lipid peroxyl radical; LOOH, lipid hydroperoxide; LPC, lysophosphatidylcholine; MS, mass spectrometry; MDA, malondialdehyde; MS/MS, tandem mass spectrometry; NR, nuclear receptors; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; Pls, phospholipids; PPARs, peroxisome proliferator-activated receptors; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; Tgs, triglycerides; UCPs, uncoupling proteins.

* Tel.: +54 221 4240967/4257430/4257291x105; fax: +54 221 425 4642.
E-mail address: catala@inifta.unlp.edu.ar.
[1] The author is Member of Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET) Argentina.

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1. The behavior of polyunsaturated fatty acids in nature

Polyunsaturated fatty acids (PUFAs) and their metabolites have a diversity of physiological roles including: energy provision, membrane structure, cell signaling and regulation of gene expression. Requirements for PUFAs cannot be met by the novo metabolic processes within mammalian tissues. Animals are absolutely dependent on plants for providing the two major precursors of the *n*-6 and *n*-3 fatty acids, C18:2 *n*-6; linoleic and C18:3 *n*-3; α-linolenic acids. In animal tissues, these precursors are transformed to fatty acids containing three to six double bonds (Poulos, 1995). In animals, some of the daily requirements in long chain PUFAs are satisfied from the diet. However, most of the long chain PUFAs found in animal tissues are derived from the biosynthetic pathway involving elongations and desaturations for conversion of essential fatty acid precursors (C18:2 *n*-6 and C18:3 *n*-3) provided by plants to their respective C20- and C22-carbon polyenoic products. Unsaturation of a fatty acid chain is a major determinant of the melting temperature of triglycerides (TGs) and the fluidity of biological membranes that are made of a bilayer of phospholipids (PLs). Thus, fatty acid desaturases that introduce a double bond into a long-chain fatty acid are conserved across kingdoms (Enoch et al., 1976; Nakamura and Nara, 2004).

Long chain polyunsaturated fatty acids such as arachidonic acid (C20:4 *n*-6) and docosahexaenoic acid (C22:6 *n*-3) play important roles in a variety of biological functions (Spector, 1999). Polyunsaturated fatty acids (PUFAs) are essential components in higher eukaryotes that confer fluidity, flexibility and selective permeability to cellular membranes. PUFAs affect many cellular and physiological processes in both plants and animals. Animal biosynthesis of high polyunsaturated fatty acids from linoleic, α-linolenic and oleic acids is mainly modulated by the delta6 and delta5 desaturases through dietary and hormonal stimulated mechanisms (Brenner, 2003).

2. Lipids in biological membranes

Lipid molecules make up between 30 and 80% of biological membranes by mass. The remainder is protein (20–60%) and sometimes carbohydrate (0–10%). The protein molecules are very much larger than the lipid molecules so although there may be similar masses of each, there are many more lipid molecules. The proteins are located such that they either entirely go through the membrane (transmembrane proteins) or just one of the two bilayers in which case they may be on the inside or outside the cell.

According to the Fluid Mosaic Model, a biological membrane is a two-dimensional fluid of oriented proteins and lipids. The lipid bilayer is the basic structure of all cell and organelle membranes. Cell membranes are dynamic, fluid structures, and most of their molecules are able to move in the plane of the membrane. Fluidity is the quality of ease of movement and represents the reciprocal value of membrane viscosity. Fluid properties of biological membranes are indispensable for numerous cell functions. Even minor changes in membrane fluidity may cause abnormal function and pathological processes. Fluid properties are determined mainly by the presence of polyunsaturated fatty acids in phospholipids molecules located in both sides of the lipid bilayer.

3. The lipid peroxidation process

Oxidative stress that occurs in the cells, because an imbalance between the prooxidant/antioxidant systems, causes injure to biomolecules such as nucleic acids, proteins, structural carbohydrates, and lipids (Sies and Cadenas, 1985). Among these targets, the peroxidation of lipids is basically damaging because the formation of lipid peroxidation products leads to spread of free radical reactions. The general process of lipid peroxidation consists of three stages: initiation, propagation, and termination (Catalá, 2006). The initiation phase of lipid peroxidation includes hydrogen atom abstraction. Several species can abstract the first hydrogen atom and include the radicals: hydroxyl ($^{\bullet}OH$), alkoxyl ($RO^{\bullet}$), peroxyl ($ROO^{\bullet}$), and possibly $HO_2^{\bullet}$ but not H_2O_2 or $O_2-^{\bullet}$ (Gutteridge, 1988).

The membrane lipids, mainly phospholipids, containing polyunsaturated fatty acids are predominantly susceptible to peroxidation because abstraction from a methylene ($-CH_2-$) group of a hydrogen atom, which contains only one electron, leaves at the back an unpaired electron on the carbon, $-^{\bullet}CH-$. The presence of a double bond in the fatty acid weakens the C–H bonds on the carbon atom nearby to the double bond and thus facilitates $H^{\bullet}$ subtraction. The initial reaction of $^{\bullet}OH$ with polyunsaturated fatty acids produces a lipid radical ($L^{\bullet}$), which in turn reacts with molecular oxygen to form a lipid peroxyl radical ($LOO^{\bullet}$). The $LOO^{\bullet}$ can abstract hydrogen from a adjacent fatty acid to produce a lipid hydroperoxide (LOOH) and a second lipid radical (Catalá, 2006). The LOOH formed can suffer reductive cleavage by reduced metals, such as Fe^{2+}, producing lipid alkoxyl radical ($LO^{\bullet}$). Both alkoxyl and peroxyl radicals stimulate the chain reaction of lipid peroxidation by abstracting additional hydrogen atoms (Buettner, 1993). Peroxidation of lipids can disturb the assembly of the membrane, causing changes in fluidity and permeability, alterations of ion transport and inhibition of metabolic processes (Nigam and Schewe, 2000). Injure to mitochondria induced by lipid peroxidation can direct to further ROS generation (Green and Reed, 1998). In addition, LOOH can break down, frequently in the presence of reduced metals or ascorbate, to reactive aldehyde products, including malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE), 4-hydroxy-2-hexenal (4-HHE) and acrolein (Esterbauer et al., 1991; Parola et al., 1999; Uchida, 1999; Kehrer and Biswal, 2000; Lee et al., 2001) (Fig. 1). In fact, the oxidation products have already detected by a simple GC–MS method, and given a series of hydroxyl-alkenals generated from low-density lipoprotein undergoing the oxidation by copper ion (Spiteller and Spiteller, 2000a,b). In addition, some recent publications also reported some novel results about the oxidative products derived from the oxidation of erythrocyte membrane in vitro (Liu and Shan, 2006).

