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REVIEW ARTICLE

Chemistry and biochemistry of lipid peroxidation products

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Abstract

Oxidative stress and resulting lipid peroxidation is involved in various and numerous pathological states including inflammation, atherosclerosis, neurodegenerative diseases and cancer. This review is focused on recent advances concerning the formation, metabolism and reactivity towards macromolecules of lipid peroxidation breakdown products, some of which being considered as 'second messengers' of oxidative stress. This review relates also new advances regarding apoptosis induction, survival/proliferation processes and autophagy regulated by 4-hydroxynonenal, a major product of omega-6 fatty acid peroxidation, in relationship with detoxication mechanisms. The use of these lipid peroxidation products as oxidative stress/lipid peroxidation biomarkers is also addressed.

Keywords: *Lipid peroxidation, alkenals, 4-hydroxy-2-nonenal, glutathione, apoptosis, adducts, volatile aldehydes*

Abbreviations: *AA, Arachidonic acid; ABAD, amyloid β peptide binding-alcohol dehydrogenase; AD, Alzheimer disease; ADP, Adenosine diphosphate; AGEs, Advanced glycation end products; AKR, Aldo/keto reductase; ALDH, Aldehyde deshydrogenase; ALEs, Advanced lipid peroxidation end products; ALDH, Aldehyde deshydrogenase; AMD, Age-related macular degeneration; AMPK, AMP-activated protein kinase; ANT, Adenine nucleotide translocator; ATP, Adenosine triphosphate; CL, Cholesterol; COX, Cyclooxygenase; Cys, Cysteine; DA-GPE, Diacyl-glycero-phosphoethanolamine; DDE, 2,4-decadienal; DHA, Docosahexaenoic acid; DHN, 1,4-dihydroxy-2-nonene; DISC, Death Inducing Signalling Complex; DODE, 9,12-Dioxo-10(E)-dodecenoic acid; EDE, 4,5-Epoxy-2(E)-decenal; EGFR, Epidermal Growth Factor Receptor; Gly, Glycine; GPx, Glutathione peroxidase; GSH, Glutathione; GST, Glutathione transferase; GS-DHN, 1,4-Dihydroxynonane-glutathione conjugate; GS-HNA, 4-Hydroxynonanoic acid-glutathione; GS-HNAL, 4-Hydroxynonanoic acid-glutathione lactone; HDDE, 4-Hydroxy-2,6-dodecadienal; HHE, 4-hydroxy-2(E)-hexenal; His, Histidine; HNA, 4-Hydroxynon-2-enoic acid; HNE, 4-hydroxy-2(E)-nonenal; HPHE, 4-hydroperoxy-2(E)-hexenal; HPNE, 4-hydroperoxy-2(E)-nonenal; HPOPE, Hydroperoxy-9,11-octadecadienoic acid; HRP, Horseradish peroxidase; HSP, Heat shock protein; isoK, Isoketals; JNK, c-Jun kinase; LDL, Low-density lipoprotein; LOOH, Lipid hydroperoxides; LPO, Lipid peroxidation; LOX, lipoxygenase; Lys, Lysine; MDA, Malondialdehyde; MPO, Myeloperoxidase; MRP, Multiresistance drug associated protein; NADPH, Nicotinamide adenine dinucleotide phosphate; OHE, 4-Oxo-2(E)-hexenal; ONE, 4-Oxo-2(E)-nonenal; PDI, Protein disulphide isomerase; PE, Phosphatidylethanolamine; PDGFR, Platelet-Derived Growth Factor Receptor; PRX, Peroxiredoxin; PS, Phosphatidylserine; PUFA, Polyunsaturated fatty acid; RDH, Retinol dehydrogenase; RLIP, Ral-interacting protein; RNS, Reactive nitrogen species; ROS, Reactive oxygen species; RPE, Retinal pigment epithelial cells; SCE, Sister chromatid exchange; SID, Streptozotocin-induced diabetic; SMC, Smooth muscle cell; SOD, Superoxide dismutase; Tcf, T-cell specific transcription factor; TNF, Tumour Necrosis Factor; TOG, Thiadiazabicyclo-ONE-GSH-adduct; TRAIL, TNF-related-apoptosis-inducing-ligand.*

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Introduction

Oxidative stress and resulting lipid peroxidation is involved in various and numerous pathological states including inflammation, atherosclerosis, neurodegenerative diseases and cancer. Free radicals produced during lipid peroxidation have some very local effects, because of their short life, but the breakdown products of lipid peroxides may serve as 'oxidative stress second messengers', due to their prolonged half-life and their ability to diffuse from their site of formation, compared to free radicals. Those breakdown products, mostly aldehydes, such as malonaldehyde, hexanal, 4-hydroxynonenal or acrolein have received a lot of attention because they are most of the time reactive compounds. They have been considered for a long time as toxic end-products of lipid peroxidation. We know now that they play a real powerful biological role in cell signalling under both pathological and physiological conditions, mainly in cell cycle regulation. Due to their chemical reactivity, those breakdown products can make covalent modifications on macromolecules such as nucleic acids, protein and lipids and exert some biological effects. They also serve as biomarkers of lipid peroxidation/oxidative stress. This review aims to give recent advances concerning those aspects.

Chemistry

Lipid peroxidation (LPO) has been studied by chemists as a degradation process of natural compounds (fats, oils) and especially as a factor in the deterioration of food quality (odour, flavour, colour, texture, toxicity) [1,2].

LPO occurs *in vivo* in plants by the activation of enzymes as a result of mechanical injury or after infection by fungi, bacteria or viruses. It is also present in germinating seeds [3–6].

In mammals, LPO is related to injury and inflammation and is often the oxidative deterioration of lipids, mainly cellular membrane lipids (phospholipids, cholesterol). This can lead to changes in the permeability and fluidity of the membrane lipid bilayer and dramatically alter cell integrity [7]. Yet, LPO has been implicated as a cause and effect of cellular damage [8,9]. The relationship between LPO and diseases is increasingly mentioned but remains to be conclusively established in most cases, because the biological and chemical conditions of LPO are complex and not fully defined [7,10,11]. Cholesterol peroxidation occurring at the lipoprotein LDL has attracted attention with its implication in atherosclerosis [10,12].

Indeed, LPO refers to different mechanisms and can be classified as enzymatic, non-enzymatic non-radical peroxidation and non-enzymatic free-radical mediated peroxidation [13].

Cholesterol and the fatty acid part of phospholipids are the most commonly referred LPO substrates [14]. Peroxidation conditions also play a major role and LPO may be induced by endogenous or exogenous factors: enzymes, radical species, metal ions, UV radiations, heat, radical-initiating chemicals, drugs and a wealth of compounds referred to as 'reactive oxygen species (ROS)' and 'reactive nitrogen species (RNS)'. The free-radical non-enzymatic peroxidation of polyunsaturated fatty acids (PUFAs) has been extensively studied. Among PUFAs, the ω6 linoleic acid (18:2 n-6) is the most abundant *in vivo* in plants and is essential in mammals and arachidonic acid (20:4 n-6) plays a crucial role in inflammation response.

LPO complexity also resides in the many compounds that can result. Lipid hydroperoxides, the primary compounds, are unstable and decompose to more stable but still reactive and potentially toxic secondary compounds.

In the following parts of this review, the proposed mechanisms of LPO with focus on PUFAs peroxidation by the non-enzymatic free radical mechanism and the resulting secondary products will be discussed with references to reviews.

Proposed mechanisms of PUFA peroxidation

The non-enzymatical free radical mechanism occurs in three phases [15]:

- *Initiation*: abstraction of $H^{\cdot}$ radical from a lipid (LH) chain to give a lipid radical; an initiator is required. The formation of lipid radicals is particularly favourable when the lipid is a PUFA because the resulting radical is resonance stabilized.
- *Propagation*: the lipid radical can react with oxygen to give a lipoperoxyl radical ($LOO^{\cdot}$) which in turn reacts with a lipid to yield a lipid radical and a lipid hydroperoxyde (LOOH). LOOH are unstable: they generate new peroxyl and alcoxyl radicals and decompose to secondary products [9,16,17].
- *Termination*: It is a combination of radical species to give non-radical or non-propagating species.