4. A great variety of compounds are formed during lipid peroxidation of membrane phospholipids

Lipid peroxidation is one of the major outcomes of free radical-mediated injury to tissue. Peroxidation of fatty acyl groups occurs

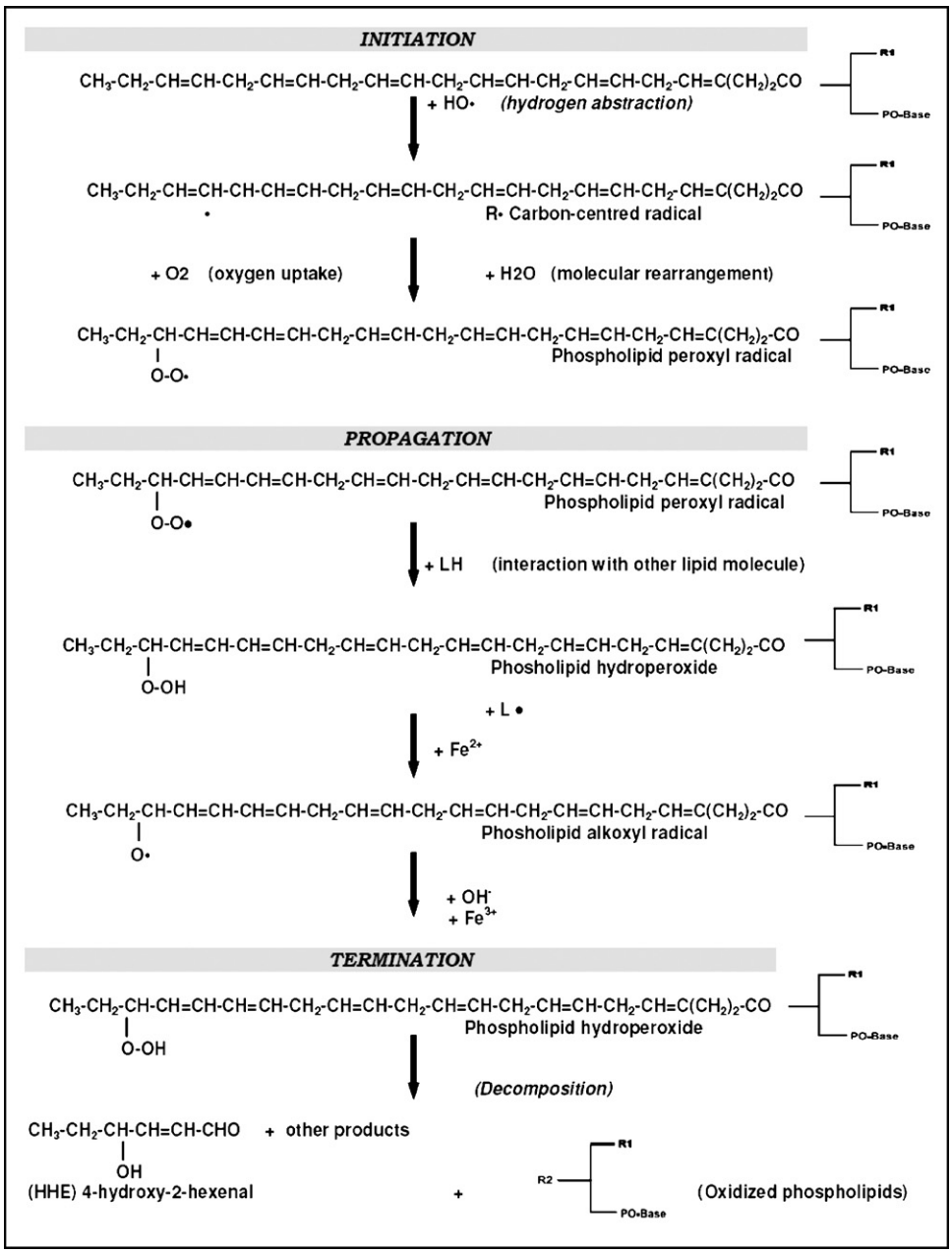

Fig. 1. Chemical diagram of the steps in lipid peroxidation of phospholipids containing docosahexaenoic acid (22:6 *n*-3), R1 = fatty acid, R2 = fragmentation products of fatty acid oxidation.

mostly in membrane phospholipids. Peroxidation of lipids can greatly alter the physicochemical properties of membrane lipid bilayers, resulting in severe cellular dysfunction. In addition, a variety of lipid byproducts are produced as a result of lipid peroxidation (Table 1), some of which can exert adverse and/or beneficial biological effects.

Several in vitro markers of oxidative stress are available, but most are of limited value in vivo because they lack sensitivity and/or specificity or require invasive methods. Isoprostanes (IsoPs) are prostaglandin (PG)-like compounds produced in vivo independently of cyclooxygenase enzymes, primarily by free radical-induced peroxidation of arachidonic acid. F(2)-IsoPs are a group of 64 compounds isomeric in structure to cyclooxygenase-derived PGF(2 alpha). Other products of the IsoP pathway are also formed in vivo by rearrangement of labile PGH(2)-like IsoP intermediates, including E(2)-, D(2)-IsoPs, cyclopentenone-A(2)-, J(2)-IsoPs and highly reactive acyclic-ketoaldehydes (isoketals). Measurement of F(2)-IsoPs is the most reliable approach to assess oxidative stress status in vivo, providing an important tool to explore the role of oxidative stress in the pathogenesis of human disease. Moreover, F(2)-IsoPs and other products of the IsoP pathway exert potent biological actions both via receptor-dependent and independent mechanisms and therefore may be pathophysiological mediators of disease. The formation of highly reactive cyclopentenone isoprostane compounds (A3/J3-isoprostanes) in vivo from eicosapentaenoic acid has been described recently (Brooks et al., 2008). Oxidation of docosahexaenoic acid, an abundant unsaturated fatty acid in the central nervous system, results in the formation of IsoP-like compounds, termed neuroprostanes.

Table 1
Compounds generated during lipid peroxidation of membrane phospholipids.

Fatty acid peroxidized	Hydroxy alkenals	Isoprostanes	Neuroprostanes	Oxidized phospholipids
C18:2 *n*-6	4-Hydroxy-2-nonenal (4-HNE)	F(2) isoPs 64 isomers		When the *sn*-2 fatty acids of phospholipids are oxidized, several different types of oxidative products are formed. These include phospholipids containing fatty acid oxidation products (oxidized phospholipids), lysophospholipids, and fragmentation products of fatty acid oxidation. Some of these products, lysophosphatidic acid or lysophospholipids, can be formed both enzymatically and nonenzymatically.
C20:4 *n*-6				
C18:3 *n*-3				
C20:5 *n*-3	4-Hydroxy-2-hexenal (HHE)	A3/J3-isoPs		
C22:5 *n*-3				
C22:6 *n*-3			(F(4)-NPs)	

5. The lipid peroxidation process generates oxidized phospholipids

Biomembranes contain different phospholipid classes (head group heterogeneity), subclasses (acyl, alkyl chains) and species (chain length and unsaturation degree). PC is the main phospholipid in all mammalian cells (40–50%) and thus, most oxidized phospholipids detected in mammalian tissues have the choline moiety. However, recently oxidized PE has been found in the retina, a tissue that contains very high amounts of ethanolamine lipids (Gugiu et al., 2006) enriched in docosahexaenoic acid (Guajardo et al., 2006). In addition, there are also reports providing evidence for the presence of oxidized PS in the surface of apoptotic cells (Matsura et al., 2005).

In eukaryotic phospholipids, the *sn*-1 position is either linked to an acyl residue via an ester bond or an alkyl residue via an ether bond, whereas the *sn*-2 position almost exclusively contains acyl residues. The highly oxidizable (*n*-3 and *n*-6 polyunsaturated fatty acids) are preferably bound to the *sn*-2 position of glycerophospholipids. Thus, most of the oxidized phospholipids are modified at this position. At the *sn*-1 position of glycerol a saturated fatty acid is usually bound. By contrast, plasmalogens (alkenylacylglycerophospholipids) contain a vinyl ether bond in position *sn*-1 and, as a consequence, they are also susceptible to oxidative modifications at the *sn*-1 position.

The differences in chemical structure of different types of phospholipids determine the physical properties of the membrane. PC tends to form bilayers with little curvature, while PE imposes a negative curvature on these lipid bilayers (Janes, 1996). Conversely, introduction of the micelle-forming LPC into a PC membrane results in a positive curvature. In addition to the polar head groups the polarity, length and unsaturation of the phospholipid acyl chains have also an impact on physical membrane properties. Thus, phospholipid oxidation products (Fig. 2) are very likely to modify the properties of biological membranes, because their polarity and shape may differ significantly from the structures of their parent molecules. Thus, they may alter lipid–lipid and lipid–protein interactions and, as a consequence, also membrane protein functions.

When the *sn*-2 fatty acids of phospholipids are oxidized by radicals, several different types of oxidative products are formed. These include phospholipids containing fatty acid oxidation products (usually referred to as oxidized phospholipids), lysophospholipids, and fragmentation products of fatty acid oxidation. Some of these products, lysophosphatidic acid or lysophospholipids, can be formed both enzymatically and nonenzymatically. Work from many laboratories have identified a variety of phospholipid oxidation products and confirmed bioactivities of these phospholipids on vascular wall cells, leukocytes, and platelets. Because of the large number of fatty acid oxidation products that have been identified, it is almost certain that many other bioactive oxidized phospholipids will be discovered. There are several important issues that confront investigators interested in bioactive phospholipids, including preparation, identification, quantification, and biological testing.

Phospholipid changes induced by oxidative reactions yield a huge number of structurally different oxidation products, which difficult their isolation and characterization. Mass spectrometry (MS) and tandem mass spectrometry (MS/MS) using the soft ionization methods (electrospray and matrix-assisted laser desorption ionization) is one of the finest approaches for the study of oxidized phospholipids. Product ions in tandem mass spectra of oxidized phospholipids, allow identifying changes in the fatty acyl chain and specific features such as presence of new functional groups in the molecule and their location along the fatty acyl chain (Domingues et al., 2008).