Reactive oxygen species (ROS) exist under normal physiological conditions and could be produced in larger amounts *in vivo* when natural defences are overwhelmed. They could play a role in LPO initiation. The hydroxyl radical $^{\cdot}OH$ is the most reactive radical and was not clearly evidenced *in vivo*. The Fenton reaction and the Haber-Weiss reaction are both supposed to form the hydroxyl radical from hydrogen peroxide and metal species (iron, copper) [7,18,19]. The superoxide radical $O_2^{\cdot -}$ is produced

during metabolism and decreases antioxidant defence, but is unable to abstract hydrogen. Hydroperoxides could be further transformed into initiating species, namely peroxyl and alkoxyl radicals (ROO$^{\cdot}$ and RO$^{\cdot}$). The role of iron and copper in free radical mediated LPO is often mentioned [7,20,21].

Hypervalent iron complexes (ferryl and perferryl) have been suggested to be the active species in the systems generated from hydrogen peroxide and iron-containing proteins (haemoglobin, myoglobin, P450 cytochromes) [7,18,19].

Reactive nitrogen species (RNS) like nitric oxide, nitrous oxide and peroxynitrite are radical species which could also induce and, in some cases, limit LPO [22]. Recently, fatty acid dimers were suggested as new key intermediates during initiation and propagation [23,24].

LPO can be inhibited enzymatically or non-enzymatically [13,25]. Glutathione peroxidases (GPx), catalases and superoxide dismutases (SOD) are antioxidant enzymes. Vitamin E and vitamin C have radical scavenging properties and are able to inhibit the free-radical mechanism of LPO. Vitamin C is a hydrophilic antioxidant. Ascorbate, in conjunction with iron species, has been used to induce LPO in microsomes [26]. Vitamin E is a lipophilic antioxidant. It has been shown to have pro-oxidant activity *in vitro* [27,28]. Glutathione (GSH) is a major antioxidant and has also been associated with inhibition of LPO. Bilirubin could scavenge peroxyl radicals [29] and increased levels of bilirubin have been associated with inhibition of LPO [30].

The primary products hydroperoxides decompose by various mechanisms to a wealth of more stable secondary products with potential toxicity.

Secondary products

The peroxidation of PUFAs is complex because many fatty acids are present in different compartments in mammals. Esterbauer [10] estimated a range of 120–150 possible hydroperoxides. LOOHs can decompose to a variety of more stable compounds. The formation of alkoxyl radicals (LO$^{\cdot}$) prone to subsequent β-scission gives rise to short-chain products (C2 to C12, with a large range of chemical functionality) and modified chain lipid (carboxylic part). LOOH can also rearrange to hydroxyl- and epoxy-acids, dimerize or polymerize [8].

LPO-generated high-molecular weight compounds are hardly ever mentioned, probably because they are more difficult to analyse than short-chain volatiles, although they could also have deleterious effects [10,31,32].

Volatile products of PUFAs peroxidation. Volatile products formed by the peroxidation of PUFAs have attracted much attention, especially those of the ω6 and ω3 series. Complex mixtures are obtained from which the identification of compounds remains partial. Classes of compounds have been identified. Alkanes and also aldehydes, ketones, alcohols and furanes with multiple functionality were reported under various reaction conditions [8,31,33,34].

Aldehydes have received much attention because they are reactive and toxic. They are less unstable than the hydroperoxides and could diffuse from their site of formation [35].

Malondialdehyde (MDA). The enzymatic and the free-radical peroxidation of PUFAs which contain at least three double bonds, like arachidonic acid and DHA, could cleave to the bis-aldehyde malonaldehyde (MDA, Figure 1). Various mechanisms have been proposed [35] and bicyclic endoperoxides were suggested as possible intermediates [36]. MDA has been extensively mentioned in LPO studies and reported as a biomarker of the peroxidation of ω3 and ω6 fatty acids [33,35], yet with some restrictions concerning the possible existence of another biochemical origin [37].

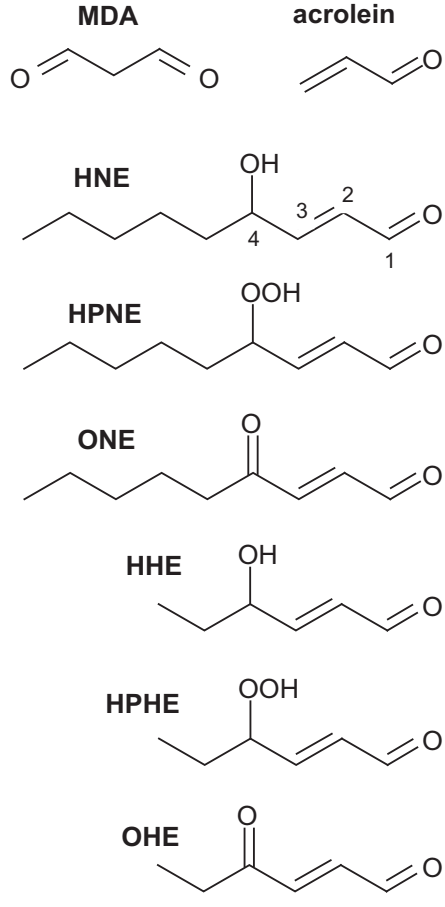

Figure 1. LPO aldehydes.

Other aldehydes. Many other aldehydes are formed during LPO: saturated, α,β-unsaturated (alkenals: acrolein, heptenal, …) [8,38], 4-hydroxy-α,β-unsaturated (4-hydroxy-alkenals), 4-hydroperoxy-α,β-unsaturated (4-hydroperoxy-alkenals), 4-oxo-α,β-unsaturated (4-oxo -alkenals), epoxy-α,β-unsaturated [16,31], conjugated dienes (2,4-dienals: 2,4-decadienal ...), ...

4-hydroxy-alkenals have three chemical functions: aldehyde (CHO), alkene (C2=C3 double bond) and secondary alcohols (OH at the chiral centre C4), which make them highly reactive. Carbon C3 is an electrophilic site for Michael-type addition, carbon C1 is also electrophilic and a redox centre (oxidation to carboxylic acid, reduction to alcohol) and carbon C4 holds the alcohol function.

4-hydroxy-2(E)-nonenal (HNE, Figure 1) is a representative compound arising from the peroxidation of ω6 fatty acids [39], while 4-hydroxy-2(E)-hexenal (HHE, Figure 1) is formed from ω3 fatty acids.

HNE was formed under various conditions like auto-oxidation and stimulated microsomal LPO [8]. LPO mechanisms of formation have been proposed [24]. HNE was found in food [40] and detected *in vivo*. Several pathologies have been associated with elevated levels of HNE. Esterbauer and Weger [41] intensively studied HNE and synthesized several 4-hydroxy-alkenals in the late 1960s. Other synthetic methods have been developed since then [35,42]. 4-hydroperoxy-2(E)-nonenal (HPNE, Figure 1) was proposed as an HNE precursor [43,44] and is also a product of plant enzymatic biotransformation [45].

HHE has been identified during the auto-oxidation of ω3 fatty acids (linolenate, DHA) [32,46] but also as a metabolite of a plant alkaloid [47]. 4-hydroperoxy-2(E)-hexenal (HPHE, Figure 1) was hypothesized as a precursor of HHE by comparison to the work on HPNE [32].

More recently, 4-oxo-2(E)-nonenal (ONE, Figure 1) was detected in the decomposition of a linoleic hydroperoxide: 13(S)-hydroperoxy-9,11-octadecadienoic acid (13(S)-HPODE). HPNE was suggested to be an intermediary compound during the *in vitro* process. ONE could be the major product of linoleic hydroperoxide decomposition [43]. ONE is electrophilic and is able to react with nucleobases [48]. It was observed *in vitro* by free radical decomposition of both (S)-regioisomers of linoleic acid hydroperoxides (9(S)-HPODE and 13(S)-HPODE) [17], but not *in vivo* in its free form. *In vivo*, only ONE metabolites have been detected [49]. This could be explained by a higher reactivity of ONE compared to hydroxyalkenals.

4-oxo-2(E)-hexenal (OHE, Figure 1) has been found in arthropods and was chemically synthesized in the late 1960s [50]. It was recently reported as a product of LPO through the identification of a deoxyguanosine adduct [51] and was detected in ω3-rich cooked food [52].