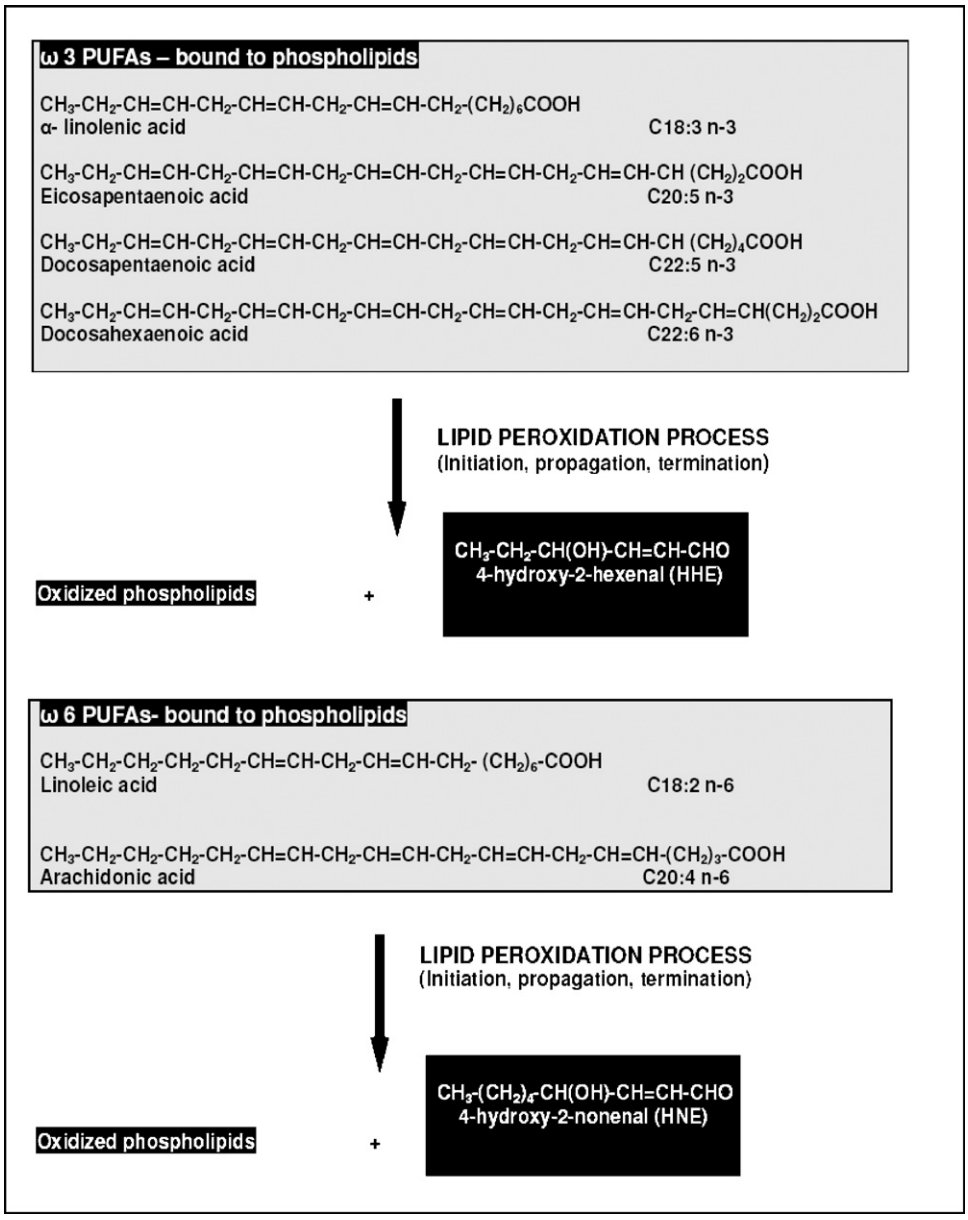

Fig. 2. Schematic diagram of reactive hydroxy-alkenals generated during lipid peroxidation of *n*-3 and *n*-6 polyunsaturated fatty acids.

6. Lipid peroxidation of *n*-3 and *n*-6 polyunsaturated fatty acids generates hydroxy-alkenals

Lipids containing polyunsaturated fatty acids are susceptible to free radical–initiated oxidation and can contribute in chain reactions that amplify damage to biomolecules as described above. Lipid peroxidation often occurs in response to oxidative stress, and a great diversity of aldehydes is formed when lipid hydroperoxides break down in biological systems. Some of these aldehydes are highly reactive and may be considered as second toxic messengers, which disseminate and augment initial free radical events. The aldehydes most intensively studied up to now are 4-hydroxy-2-nonenal, 4-hydroxy-2-hexenal, and malondialdehyde. 4-Hydroxy-2-nonenal (HNE) is known to be the main aldehyde formed during lipid peroxidation of *n*-6 polyunsaturated fatty acids, such as linoleic acid C18:2 *n*-6 and arachidonic acid C20:4 *n*-6.

On the other hand, lipid peroxidation of *n*-3 polyunsaturated fatty acids such as α-linolenic acid C18:3 *n*-3 and docosahexaenoic acid C22:6 *n*-3 generates a closely related compound, 4-hydroxy-2-hexenal (HHE), which is a potential mediator of mitochondrial permeability transition (Kristal et al., 1996). 4-Hydroxy-2-alkenals represent the most prominent aldehyde substances generated during lipid peroxidation. Among them, 4-hydroxy-2-nonenal (HNE) is known to be the main aldehyde formed during lipid peroxidation of *n*-6 polyunsaturated fatty acids, such as linoleic acid and arachidonic acid (Fig. 2).

HNE was identified three decades ago as a cytotoxic aldehyde formed during the NADPH–Fe^{2+} induced peroxidation of liver microsomal lipids (Benedetti et al., 1980). Since then, a vast number of reports have been available, which sustain a function for this compound in a diversity of disease processes. HNE is considered as an indicator of oxidative stress and a probable contributing agent of several diseases.

7. Reactive hydroxy-alkenals modify protein structure

The oxidation of proteins can lead to hydroxylation of aromatic groups and aliphatic amino acid side chains, nitration of aromatic

amino acid residues, nitrosylation of sulfhydryl groups, sulfoxidation of methionine residues, chlorination of aromatic groups and primary amino groups, and to conversion of some amino acid residues to carbonyl derivatives. Oxidation can lead also to cleavage of the polypeptide chain and to formation of cross-linked protein aggregates. Furthermore, functional groups of proteins can react with oxidation products of polyunsaturated fatty acids and with carbohydrate derivatives (glycation=glycoxidation) to produce inactive derivatives.

Because the generation of carbonyl derivatives occurs by many different mechanisms, the level of carbonyl groups in proteins is widely used as a marker of oxidative protein damage (Guajardo et al., 2006). The level of oxidized proteins increases with aging and in several diseases. However, the accumulation of oxidized protein is a complex function of the rates of ROS formation, antioxidant levels, and the capacity to eliminate oxidized forms of proteins by proteolysis (Stadtman and Levine, 2003).

It is well established that end-products of lipid peroxidation, such as malondialdehyde (MDA), 4-hydroxynonenal (HNE), and 4-hydroxyhexenal (HHE), cause protein damage by reactions with lysine amino groups, cysteine sulfhydryl groups, and histidine imidazole groups.

In a very careful study, Liu and Wang have analyzed the ability of unsaturated fatty acid methyl esters to modify bovine serum albumin (BSA) in the presence of a metal-catalyzed oxidation system [ascorbic acid/Fe^{2+}/O_2]. The fatty acid esters studied include methyl linoleate (LA) and methyl arachidonate (AA) as *n*-6 PUFA esters, and methyl linolenate (LN) and methyl docosahexaenoate (DHA) as *n*-3 PUFA esters.

There is abundant evidence that lipid peroxidation products (radicals, lipid hydroperoxides, and reactive aldehyde derivatives) are able to modify proteins both in vivo and in vitro (Esterbauer et al., 1991; Calingasan et al., 1999; Tsai et al., 1998; Musatov et al., 2002; Wataya et al., 2002; Metz et al., 2003). Liu and Wang have directly analyzed, under comparable experimental conditions, the oxidative stabilities of LA, LN, AA, and DHA in an ascorbic acid/Fe^{2+}/O_2 metal-catalyzed oxidation system, and the abilities of these lipids to promote metal-catalyzed formation of protein carbonyl derivatives of BSA and to produce high-molecular-weight proteins (Liu and Wanga, 2005). Metal-catalyzed oxidation of PUFAs leads to formation of lipid hydroperoxides and it is also believed that such hydroperoxides are highly susceptible to one-electron reduction (e.g. by Fe^{2+}) to give further oxygen-centered radicals that can react directly with the side chains of some amino acid residues in proteins (Kato et al., 1999).

8. Proteins and amino lipids can be covalently modified by lipid peroxidation products

Oxidative stress is implicated in many pathophysiological states, such as, atherosclerosis, diabetes, aging and neurodegenerative disorders. Reactive oxygen species initiate injurious effects on diverse biological components, particularly polyunsaturated fatty acids, which lead to lipid hydroperoxide formation (Terrasa et al., 2008). Those hydroperoxides are normally reduced to their corresponding alcohols by glutathione peroxidases (Bryant and Bailey, 1980). Nevertheless, glutathione peroxidase activities have been shown to be decreased in aging (Véricel et al., 1992) and diabetes (Muruganandam et al., 1992; Véricel et al., 2004), resulting in a temporary accumulation of lipid hydroperoxides that favors their degradation into several compounds, including hydroxyalkenals. The best known of these are: 4-hydroxy-2-nonenal (4-HNE) (Esterbauer et al., 1991) and 4-hydroxy-2-hexenal (4-HHE) (Van Kuijk et al., 1990), which derive from *n*-6 and *n*-3 fatty acid peroxidation, respectively.

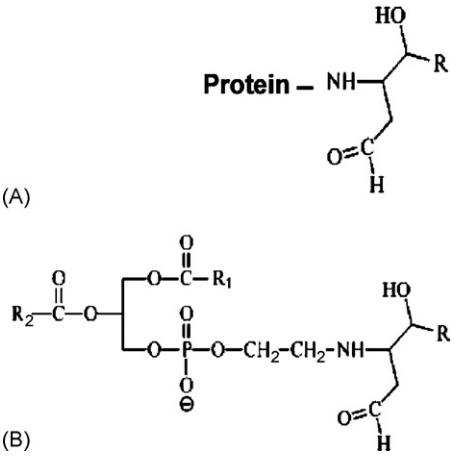

Fig. 3. Scheme of alkenal Michael adducts. (A) protein–alkenal adducts and (B) ethanolamine phospholipid–alkenal adducts.

During lipid peroxidation, biomolecules such as proteins or amino lipids can be covalently modified by lipid decomposition products (Fig. 4). For the case of aliphatic aldehydes (alkanals) such as 1-hexanal or 1-nonanal, the N^{ε}-amino groups of the lysine residues in proteins can be modified through the formation of a Schiff base. α, β-unsaturated aldehydes (alkenals) such as acrolein or 4-hydroxy-2-nonenal react with lysine, cysteine, and histidine through a Michael-type addition (Esterbauer et al., 1991; Uchida et al., 1998) (Fig. 3A). Conversely, lipid hydroperoxide might covalently react with protein without serious decomposition of its structure (Fruebis et al., 1992). Keto fatty acid (Kühn et al., 1991), which is one of the products by lipoxygenase reaction, can also react with proteins and amino acids as previously suggested (Fukuzawa et al., 1985; Bull et al., 1996; Blackburn et al., 1997). In addition, the pyrrole compounds from long chain epoxides and lysine have been identified (Hidalgo and Zamora, 1995). However, the mechanism of lipid hydroperoxide-derived protein modification is not so clear. Evidence for in situ ethanolamine phospholipid adducts with hydroxy-alkenals has been recently described (Bacot et al., 2007). Ethanolamine phospholipid–alkenal adducts are shown in Fig. 3B.

9. The lipid peroxidation process damage membrane structure modifying its physical properties

Free radicals, formed via diverse mechanisms, induce peroxidation of membrane lipids. This process is of great significance since it modifies the physical properties of the membranes, including its permeability to diverse solutes and the packing of lipids and proteins in the membranes, which in turn, influences the membranes' function. Therefore, much research has been dedicated to the understanding of the factors that rule lipid peroxidation, including the composition and properties of the membranes and the inducer of peroxidation.

Lipid peroxidation is a degenerative process that affects unsaturated membrane lipids under conditions of oxidative stress (Girotti, 1998). This complex process is believed to contribute to human aging and disease by disrupting the structural conformation, the packing of lipid components and, ultimately, the function of biological membranes (Catalá, 2007). The polyunsaturated fatty acids of membrane phospholipids are particularly susceptible to peroxidation and undergo significant modifications, including the rearrangement or loss of double bonds and, in some cases, the reductive degradation of lipid acyl side chains (Leibowitz and Johnson, 1971; Gardner, 1975; Buege and Aust, 1978). Lipid hydroperoxides, prominent intermediates of peroxida-