Other aldehydes with various functionalities have been mentioned as a result of linoleic acid peroxidation [17]. 4,5-epoxy-2(E)-decenal (EDE) was formed from the auto-oxidation of arachidonic acid [53] and was one of the detected odourant aldehydes [54]. 9,12-dioxo-10(E)-dodecenoic acid (DODE) was suggested as a carboxylic acid break-down product from the *in vitro* peroxidation of a linoleic hydroperoxide as the (E)-isomer [55] and was earlier reported in lentil seed as the (Z)-isomer [56].

In conclusion, lipid peroxidation represents a set of complex enzymatic and non-enzymatic suite of reactions, which can lead to a wealth of compounds with different chemical functions, molecular weight, physico-chemical properties, reactivity, toxicity, etc, …

The biological implications are of major importance and range from food quality to health concerns, with possible implications in the occurrence of various diseases. Many studies report on the formation and subsequent reactions of alkenals, as these are highly reactive compounds, which could further exert toxicity by biotransformation and adduction to biomolecules (proteins, DNA).

Biotransformations of LPO product alkenals

Biotransformations of LPO secondary products has been reported previously in the excellent review written by Esterbauer et al. [35] in 1991. More recently, reviews focused on HNE *in vitro* and *in vivo* biotransformations have been published [57,58] in the special issue of *Molecular Aspects of Medicine* on '4-hydroxynonenal: a lipid degradation product provided with cell regulatory functions'. A review on acrolein has been written in 2008 by Stevens and Maier [38]. We provide here a quick overview on biotransformations and transport of LPO secondary products, mainly 4-hydroxy- and 4-oxo-alkenals, MDA and acrolein with a specific focus on recent advances in this field.

Metabolism

LPO secondary products can, for most of them, travel across membranes by passive diffusion. Most of LPO secondary products such as HNE, MDA or acrolein are reactive compounds because they bear electrophilic properties.

4-hydroxy- and 4-oxo-alkenals. Most of them readily react with the nucleophilic tripeptide glutathione (GSH) by Michael addition to form GSH conjugates. This conjugation takes place in most cells and tissues, but it seems that liver is more efficient than other tissues. This reaction can occur spontaneously but it is several hundred times faster when catalysed by glutathione-*S*-transferases (GSTs). It seems that

some of the GST isoforms have had a specific evolution towards LPO products, as is the case for GST A4–4. GST A4–4 presents high substrate chemospecificity, but an intriguingly low stereoselectivity, as HNE is formed as a racemic mixture [59].

Michael additions can occur also to cellular proteins, cysteine sulphydryl groups being the primary soft nucleophilic targets of those compounds. This is in the case of HNE, but it is likely to occur for other 4-hydroxyalkenals, the double bond between C2 and C3 can be saturated giving 4-hydroxynonanal that further rearranges in its hemi-acetal form [60]. The enzyme involved is the NAD(P)H-dependent alkenal/one oxidoreductase, also known as leukotrieneB4 12-hydroxydehydrogenase/15-oxoprostaglandin 13-reductase.

Other modifications can concern the aldehyde function that can be either reduced into alcohol or oxidized into acid, involving alcohol dehydrogenase or aldo/keto reductases (AKR) and aldehyde dehydrogenase, respectively, for the formation of 1,4-dihydroxynonene (DHN) and 4-hydroxynonenoic acid (4-HNA) in the case of HNE. The acidic metabolite of HNE can undergo beta-oxidation. Aldose reductase was shown to reduce both HNE and its GSH conjugate [61]. Very recently, Zhong et al. [62] reported that the enzyme AKR1B10, present in the intestinal tract, was able to reduce dietary aldehydes [62]. Marchette et al. [63] reported the involvement of retinol dehydrogenase 12 for the reduction of HNE in photoreceptor inner segments of the retina. The defect of this enzyme is implicated in the pathogeny of Leber congenital amaurosis. Demozay et al. [64] reported the role of fatty aldehyde dehydrogenase in HNE-induced modifications in insulin signalling.

Metabolization of secondary lipid peroxidation products such as HNE in most cells and tissues is rapid and complete. GSH conjugation seems to be the primary and major step. If GSH is depleted by buthionine sulphoximine for instance or by a concomitant oxidative insult, there is a reduction in GS-HNE conjugate together with an increase in unmetabolized HNE and in HNE toxicity [65]. On the other hand, when the oxidative insult occurs some time before treatment of cells by HNE, preconditioned cells acquire resistance to HNE-induced apoptosis by metabolizing and excluding HNE at a higher rate when compared to non-preconditioned cells [66,67]. This primary conjugation step can be further completed by reduction of the aldehyde function by AKR, giving 1,4-dihydroxynonane-glutathione (GS-DHN) conjugate, or by oxidation of the aldehyde by aldehyde deshydrogenase (ALDH), giving 4-hydroxynonanoic acid-glutathione (GS-HNA), which can also exist in the lactone form (GS-HNAL). The oxidized derivatives GS-HNA and GS-HNAL can be further metabolized by cytochrome P450 4A, giving omega-hydroxylated and carboxylated metabolites [57,68–70]. All these metabolites can serve as biomarkers of lipid peroxidation in disease states [71]. The glutathione moiety of all these adducts is further metabolized into mercapturic acid in the kidney and the resulting metabolites are excreted into urine [38,72–74]. The reduced mercapturic acid of HNE, namely 1,4-dihydroxynonane-mercapturic acid (DHN-MA), is the major metabolite found in urine [73,75]. Those mercapturic acids can be used as biomarkers of oxidative stress/LPO available by non-invasive sampling techniques [49,76,77]. They are also used to reflect consumption of 'peroxidable' diet associated with colon cancer risk [78]. Recently, Kuiper et al. [79] reported the presence of mercapturic acids of HNE and ONE in the urine of smokers upon smoking cessation.

It is noteworthy that alkenal conjugation to GSH is a reversible reaction and that conjugates can be considered as 'transport forms' which can give back the parent compound possibly far away (other cell, other tissue) from its generation place. This reversible reaction is suppressed if the aldehyde function is reduced, underlining the importance of the enzymes involved.

In vivo biotransformation studies of dietary aldehydes such as HNE are still lacking. However, Goicoechea et al. [80] reported recently the use of an *in vitro* digestion model to study the bioaccessibility of oxygenated α,β-unsaturated aldehydes. Those compounds remained unaltered after digestion and could then be bioaccessible in the gastrointestinal tract and eventually reach the systemic circulation. This has been indirectly confirmed by the fact that enormous quantities of DHN-MA have been found in the urine of rats fed on a HNE-rich diet [78].

Conjugation of HNE to GSH is considered as a detoxication step, facilitating urinary excretion. However, Enoiu et al. [81] have reported that the metabolization of GS-HNE by gamma-glutamyl-transpeptidase giving the CysGly-HNE adduct induced a considerable increase in cytotoxicity. The authors attribute this cytotoxicity to a retro-Michael cleavage of the adduct, Cys-Gly-HNE being less stable than GS-HNE. Such a mechanism has also been reported for acrolein-mercapturic acid adducts in the kidney [82].

Acrolein. Acrolein is much more electrophilic than HNE and reacts with GSH much faster [35]. Conjugation with GSH, followed by mercapturic acid transformation of the GSH moiety, is the main pathway for its elimination. Mercapturic acids of acrolein can be reduced or oxidized, as is the case for HNE [38]. It seems that GST A4–4 can also catalyse the conjugation of GSH to acrolein [83]. Reduced mercapturic acid of acrolein is the major metabolite in urine, as is the case for HNE. As described above for other alkenals, acrolein can make adducts with proteins.

MDA. MDA is metabolized to CO_2 and water via transformation into acetaldehyde by ALDH, but it can be found unmodified in urine [77] or in plasma [84,85]. MDA can make adducts with lysine and serine residues.

Transport

Several studies have reported the transport of GS-HNE by transport proteins such as Multi Drug Resistance protein MRP1 [86] using cells over-expressing this protein or MRP2 [87] in freshly isolated hepatocytes. Recently, Miranda et al. [88] reported a protective role of ascorbate against HNE effect through the modulation of its MRP-mediated transport in human THP-1 monocytic leukaemia cells.

RLIP76 (Ral-interacting protein), an ATP dependent non-ABC multifunctional protein, is also involved in the transport of glutathione-electrophile conjugates in mammalian cells. This enzyme is believed to provide protection against different stressors including oxidant chemicals. This enzyme accounts for 80% of the GS-HNE conjugate transport, thereby counteracting the pro-apoptotic effect of HNE. This enzyme is frequently over-expressed in malignant cells [89–91]. In RLIP76 null mice, the transport of GS-electrophilic compound adducts is impaired by ~ 80%. In those mice, there is an accumulation of HNE and its GSH conjugate in tissues [91,92].