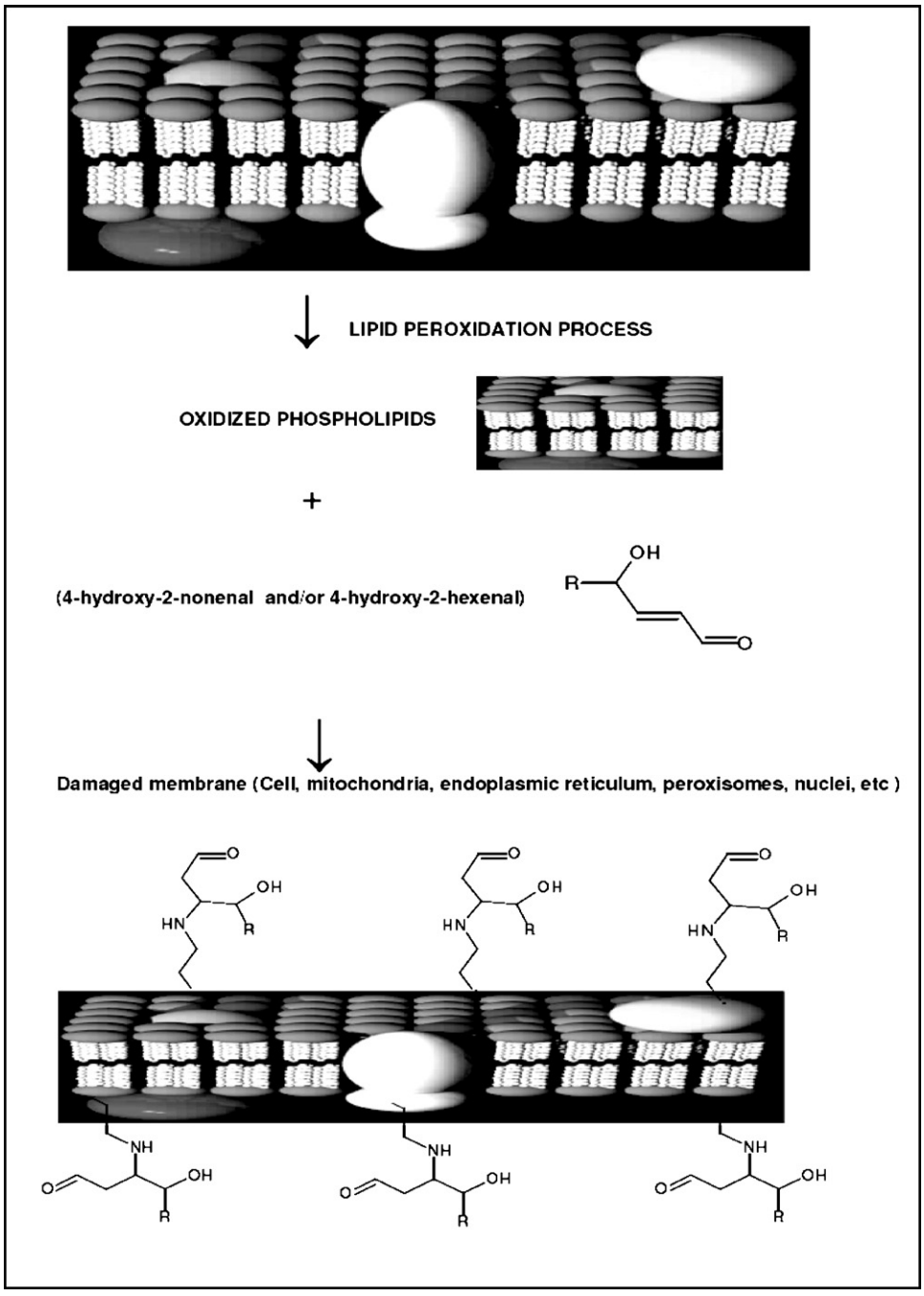

Fig. 4. Covalent modifications of amino-phospholipids and proteins by hydroxy-alkenals during lipid peroxidation of biological membranes.

tive reactions, also accumulate in the bilayer and further contribute to changes in the structural organization and packing of membrane lipid components (Girotti, 1998). Many of the biophysical consequences of these structural modifications have been well characterized and include changes in membrane fluidity (Petrescu et al., 2001; Chatterjee and Agarwal, 1988; Borchman et al., 1992), increased membrane permeability (Goldstein and Weissmann, 1977; Mandal and Chatterjee, 1980; Kunimoto et al., 1981), alteration of membrane thermotropic phase properties (Chia et al., 1984; Van Duijn et al., 1984; Verma, 1986), and changes in membrane protein activity (Yukawa et al., 1983; Dinis et al., 1993; Goel et al., 1993; Kourie, 1998; Mattson, 1998; Mattson et al., 1999; Sevanian and Ursini, 2000).

In view of the complexity of biological membranes, a large amount of work was devoted to the latter issues in basic model systems, frequently lipid vesicles (liposomes). Although peroxidation in model membranes may be very different from peroxidation in biological membranes, the results obtained in model membranes may be used to advance our understanding of issues that cannot be analyzed in biological membranes.

The damaging effects of lipid peroxidation on membrane structure and function are well documented (Gavazza and Catalá, 2003; Marmunti and Catalá, 1998; Girotti, 1998; Guajardo et al., 1999). Among cellular macromolecules, polyunsaturated fatty acids (PUFAs) exhibit the highest sensitivity to oxidative damage. Many studies have shown that free radical damage and lipid peroxida-

tion increase as a function of the degree of unsaturation of the fatty acids present in the phospholipids of biological membranes. In this regard it has been demonstrated that the number of bis-allylic positions contained in the cellular lipids of intact cells determines their susceptibility, i.e. oxidizability, to free radical mediated peroxidative events (Wagner et al., 1994). Membrane phospholipids are particularly susceptible to oxidation not only because of their highly polyunsaturated fatty acid content but also because of their association in the cell membrane with nonenzymatic and enzymatic systems capable of generating prooxidative-free radical species. There are two broad outcomes to lipid peroxidation: structural damage to membranes and generation of secondary products. Membrane damage derives from the generation of fragmented fatty acyl chains, lipid–lipid cross-links, lipid–protein cross-links and endocyclization to produce isoprostanes and neuroprostanes. These processes combine to produce changes in the biophysical properties of membranes that can have profound effects on the activity of membrane-bound proteins. The consequence of peroxidation of unsaturated fatty acids is severe: damage of membranes function, enzymatic inactivation, toxic effects on the cellular division, etc. (Halliwell and Gutteridge, 1990; Palmer, 1985; Aruoma, 1994).

10. Reactive hydroxy-alkenals generated during lipid peroxidation of *n*-3 and *n*-6 polyunsaturated fatty acids are bioactive molecules in physiological conditions

Aldehydic molecules generated during lipid peroxidation have been implicated as causal agents in many cellular effects. Compared to free radicals, the aldehydes are moderately stable and can disperse within or even escape from the cell and attack targets distant from the site of the original event. Because a conjugated double bond between the α and β carbons, the γ carbon of these aldehydes is electron deficient and reacts readily with nucleophilic molecules such as thiols and amines. Due to their high reactivity, these aldehydes exhibit effective biological effects, which, depending upon their concentrations, cause selective modification in cell signaling, protein and DNA damage, and cytotoxicity (review Esterbauer et al., 1991), including induction of apoptosis.

These aldehydes exhibit great reactivity with biomolecules, such as proteins, DNA, and phospholipids, generating a variety of intra and intermolecular covalent adducts. These aldehydes can also act as bioactive molecules in physiological and/or pathological conditions. They can affect and regulate, at very low and nontoxic concentration, several cell functions, including signal transduction, gene expression, cell proliferation, and, more generally, the response of the target cell(s) (Uchida, 2003).