Reactivity of lipid oxidation products with macromolecules

DNA

LPO, the oxidative degradation of PUFAs of membrane lipids, leads to the formation of a variety of aldehydic breakdown products such as MDA, alkanals, 2-alkenals, 2,4 alkadienals and 4-hydroxyalkenals [8]. Since it is generated from most fatty acids with more than two double bonds [93], MDA quantitatively is the major product of lipid peroxidation, whereas the formation of other aldhydic LPO products is dependent on the parent ω-3, ω-6 and ω-9 polyunsaturated fatty acids, i.e. HHE arises from breakdown of ω-3 PUFAs, 4-hydroxynonenal (HNE) from ω-6 PUFAs and 4-hydroxyundecenal from ω-9 PUFAs [94,95]. Since ω-6 PUFAs are most abundant, HNE by far exceeds the levels of HHE and 4-hydroxyundecenal [96]. Compared to MDA, HNE is formed in up to 80-fold lower concentrations [21]. In toxicological terms, however, HNE appears to be more significant than MDA due to its higher electrophilicity [97] and its implication in pathological developments such as cancer, neurodegenerative diseases, arteriosclerosis and others (for a review see [98]). Due to their significant formation and their toxicological potential this contribution focuses on both MDA and HNE and briefly summarizes the findings on DNA adduct formation and chromosomal damage.

MDA. Mutagenicity of MDA to Salmonella typhimurium was first demonstrated in 1976 by Mukai and Goldstein [99] and later confirmed by other researchers [100,101]. The mutations observed were base pair substitutions as well as frameshift mutations [102]. By transfecting human fibroblasts with a shuttle vector, which had been allowed to react with MDA, and sequence analysis of the reporter gene after replication, Niedernhofer et al. [103] observed that the majority of mutations occurred at GC base pairs and that the most frequent mutations were large insertions and deletions and base pair substitutions.

MDA has further been shown to induce genotoxicity in mammalian cells, i.e. mutations in the mouse lymphoma assay [104], chromosomal aberrations and micronucleus formation in rat skin fibroblasts [105] and both single strand breaks and sister chromatid exchanges in CHO cells [106].

The mutational spectrum of MDA is related to its reaction with DNA at physiological pH to form guanosine, adenosine and cytidine adducts [107–109]. When reacting with guanosine both carbonyls react with nitrogen (N^2 and N1) to form pyrimido[1,2α] purin-10(3*H*)-one (M_1G), which is the most abundant MDA adduct. Adenosine and cytidine adducts arise from addition of one carbonyl with the exocyclic amino groups to form N^6-(3-oxopropenyl)deoxyadenosine (M_1A) and N^4-(3-oxopropenyl)deoxycytidine (M_1C), respectively. While M_1C is formed only in trace amounts, M_1A yields ~ 20% of M_1G [110]. M_1G adducts are detected in tissues from healthy humans [111,112], are mutagenic in bacteria and are a substrate for nucleotide excision repair [113].

HNE. After its identification as a cytotoxic product of the oxidation of liver microsomal lipids [94] a large body of evidence accumulated concerning the genotoxic action of HNE. It was demonstrated to induce significant amounts of DNA fragmentation [114], sister chromatid exchanges [114] and a dose-dependent increase in the number of mutations to 6-thioguanine resistance in Chinese hamster cells [115]. Furthermore, HNE proved to be a very potent inducer of the SOS response in the *Salmonella typhimurium* assay [116].

Further investigations carried out with metabolically competent primary hepatocytes revealed a very high sensitivity of this cell type, at least one order of magnitude higher than other mammalian cell types utilized before. The most sensitive indicator of genotoxic action was demonstrated to be sister chromatid

exchanges, followed by micronuclei and chromosomal aberrations [117]. In the same study also the genotoxic potential of 4-hydroxyhexenal and 4-hydroxyundecenal and the analogous aldehydes 2-*trans*-nonenal and nonanal were tested and turned out to be less genotoxic. Based on the structure of the aldehydes the authors concluded that the length of the lipophilic tail had no influence on chromosomal aberration induction, but appeared to influence the yield of SCE and micronucleus formation. Furthermore, the lack of the OH group (2-*trans*-nonenal) reduced the SCE-inducing potential to higher concentrations and the lack of both the OH group and the CC double bond did not result in a complete loss of SCE induction, but did not cause the formation of micronuclei and chromosomal aberrations. Based on these observations HNE appears to be the most potent mutagenic aldehydic lipid peroxidation product.

Studies with brain endothelial cell clones expressing different functions such as the blood–brain barrier resulted in a significant dose-dependent induction of micronuclei and chromosomal aberrations [118] at concentrations $\geq$ 1 μM, and a more recent study observed dose-dependent genotoxic effects in human adenoma cells as evidenced by the COMET assay [119].

Since HNE occurs in two stereoisomeric forms, (S)-HNE and (R)-HNE, and these are enantioselectively metabolized [120], both isoforms were tested in the primary rat hepatocyte assay, however, did not cause any differences of the dose response of chromosomal aberration induction [121].

When comparing the concentrations applied in the different investigations it becomes evident that the effects observed are dependent on the cell type utilized and that primary hepatocytes react most sensitively towards HNE, causing significant levels of sister chromatid exchanges, even at concentrations as low as 0.1 μM, a concentration which can be achieved under physiological conditions [96]. The reason for this high sensitivity most likely is associated with the two distinct pathways of HNE interaction with DNA.

When HNE directly interacts with the guanosine moiety of DNA, four isomeric 1,*N*-2-propano adducts are formed [122]. Second, 2,3-epoxy-4-hydroxy-nonanal can be generated from *trans*-4-hydroxy-2-nonenal by auto-oxidation or incubation with fatty acid hydroperoxides or hydrogen peroxide [123] and causes the formation of etheno adducts, i.e. 3,N^4-ethenodeoxycytidine, 1,N^6-ethenodeoxyadenosine and N^2,3-ethenodeoxyguanosine. Interestingly, 2,3-epoxy-4-hydroxy-nonanal is not a substrate of epoxide hydrolase [124] and could thus favour the formation of etheno adducts. In this respect the high sensitivity of hepatocytes may eventually be explained by the formation of 2,3-epoxy-4-hydroxy-nonanal in the course of biotransformation by cytochromes P-450 [123]. Moreover, the observation that the background level of propano adducts are particularly high in the liver [125] is in support of the high sensitivity of this organ. In fact, Hu et al. [126] demonstrated that propano adducts may even be causatively involved in hepatic carcinogenesis, since HNE preferentially forms adducts at codon 249 of the human p53 gene.

Both propano and etheno adducts of HNE appear as endogenous lesions in liver and other tissues of untreated rodents and humans [127]. In this context it appears of interest that studies by Nair et al. [128] demonstrated that the high intake of ω-6 polyunsaturated fatty acids increased malondialdehyde adducts in male and female subjects, while the levels of ethenodeoxyadenosine and ethenodeoxycytidine were not affected in male subjects; however, they were ~ 40-times higher in females.

Both MDA and HNE adducts are found in tissues of untreated rodents and humans. According to Burcham [93] the levels of these adducts can be as high or even higher than adducts induced by exogenous mutagens/carcinogens. The high sensitivity of certain cells and tissues such as liver and endothelial cells [117,118] together with the observation that there appear to be mutational hotspots for HNE adducts in the human p53 gene [126] therefore may reflect the causative contribution to the development of degenerative diseases such as cancer.

Oxidative modification of proteins with lipid peroxidation by-products

Mechanism of protein modification by reactive aldehydes. Oxidative modifications of metabolic and structural proteins play a significant role in the protein dysfunction, altered trafficking, processing of proteins, tissue damage and pathogenesis of several human diseases [129]. Protein carbonyl content is the most common biomarker of the oxidative damage of proteins [129]. Lipid peroxidation products can diffuse across membranes, allowing the reactive aldehyde-containing lipids to covalently modify proteins localized throughout the cell and relatively far away from the initial site of reactive oxygen species (ROS) formation [130–132]. Therefore, protein carbonyls are formed endogenously during lipid peroxidation and during the glycoxidation of carbohydrates, which are precursors of advanced glycation end products (AGEs) and advanced lipid peroxidation end products (ALEs) [133]. Protein-based carbonyls, key indicators of oxidative stress, reflect adduction of bifunctional lipid peroxidation-derived aldehydes, ketones and, perhaps, sugar oxidation products, rather than metal-catalysed oxidation of protein side-chains [134]. Major toxic effects of LPO-derived aldehydes are suggested to be caused by the modification and cross-linking of amino acid

proteins and peptides by these reactive aldehydes [135,136]. Mildly cross-linked, HNE-modified proteins are preferentially degraded by the proteasome, but extensive modification with this cross-linking aldehyde leads to the formation of protein aggregates. Interestingly, such cross-linked proteins are able to inhibit the proteasome and further impair cellular protein turnover [135]. The consequences of adduct formation at the protein level is associated with numerous cytotoxic consequences including the disruption of cell signalling, altered gene regulation, inhibition of enzyme activity, mitochondrial dysfunction, impaired energy metabolism, altered tertiary structure and finally loss of cytoskeletal formation [97].

Among several LPO-derived aldehydes, HNE is the most studied cytotoxic product of lipid peroxidation [137]. HNE readily modifies and causes cross-linking of proteins [138]. The chemical reactions leading to the formation of HNE-protein adducts involve Schiff base formation and Michael addition. Schiff base formation on primary amine (for example, lysine) results in the generation of more complex compounds, such as pyrrole-Lys adducts and fluorescent cross-links. Michael addition of HNE on amino groups (Lys and His) or biological thiols including cysteine, glutathione, are followed by cyclization and hemi-acetal or hemithioacetal formation [139]. Moreover, simple lysine Michael adducts are suggested to be formed reversibly [140]. Among the protein residues which react with HNE, Cys has the highest reactivity, followed by His and Lys [141], but Cys–HNE adducts are less stable than His–HNE adducts [137]. Regarding the modification of apolipoprotein B, HNE attacks mainly Lys and to a lesser extent His and Cys residues [142].

4-oxo-2-nonenal (ONE), a direct product of lipid oxidation [43], has been proposed as a more reactive protein modification and cross-linking agent than HNE [143]. The Cys-Lys and His-Lys cross-links are quite stable, although the Lys-Lys version readily suffers oxidation (require characterization by trapping with Ac_2O). Surprisingly, although ONE is more reactive than HNE towards imidazole and thiol nucleophiles, it is less reactive than HNE towards Lys/amine conjugate adduction.

MDA, one of the most abundant aldehydes formed during lipid peroxidation, reacts with Lys residues by forming Schiff bases [97] and plays a major role in low-density lipoprotein (LDL) modification and their affinity towards macrophages [144]. MDA has cross-linking activity similar to that of HNE and DDE (2,4-decadienal), it is virtually inactive at protein-carbonyl formation. It has been reported [130] that the low carbonyl incorporation at pH 7.4 increases dramatically at lower pH (pH 4–5). This property can be attributed to the character of MDA which exists predominantly at its non-electrophilic anionic conjugate base (enolate) and its carbonyl incorporation property. The accumulation of MDA adducts on proteins is involved in the formation of the fluorescent pigment lipofuscin, which accumulates progressively during ageing [145]

Other major lipid peroxidation products capable of modifying proteins are simple 2-enals, 2,4-dienals, 2-hydroxyaldehydes and 4,5-epoxy-2-enals. Acrolein is also formed during lipid peroxidation and is a strong electrophile exhibiting high reactivity with Cys, His and Lys nucleophile residues [146]. In the series of aldehydes studied at early times the rank order of carbonyl incorporation was acrolein > ONE > HNE > DDE > MDA. However, further data showed that acrolein is more potent than ONE for carbonyl incorporation and ONE has more affinity for protein cross-linking [132].

It has been recently hypothesized that the toxic effects of protein adduct not only depend on residue selectivity or affinity, but are also based on the importance of the targeted amino acid in protein function or structure [147]. Mainly acrolein and HNE, but also other lipid peroxidation by-products including ONE and HHE, are characterized by an α,β-unsaturated carbonyl structure, that is a conjugated system and contains mobile π-electrons.. The carbonyl oxygen atom is electronegative and can cause regional electron deficiency. On the basis of electron polarizability, both acrolein and HNE are considered to be soft electrophiles that form 1,4-Michael type adducts with soft nucleophilic sulphydryl thiolate groups of cysteine [148]. These anionic residues are the central nucleophilic components of catalytic sites of several key proteins of the cellular metabolism. Therefore, adduction of these regulatory thiolate groups by acrolein or HNE will disrupt redox control of protein function and, thereby, produce cytotoxicity [146,149]. Lys and His residues, main targets of nitrogen groups, are also targets for type 2 alkenes. Because these residues are relatively harder biological nucleophiles with significantly slower adduction kinetics, the toxic consequences of lysine or histidine adduction are more likely to develop during high-dose intoxication or during the late stages of chronic diseases when adduction of the cysteine thiolate pool has been saturated [146,148].

Formation of AGEs is also another type of interaction of lipid peroxidation by-products with proteins during diabetes and hyperglycaemia. A class of reactive ALE precursors, namely α-oxoaldehydes (methylglyoxal and glyoxal), are basically generated, from the Maillard reaction, resulting in the formation of a Schiff base. This reaction is followed by a non-enzymatic glycation and generates the Amadori product, where α-oxoaldehydes (methylglyoxal and glyoxal) are formed and react with Lys and arginine residues to form AGEs [150]. These α-oxoaldehydes are also produced from lipid peroxidation (glyoxal),

the catabolism of ketone bodies and the fragmentation of triosephosphates (methylglyoxal) in the early stages of glycation in hyperglycaemic conditions [151]. Glycation in *vivo* is slow and reversible at physiological glucose levels, tending to affect proteins with very slow turn-over including connective tissue collagen and lens cyrstallines. Glycation is faster at elevated glucose levels and therefore implicated with the progress and complications of diabetes. Glycated haemoglobin (HBA_{1C}) which contains a glucose Amadori product is, indeed, a marker used to monitor hyperglycaemia [150].

Recent studies provide more evidence on the mechanisms of how the modification of proteins by reactive aldehydes may lead to alterations of function of target proteins linked to disease states, signalling systems and age-related conditions. Some of the major functional protein modifications by lipid peroxidation end product are listed in this section.

Heat shock protein adducts. Heat shock protein 72 (HSP72) is the major inducible HSP found in the nucleus and cytosol [152]. Stress-induced HSP72 protects proteins against aggregation of denaturation. Moreover, HSP72 is also capable of inhibiting stress-induced apoptosis [153], even after the activation of effector caspases. Impaired synthesis of HSP72 may result in disturbances of cell proliferation [154,155]. Over-expression of HSP72 increases the survival and reduces oxidative damage and suppresses the inflammatory response [156]. On the other hand, HSP72 effect was not blunted in the absence of reactive oxygen radicals [156]. HSP72 requires ATP for its chaperone activity [157] and minimizes aggregation of newly synthesized proteins. HSP72 has an ATP-binding domain which exhibits low intrinsic ATPase activity [152,158]. Primarily ADP-bound state, but also ATP-bound state, demonstrate high affinity for the substrate [159]. Many of the molecular co-chaperones that influence the function of HSP72 do so through manipulation of the intrinsic ATPase activity. In a previous study it was tested whether major inducible stress protein HSP72 was susceptible to modification and inactivation by reactive lipid aldehydes [160]. An increase in the number and severity of HNE protein adducts was clearly demonstrated in cytosolic fractions obtained from ethanol fed animals when compared to their respective pair-fed controls [160]. Proteomic assays identified that HSP72 was one of several proteins consistently modified by HNE. Furthermore, HSP72 induction was impaired in hepatocytes isolated from ethanol-fed animals when compared to their respective control animals [160]. Interestingly, heat-denatured recombinant firefly luciferase assays demonstrated that HNE is a potent inhibitor of HSP72-mediated protein refolding. Because Cys267 adducts at ATPase domain of HSP72 were found after HNE administration, in the same set of experiments, the importance of oxidative modification on this particular residue in HNE-mediated inhibition of the chaperone was further confirmed [160]. Consistently, other studies show Cys modification and HSP72 inhibition by treatments with HNE and ONE, which are reactive to thiols, but not after MDA treatment, which does not react with thiols [35,161]. All these reports together emphasize that lipid peroxidation end products are potent inhibitors of the chaperone's refolding ability, which is possibly mediated through that Cys modification of the ATPase domain of HSP72.