The central nervous system is particularly vulnerable to oxidative stress because the high rate of oxygen utilization and high amount of unsaturated lipids. Thus, there has been much focus on possible roles of lipid peroxidation-derived aldehydes in contributing to neuronal dysfunction in neurodegenerative diseases associated with oxidative stress. Among such aldehydes, HNE has become the most studied cytotoxic product of lipid peroxidation (Uchida, 2003) and appears a main signaling molecule in the pathogenesis of neurodegenerative diseases (e.g. it may mediate A^{β} toxicity). While modest concentrations of HNE can induce apoptosis (Kruman et al., 1997; Ji et al., 2001), induce differentiation, damage proteasomal function (Friguet and Szweda, 1997; Ferrington and Kapphahn, 2004), and modify signal transduction, including activation of adenylate cyclase, JNK, PKC, and caspase 3, lower concentrations of HNE appear to promote cell proliferation (Awasthi et al., 2004; Uchida, 2003). As there has been no evidence to propose that HNE can exert biological activity through a noncovalent process, one must assume that the different deleterious biological activities attributed to HNE reflect its capacity to bind covalently to protein targets, such as in signaling cascades. It will be crucial to verify the nature of HNE covalent binding that results in modulating the enzymatic activity of, e.g. various protein kinases (Kutuk and Basaga, 2007).

11. 4-Hydroxynonenal regulates mitochondrial uncoupling

Mitochondria are the main intracellular producers of reactive oxygen species (ROS) in most cells and are in addition important targets for their damaging effects. Mitochondrial oxidative damage can be a main cause of a large amount of pathologies, including neurodegenerative diseases, ischaemia/reperfusion injury and inflammatory disorders (Beal et al., 1997; Esterbauer et al., 1991). Although there are several manifestations of oxidative damage to biological molecules, lipid peroxidation may be especially injurious (Esterbauer et al., 1991; Halliwell and Gutteridge, 1999). The polyunsaturated fatty acyl groups of membrane phospholipids are highly vulnerable to peroxidation by oxygen radicals, and a self-propagating chain of free radical reactions produces a variety of aldehydes, alkenals and hydroxyalkenals, such as malondialdehyde, 4-hydroxy-2-nonenal (HNE) and 4-hydroxy-2-hexenal (HHE). Many of these products are cytotoxic, probably because of their reactivity toward proteins. Hydroxynonenal is thought to be one of the most reactive and significant mediator of free-radical damage (Esterbauer et al., 1991).

As described in Sections 6 and 7, hydroxynonenal can chemically modify several amino acid residues of proteins: the sulphydryl group of cysteine, the imidazole moiety of histidine and the ε-amino group of lysine (Esterbauer et al., 1991; Uchida and Stadtman, 1992; Nadkarni and Sayre, 1995; Cohn et al., 1996). It has wide biological toxicity: inhibition of DNA and protein synthesis, inactivation of enzymes, change of low density lipoprotein and modulation of gene expression (Esterbauer et al., 1991). Mitochondrial proteins are targets of hydroxynonenal adduct formation following oxidative stress in vivo and in vitro, and hydroxynonenal inactivates the 2-oxoglutarate dehydrogenase and pyruvate dehydrogenase complexes, cytochrome *c* oxidase and NADH-linked respiration in isolated mitochondria (Humphries and Szweda, 1998; Humphries et al., 1998; Musatov et al., 2002).

Superoxide formed from oxidative stress frequently leads to peroxidation of membrane phospholipids and production of highly reactive aldehydes, particularly 4-hydroxy-2-nonenal (HNE).

Superoxide ($O_2^{\bullet -}$) and hydroperoxyl radical ($HO_2^{\bullet -}$) are generated primarily by the electron transport chain (ETC). Superoxide is dismutated to hydrogen peroxide (H_2O_2) by Mn-superoxide dismutase (MnSOD). Some superoxide inactivates enzymes (e.g. aconitase) that include iron–sulfur centers, releasing ferrous iron, which catalyzes production of hydroxyl radicals ($^{\bullet}OH$) from the H_2O_2 by the Fenton reaction. The hydroxyl and hydroperoxyl radicals can both remove hydrogen atoms from polyunsaturated fatty acyl chains (linoleic, α-linolenic, arachidonic, docosahexaenoic, others) of membrane phospholipids, generating carbon-centered fatty acyl radicals that react with oxygen to form peroxyl radicals (Catalá, 2007). These propagate lipid peroxidation cascades that produce a complex mixture of species including, reactive alkenals such as 4-hydroxy-2-nonenal (see Fig. 2).

A model for activation of mitochondrial carriers (UCPs and ANT) by superoxide, through initiation of lipid peroxidation has been proposed by (Echtay et al., 2003; Brand et al., 2004). In this model, hydroxynonenal induces mitochondrial uncoupling by specific interactions with the uncoupling proteins UCP1, UCP2 and UCP3, and with the adenine nucleotide translocase (ANT). These proteins are members of a large family of at least 35 anion carriers present in the mitochondrial inner membrane (Bouillaud et al., 2001), mild uncoupling, decreases mitochondrial production

of ROS, which can subsequently cause hydroxynonenal production (Papa and Skulachev, 1997). This suggests a negative feedback loop in which hydroxynonenal signals damage by ROS and decreases ROS production through induction of uncoupling by UCPs and ANT. Reactive alkenals activate UCPs and ANT, increasing the proton conductance of the mitochondrial inner membrane. This induced uncoupling is part of the proposed regulatory mechanism to reduce mitochondrial production of superoxide when it is too high.

The effects of a mitochondrially targeted spin trap were informative. PBN (phenylbutylnitrone) was covalently attached to triphenylphosphonium to form a mitochondrially-targeted spin trap (mitoPBN). This compound, which reacts rapidly with carbon-centered radicals but not superoxide or lipid peroxidation products, prevented activation of UCPs by superoxide but did not block activation by hydroxynonenal. This observation suggests that superoxide and lipid peroxidation products share a common pathway for the activation of UCPs (Murphy et al., 2003).