Another key member of stress proteins 90-kDa heat shock protein (HSP90) accounts for nearly 2% of total protein in most unstressed cells and is involved in essential physiological processes, including protein trafficking and signal transduction, in specific steroid hormone signalling [162]. Because HSP90 together with other HSPs maintains protein homeostasis and cell survival, it was hypothesized that lipid aldehyde modification of HSP90 results in decreased chaperoning efficiency, vulnerability to cellular insults and linked to progression of disease. In a rat model of alcohol-induced oxidative stress, it was shown that HNE consistently modified HSP90, impaired the chaperoning activity through thiol modification and finally contributed to disease progression [163]. Consistent with the previous reports, in our studies we observed that pathological conditions which are linked to oxidative stress apparent with increased HNE adducts and protein carbonyl content, also impaired tissue HSP responses [164,165]. In streptozotocin-induced diabetic (SID) rats, induction of diabetes decreased HSP72 levels in liver and vastus lateralis muscle and HSP90 in liver tissue. Respectively protein carbonyl levels were increased in both tissues examined and higher levels of HNE protein adducts were observed in liver [164,165].

Cysteine adducts. Many of the LPO-derived aldehydes are capable of modifying proteins and other cellular nucleophiles through Michael addition, typically at Cys residues, causing impaired protein function [161,166, 167]. The enzyme protein disulphide isomerase (PDI) (E.C. 5.3.4.1.) is an endoplasmic reticulum protein and a molecular chaperone, which promotes the repair of incorrectly formed disulphide bonds and is thus an important component of protein maturation. PDI is one of several proteins found to be consistently modified by HNE in response to alcohol administration in the mitochondrial fractions of liver [168,169]. Two-dimensional gels assays revealed that HNE modification was occurring in the N-terminal thioredoxin-like domain, which is the active site for the repair of disulphide bonds of the damaged or newly synthesized client proteins [170]. Modification of these active N-terminal thiol sites by HNE results in impaired substrate binding and decreased enzyme

activity [170,171]. Interestingly, despite the susceptibility of PDI to inactivation by HNE, this inhibition was considerably lower compared to the inhibition of other chaperones in response to HNE modification [168,169]. Other lipid aldehydes including MDA, acrolein and ONE cause a considerable amount of inhibition of PDI upon modifying these enzymes. While PDI was almost completely resistant to MDA inhibition, acrolein results in similar PDI inhibition to that of HNE. Treatments with comparable concentrations of ONE end up with higher enzyme inactivation compared to the other aldehydes and at higher doses ONE completely inhibit PDI activity [168].

It has been recently hypothesized that biological activities of ONE, including its apoptotic and immune responses, are dependent on the ONE sulphydryl reactivity [172]. ONE-cysteine adducts, including the most prominent 2-cyclopentenone derivatives that originated from the initial Michael addition, exhibit carbonyl function [133]. Therefore, carbonyl property of ONE-cysteine adducts may explain the potent biological and toxic activities of ONE.

Another thiol protein target for HNE and ONE modification is Peroxiredoxin-6 (PRX6). Peroxiredoxins are an emerging class of thiol-specific antioxidant enzymes, exerting peroxidase activity against hydrogen peroxide, peroxynitrite, organic hydroperoxides and phospholipid hydroperoxides [173]. PRX6 is a 1-Cys class peroxiredoxin and exists as a homodimer with a critical redox-active Cys residue, which is involved in the peroxidase activity. Recent studies demonstrated that both HNE and ONE caused cross-linkage of PRX6 via Cys-Lys and Lys-Lys cross-links, resulting in the inactivation of the enzyme [174].

Mitochondrial aldehyde dehydrogenase (ALDH2; EC 1.2.1.3), an enzyme containing Cys nucleophile in its catalytic site, catalyses oxidation of the lipid aldehyde to the non-electrophilic 4-hydroxynon-2-enoic acid (HNA) in a NAD-dependent manner. HNE and ONE are both inhibitors and substrates for the enzyme. It was previously reported that the enzyme was modified reversibly by low micromolar concentrations of HNE [167]. Mass spectral analysis data demonstrated aldehyde dehydrogenase active sites were covalently modified by high concentrations of ONE [175].

Glutathione adducts. GSH is the most abundant intracellular thiol, present in virtually every animal cell in millimolar concentrations [176]. Directly or indirectly GSH plays a key role in many physiological functions, including antioxidant defense, storage and transport of cysteine, synthesis and degradation of proteins, transportation of amino acid, regulation of enzymes, synthesis of deoxyribonucleotide precursors of DNA, in conjugation with exogenous and endogenous compounds; detoxification of electrophilic xenobiotics, regulation of prostaglandin metabolism, modulation of redox regulated signal transduction and regulation of cell proliferation and immune functions [177,178]. GSH is a major antioxidant which provides an appropriate reducing milieu inside the cell [179]. GSH can directly scavenge ROS or enzymatically via GSH peroxidases and GSH transhydrogenases [177]. GSH-adducts of a wide range of reactive intermediates have been studied intensively *in vitro*, including Michael acceptors and HNE (from lipid hydroperoxides) [180]. HNE is more reactive towards GSH than other α,β-unsaturated aldehydes. Reaction of HNE with thiols ends up with the formation of HNE cyclic hemiacetal adducts which were observed as four isomeric adducts (H1-H4) by LC-ESI/SRM/MS analysis [181]. Recently GSH-adducts derived from ONE have been discovered as major GSH-aldehyde adducts. GSH addition to ONE leads to the formation of an unusual thiadiazabicyclo-ONE-GSH-adduct (TOG), a major endogenous GSH-adduct formed during oxidative stress [182,183]. Recent studies have also shown that TOG (as its dimethyl ester derivative) can induce endothelial cell apoptosis when intracellular GSH levels are lower than 1 mM [181]. In addition, GSH-adducts of ONE and its structural analogues are more abundant and more potent than GSH-adducts of HNE (GS-HNE) at inducing apoptosis [55]. Nevertheless, GS-HNE adducts were rapidly formed when cells were treated with HNE and resulted in an augmented apoptotic response [184]. Similarly, ít has been shown that the GS-HNE adducts rather than HNE may be involved in the signal transduction pathways that mediate increased COX-2 and lipoxygenase expression [183,185]. This evidence suggests that lipid hydroperoxide-derived endogenous GSH-adducts are involved in signal transduction pathways. Moreover, there is growing evidence that endogenous aldehyde-GSH adducts and their metabolites can be used as quantitative biomarkers of oxidative stress using high sensitivity APCI/MS systems, where TOG has a clear advantage over GS-HNE adducts [186]. In contrast to GS-HNE adducts, which are detected as four different diastereomers, in LC-MS analyses, TOG is eluted as a single isomer. Furthermore, sensitivity of TOG detection can be increased by 30-fold in LC-MS assays using PFB [181].

Interaction of LPO products with biomembranes

Biomembranes are the barrier of the cell towards its environment, but its changes in structure and function due to stress conditions are often neglected. Still, the fact is that the first contact and defence against environmental stress lies in cell membranes, together with the energy production processes which are bound to endogenous membranes. Biomembranes

are consisted mainly of lipids, which make between 30–80% by mass, proteins making 20–60% and carbohydrates 0–10% [187].

Membranes are described by Singer and Nicolson [188] with the Fluid Mosaic Model. The model presents membrane as a fluid due to hydrophobic integral components, like lipids, which move laterally or sideways through the membrane. The membrane are lipid bilayers in which protein moieties are inserted going either entirely through the membrane or just being located inside or outside the membrane. Phospholipid components of the membrane fold themselves, creating a double layer in polar surrounding like water. Membrane is the basic structure which segregates cell content from the surrounding and also surrounds cell organelles, defining their function. Alternation of all these factors, structure, content and fluidity causes abnormal function and pathological processes. Fluidity is determined by the presence of PUFAs in phospholipid molecules, in both sides of the lipid bilayer. The current lipid raft hypothesis suggests that the lipid bilayer is not structurally passive, but has 'patchy' structure with spatially organized structure and, consequently, function [189]. The model proposes the existence of lipid rafts, enriched in cholesterols, raft-associated proteins, saturated lipids (i.e. sphingolipids) and the 'non-raft' matrix. These rafts are implicated in many cellular processes, such as signal transduction, membrane trafficking and protein sorting [190]. These rafts are highly dynamic microdomains, with dimensions 20–200 nm, and lifetime from 10–2 to 10^3 s. Proteins of the microdomains are often coupled to the cytoskeleton, thereby defining the spatial distribution of the domain [190].