12. Hydroxy-alkenals serve as subcellular messengers in gene regulatory and signal transduction pathways

Due to high reactivity, ROS can injure any macromolecule (proteins, DNA, and lipids); however, the presence of an antioxidant protection system under normal conditions maintains intracellular concentration of antioxidants at a secure level. Under certain circumstances, ROS production rate exceeds the rate of its detoxification, resulting in cell damage and death due to oxidative stress. At the same time, at low ROS concentrations take part in the regulation of diverse functions in eukaryotic cells, such as proliferation, hormone biosynthesis, chemotaxis, oxidative burst, aggregation, apoptosis, and others (Gamaley and Klyubin, 1999).

The discovery of specific genes and pathways affected by oxidants led to the hypothesis that reactive oxygen species serve as subcellular messengers in gene regulatory and signal transduction pathways.

Lipid peroxidation produces several toxic carbonyls, including alpha-beta unsaturated aldehydes. HNE (one of the major end products of lipid peroxidation) exerts several biological effects. Lipid peroxidation also occurs under physiological conditions, predominantly in cells that are not rapidly proliferating. Under physiological conditions the cellular concentration of 4-HNE ranges from 0.1 to 0.3 mM. However, it accumulates in membranes at concentrations of 10 μM to 5 mM in response to oxidative insults (Uchida, 2003).

Hydroxynonenal (HNE), the aldehyde most represented in the 4-hydroxy-2,3-trans-alkenal class, has extensive been investigated, because, at concentrations close to those "physiologically" found in normal cells and plasma, it modulates cellular functions, gene expression and biochemical pathways, without cytotoxic effects (Awasthi et al., 2005). For this reason, HNE has been proposed by numerous authors as an intracellular signaling intermediary, rather than a toxic product of lipid peroxidation (Uchida, 2003) and (Yang et al., 2003). Earlier results have shown the antiproliferative and differentiative effects of HNE in leukemic cells (Barrera et al., 1991, 1996) and the antiproliferative and proapoptotic effects in other cell models (Awasthi et al., 2003).

The most important group of transcription factors involved in mediating the effect of nutrients and their metabolites on gene transcription is the super family of nuclear receptors, which consists of 48 members in the human genome (Zhang et al., 2004). This super family is subdivided into six families (Germain et al., 2006), of which the NR1 family is most relevant to nutrition.

Nuclear receptors control gene expression via several different mechanisms that involve both activation and repression of DNA transcription. After site-specific DNA binding, their final transcriptional activity depends on physical interactions with a set of related proteins, named coactivators and corepressors. These coregulators are not exclusive to nuclear receptors and are recruited in a similar manner by numerous other DNA-binding transcription factors (Germain et al., 2006; Nettles and Greene, 2005; Bain et al., 2007).

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor super family (Nuclear Receptor Nomenclature Committee, 1999). After the isolation of PPARα as the receptor mediating peroxisome proliferation in rodent hepatocytes in 1990 (Issemann and Green, 1990), two related isotypes, PPARβ and PPARγ have been characterized (Dreyer et al., 1992). PPARs are sensors capable of adapting gene expression to integrate various lipid signals. The multiplicity of functions in which they are concerned is also reflected by the diversity of ligands that can be accommodated within their ligand binding pocket. Besides upregulating gene expression, PPARs are also able to repress transcription by directly interacting with other transcription factors and interfere with their signaling pathways, a mechanism usually called transrepression (Ricote and Glass, 2007).

The first evidence of the participation of a product of lipid peroxidation in the modulation of PPAR ligand activity was described by (Pizzimenti et al., 2002), these authors suggested a connection between HNE and PPAR ligand pathways in leukemic cell growth and differentiation. Cerbone et al. (2007) have recently demonstrated that 4-hydroxynonenal and PPAR ligands affect proliferation, differentiation, and apoptosis in colon cancer cells.

Similar to other nuclear receptors, the PPARs are phosphoproteins and their transcriptional activity is affected by cross-talk with kinases and phosphatases. Phosphorylation by the mitogen-activated protein kinases (ERK- and p38-MAPK), Protein Kinase A and C (PKA, PKC), AMP Kinase (AMPK) and glycogen synthase kinase-3 (GSK3) affect their activity in a ligand-dependent or -independent manner. The effects of phosphorylation depend on the cellular context, receptor subtype and residue metabolized can be manifested at several steps in the PPAR activation sequence, including ligand affinity, DNA binding, coactivator recruitment and proteasomal degradation (Burns and Vanden Heuvel, 2007). The expression of oncogenes, c-myc, and c-jun, which regulate cell proliferation and survival, has been shown to be modulated by HNE in K562 Erythroleukemic cells and HL-60 (Fazio et al., 1992; Barrera et al., 2005) suggesting a role of these aldehydes in the control of gene expression.

13. Conclusions

This review describes the behavior of polyunsaturated fatty acids in nature with emphasis on biological membranes. Membrane phospholipids containing polyunsaturated fatty acids are particularly susceptible to oxidation and can contribute in chain reactions that amplify damage to bio molecules. Lipid peroxidation often occurs in response to oxidative stress, and a great diversity of phospholipid oxidation products and aldehydes is formed when lipid hydroperoxides break down in biological systems. Bioactivities of these phospholipids on vascular wall cells, leukocytes, and platelets have been described. Some of these aldehydes are highly reactive and may be considered as second toxic messengers which disseminate and augment initial free radical events. The aldehydes most intensively studied up to now are 4-hydroxy-2-nonenal and 4-hydroxy-2-hexenal. 4-Hydroxy-2-nonenal (HNE) is known to be the main aldehyde formed during lipid peroxidation of *n*-6 polyunsaturated fatty acids, such as linoleic acid C18:2 *n*-6 and arachidonic acid C20:4 *n*-6 whereas lipid peroxidation of *n*-3 polyunsaturated fatty acids such as α-linolenic acid C18:3 *n*-3 and docosahexaenoic acid C22:6 *n*-3 generates a closely related

compound, 4-hydroxy-2-hexenal (HHE). During lipid peroxidation, biomolecules such as proteins or amino lipids can be covalently modified by these lipid decomposition products, which damage membrane structure modifying its physical properties. In addition this review provide a synopsis of identified effects of hydroxy-alkenals and oxidized phospholipids on cell signaling, from their intracellular production, to their action as intracellular messenger, up to their influence on transcription factors and gene expression.

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