One of the most challenging topics in lipid peroxidation is its consequence on membrane structure and function considering lipid peroxidation products. Reactive oxygen species cause lipid peroxidation having as a consequence formation of reactive aldehydes. These reactive aldehydes are implicated in many pathological as well as physiological states. Still, their interactions with membrane structures, especially with membrane phospholipids, are not nearly understood. Major product of n-6 polyunsaturated fatty acid (arachidonic and linoleic acid, especially) peroxidation is HNE. Also, HNE is one of the reactive aldehydes with high biological significance by taking part in cell growth modulation, signal transduction, induction of apoptosis [137,191,192]. As a product of PUFA peroxidation, the first molecules to be modified with HNE are membrane molecules, phospholipids and membrane proteins. Therefore, modification of membrane lipids and proteins consequently causes modification of membrane function and fluidity [187,193]. Also, it is essential to emphasize that lipophilic properties of HNE are much more pronounced than its hydrophilic properties. Thus, HNE tends to be distributed in biomembranes rather that in aqueous compartments of the cells, which is important for its biological effects [194].

One of the important characteristics of biomembranes is distribution of phospholipids in the inner and outer leaflet of plasma membrane. Aminophospholipids, phosphatidyl ethanolamine, PE, and phosphatidyl serine, PS, are located in the inner leaflet of the cell plasma membrane. Phosphatidyl serine is kept in the inner leaflet by flippases. When the cell is designated to apoptosis, flippases are inactivated and PS could also be found in the outer leaflet. Both of these phospholipids contain a primary amino group and can therefore react with HNE [195]. Still, PS reacts poorly with HNE, possibly due to the presence of a carboxyl group in the close surrounding of amine group. On the other hand, reactions of PE with HNE seem to have biological significance.

Ethanolamine phospholipids are composed of two main sub-classes, diacyl-glycero-phosphoethanolamine (DA-GPE) and alkenyl acyl-glycero-phosphoethanolamine (plasmalogen PE). HNE binds to PE covalently, forming at least three different adducts: Michael adduct, which is the major reaction product and the result of addition of HNE via its double bond to the primary amine of PE; Schiff base adduct, the result of condensation of HNE's carbonyl group with the primary amine of PE; and a pyrrole derivative, cyclization product of the latter adduct [193,196]. The same type of reactions occurred in other α,β-aldehydes, HHE, peroxidation product of n3-PUFA, and 4-hydroxy-2,6-dodecadienal (HDDE), issued form of 12-lipoxygenase reaction with arachidonic acid (AA) [137]. Hydrophobicity of these three aldehydes, HHE, HNE and HDDE, defines their reactivity toward PE, making HDDE the most reactive, followed by HNE, and HHE being the least reactive of the three.

HNE binds to both PE sub-classes with no difference in binding capacity, but alkenyl acyl-GPE are further degraded when HNE is bound [195,196]. In addition to making covalent adducts with PE, HNE may alternate the alkenyl chain of plasmalogen PE. This alternation might be important in antioxidant potential of plasmalogen PE. Namely, studies using plasmalogen-deficient cells and *in vitro* model systems have supplied evidence that plasmalogens may have a protective role during oxidant-induced stress [194].

Biological importance of interactions between HNE and PE is easily understood when put in context of PE as a source of AA. AA metabolizes to prostaglandins, leukotriens, thormboxanes and eicosanoids. In platelets, PE appears to be a major source of AA, which is released by phospholipase A_2 (PLA_2) [197]. If bound to PE, HNE decreases 2-fold activity of PLA_2 and completely diminishes activity of phospholipase D (PLD) [194]. In this way HNE may regulate inflammation and the cell response to the same.

Another example of the importance of HNE-PE interactions is reflected in inhibition of platelet aggregation by these adducts [198], once again strengthening the bimodal role of HNE in the cell.

Michael adducts may alter membrane fluidity and HNE bound to membrane anchored proteins may alter their functions. An example of membrane protein modification is augmentation in Ca^{2+} current in dentate granulate cells exposed to HNE. In addition, the effect is specific to L-type Ca^{2+} channels, which are associated with amyloid β protein (A,β)-induced cell death [199]. The mechanism showed that HNE triggers $Ca^{2}+$ influx via L-type calcium channels by interacting with its thiol groups [200]. It is important to emphasize that HNE and other α,β-aldehydes react with proteins, including especially their lysine, cysteine and His residues. These reactions may cause protein cross-linking, leading to the formation of fluorophores [191]. Biological consequence of protein cross-linking may be either inactivation of function or activation of certain receptors with consequence in signal transduction. Physically, protein cross-linking rearranges protein moieties in membrane, thereby changing physical properties of the membrane.

One of the first described effects of lipid peroxidation on membranes was plasma membrane blebs. Blebs are plasma membrane distortions, which can rupture and discharge cell content [201]. Blebs are associated to apoptosis, together with cell shrinkage, dynamic membrane blebbing, chromatin condensation, DNA laddering, loss of plasma membrane phospholipid asymmetry, reduction of ATP, mitochondrial oxyradical generation and mild calcium overload [202]. It is generally believed that blebs appearance is associated to oxidation of cytoskeleton-associated protein thiols. Furthermore, blebs are not only consequence of apoptosis, but represent novel sources for biologically active oxidized phospholipids [199], contributing to the initiation and progression of chronic inflammatory processes in the organism [203]. Still, the exact mechanism of blebs formation is not yet undersood.

When discussing lipid peroxidation products, mostly reactive aldehydes are elaborated. Still, there are reports about isoketals (isoK), highly reactive γ-ketoaldehydes formed by rearrangement of the isoprostane pathway of lipid peroxidation [204]. IsoK are highly reactive and rapidly form protein adducts and underlie proclivity to cross-linked proteins. Indeed, IsoK form Schiff base and pyrrole adducts with PE *in vitro*. Still, there is a need to explore their formation *in vivo* and their biological relevance.

Finally, interactions of LPO products with biomembranes are a new and insufficiently explored field. The complexity of these interactions is demonstrated through reactions of LPO products with both proteins and lipids in membrane and coupling of membrane rafts to cytoskeleton and plasmatic proteins. Modulation of signal transduction and cellular processes of HNE, one of the most intriguing LPO products, may be either by interactions with PE, thereby interfering with lipid rafts, or by interactions with membrane proteins. Therefore, progress in LPO research may be achieved by interdisciplinary collaborations which comprehend complex (bio) chemistry and biology of HNE and other reactive aldehydes.

Lipid oxidation products in cell signalling

Basic principles of lipid oxidation product reactivity into cells

Basal HNE concentration in human blood and serum is ~ 0.05–0.15 μM and it increases with age [205]. Nutrition influences these values since HNE is a lipoperoxidation product derived from linoleic acid n-6 PUFA [78]. Antioxidants, intestinal flora can also affect HNE circulating concentration [206,207]. Under conditions of oxidative stress (rheumatologic diseases for example), HNE concentrations increase up to 3–10-fold of physiological concentrations [208].

In mammalian cells, HNE is highly reactive and rapidly metabolized (3–5 min) depending on the battery of detoxifying enzymes expressed in the cell type [209]: alcohol dehydrogenase, GST, ALDH, respectively, leading to DHN, GS-HNE and HNA; 1–8% of HNE is conjugated to proteins, on histidine, cystein and lysine. The metabolites have a low reactivity and the majority of the biological effects depend on HNE itself [69,98].

HNE has several intracellular biochemical targets: proteins, lipids and nucleic acids. When it reacts with proteins it can increase proteolysis by proteasome [58]. HNE-protein adducts impair protein function and, so, trigger signalling and enzymatic disturbances. The formation of GS-HNE is reversible; a retro Michael cleavage could occur and liberate bioactive HNE in another site, as was shown in liver and kidney [69]. HNE can also react with phospholipids of the plasma membrane, leading to potential modifications of its biophysical properties [210] (see above). HNE is genotoxic, by interacting directly with guanine or through its oxidative epoxide product [211–213]. These reactions could induce a nuclear stress and affect cell cycle and growth. Finally, HNE can also have epigenetic effects, since HNE can bind histones and, so, control chromatin condensation and gene expression [214].

As a major highly reactive aldehydic product of lipoperoxidation (LPO), HNE is able to exert cytotoxic, mutagenic and carcinogenic effects. Its appropriate and rapid catabolism could significantly contribute to modulate cellular defence system and condition cellular responses.

Cell death induced by lipid oxidation products: A stressful story

HNE is produced under stress situations, its formation is directly proportional to reactive oxygen species (ROS) production. Accumulation of reactive components leads to induction of cell death if cells cannot eliminate them quickly. So at physiological concentrations, we observe an apoptosis, mainly triggered according to intrinsic type I cell death. HNE was demonstrated to be able to alter mitochondrial respiratory complexes in PC12 cells and mediate apoptosis [215]. More precisely, cytochrome *c* oxidase and aconitase activities were strongly decreased upon HNE treatment. Moreover, HNE and HHE directly inhibit adenine nucleotide translocator (ANT), which has a key role in mitochondria-dependent apoptosis [216]. These events could amplify the oxidative stress, by induction mitochondrial ROS [217]. However, HNE can also trigger mitochondria-dependent apoptosis by targeting Bcl2 anti-apoptotic members, principally located in the external mitochondria membrane [218]. Then, in colon cancer RKO cells, activation of HSF1 (heat shock factor 1) leads to BAG3 (Bcl2-associated athanogene domain 3) upregulation, which stabilizes BclXL [219] by the formation of a complex BAG3-BclXL and HSP70. This event is crucial in survival processes in cancer cells and the silencing of HSF1 allows a decrease of many Bcl2 anti-apoptotic members like Bcl2, BclXL and Mcl1 [220]. This HSF1 inhibition would constitute a strategy to sensitize cancer cells to HNE-mediated toxicity, useful in anti-cancerous therapeutics. Mitochondria-dependent apoptosis can also be promoted by oxidative nuclear stresses. p53, as a tumour suppressor protein, is usually activated by ROS and DNA damage [221]. It can act as a transcription factor and regulate in a first step cell cycle and DNA repair enzymes. Among them, p21, GADD45 or XPC are activated to arrest cell proliferation and repair DNA damage [222]. Whether damages persist, p53 secondary induces pro-apoptotic proteins, like Bax, Fas or PUMA, and represses anti-apoptotic proteins like Bcl2 and, so, triggers apoptosis [223]. HNE, as a pro-oxidant compound, favours p53 activation, with its phosphorylation, its accumulation and its nuclear translocation [224]. In T-cell leukaemia Jurkat cell line, HNE induces p53-dependant apoptosis, with p21 and Bax induction, that finally leads to caspase activation [225]. This process was also identified in neuroblastoma SH-SY5Y cells [226], in neuronal PC12 cells [227], in human chondrocytes [228] and in retinal pigment epithelium RPE cells [229]. Thus, HNE is a major p53 activator and accumulation of this protein was correlated to an accumulation of HNE in Alzheimer's disease (AD) [230]. However, HNE can stabilize p53 by direct binding and, so, amplify neuronal apoptosis [231]. Even if HNE was described as a genotoxic compound, we have no information about the fact that p53 could be activated by HNE via DNA damage. It seems that oxidative stress and direct binding to p53 should constitute the main pathway of p53 activation by HNE. The use of antioxidants underlines this phenomenon: JNK (c-Jun kinase) appears to be upstream p53. This MAP kinase is highly sensitive to oxidative stress and more particularly to HNE. It regulates p53 expression and so triggers HNE-dependent apoptosis [225,226,229,232,233].

LPO is a process which can induce organelles stress. We have previously commented about mitochondria and nuclear stresses, but we cannot exclude lysosome stress. Actually, it was shown in hepatocytes that ferritin-induced apoptosis was mediated by an increase of LPO, which promoted lysosomal membrane permeability and allowed the release of lysosome content, like proteases. Consequently, global cell damaging was observed with DNA and protein adducts, micronuclei, p53 activation and the drop of the mitochondrial transmembrane potential $\Delta\psi M$ [234]. This cell death depends on an early lysosome disturbance, so is qualified as an apoptotic process. It is worth noting that the cross-talk between lysosome and mitochondria is relevant in the ageing field [235], a process in which HNE is strongly involved. Future studies must then focus on this axis in order to determine the HNE-related crucial events.

Extrinsic type I cell death is an apoptotic pathway dependent on death receptors, like Fas, TNF (Tumour Necrosis Factor) receptors and TRAIL (TNF-related-apoptosis-inducing-ligand) receptors [236]. Basically, this signalling is activated by cytokines (FasL for Fas ligand, TNFα, TRAIL). For example, the binding of FasL on Fas triggers the aggregation of Fas and the formation of a pro-apoptotic complex, called DISC (Death Inducing Signalling Complex). After death domain aggregation, the receptor complex is internalized via the cellular endosomal machinery. This allows the adaptor molecule FADD to bind the death domain of Fas. FADD also contains a death effector domain near its amino terminus, which facilitates binding to caspase-8. Active caspase-8 is then released from the DISC into the cytosol, where it cleaves other effector caspases, leading to major hallmarks of apoptosis. HNE was shown to induce Fas expression, which sensitizes cells to FasL-dependent apoptosis if soluble FasL is present in the extracellular medium or transmembrane FasL is expressed in neighboured cells [237]. Fas expression can also be regulated by p53 [228,234]. However, another mean to activate death receptor pathway was described: HNE can mimic FasL and activate Fas without formation of DISC [225]. So, in HNE-triggered cell death, both intrinsic and extrinsic pathways are involved, which facilitates death signal amplification [225]. However, some cells deficient for Fas could be resistant to HNE [237], meaning that, according to the cell type, extrinsic or

intrinsic pathway would be predominant and it is barely predictable.

Depending on the cell specificity, apoptosis requires particular pathways. In neurons, recent studies have demonstrated that Toll-like receptors (TLR) were induced following energy deprivation and triggered apoptosis [238]. Tang et al. [239] have shown that HNE was able to upregulate TLR4 expression and activate JNK-dependent apoptosis. Neurons mutated for TLR4 exhibit a protection regarding HNE exposure, underlying that this expression directly affects neuronal sensitivity. This discovery is relevant in terms of AD, since a decrease of TLR4-positive cells in end-stage AD patients was observed, probably due to a specific loss of TLR4 expressing neurons.

HNE induces apoptosis by disturbing organelles (mitochondria, lysosome, nucleus), by activating stress-sensors proteins (p53, JNK) and by directly binding of key regulators (Fas, p53). HNE-dependent genotoxicity was not described yet as a process involved in cell death, but the identification of caspase 2 in HNE-induced apoptosis [232,240] could put back into question the importance of DNA damage following LPO [241] (Figure 2).

Survival processed triggered by lipid oxidation products: A balanced question?

Balance survival/proliferation. HNE, by its ability to bind proteins, can activate growth factor receptors, as was shown for EGFR (Epidermal Growth Factor Receptor) [242] and PDGFR (Platelet-Derived Growth Factor Receptor) [243]. These tyrosine kinase receptors are then autophosphorylated and trigger survival pathways involved in the proliferation of smooth muscle cells (SMC) in atherogenesis [244]. In this precise disease, the induction of apoptosis by HNE could constitute a way to avoid SMC proliferation and atherome formation. Among cardiovascular diseases, cardiac hypertrophy is characterized by increased myocardial cell size, via an important protein synthesis [245]. The mammalian target of rapamycin (mTOR)/p70S6 kinase pathway is primordial in the regulation of cell growth. It is inhibited by the system LKB1/AMP-activated protein kinase (AMPK), which acts as an energy sensor, limiting anabolism [246]. A recent study has reported that HNE levels are higher in the blood and heart of hypertensive rats. In isolated cardiomyocytes, HNE forms adducts with LKB1 that inhibits the LKB/AMPK pathway and activates the mTOR pathway. Treatment with resveratrol prevents such events *in vitro* and reduces hypertrophy *in vivo*, suggesting the preponderant role of HNE in the development of cardiac hypertrophy [247]. However, the regulating role of HNE on energy sensing systems was also described in tumour growth [248], stressing the importance of LPO in anabolic homeostasis.

In other pathologies like age-related bone loss, HNE accumulation was associated with a decrease of osteoblast number. Osteoblastogenesis is related to the activation of Wnt/β catenin/Tcf (T-cell specific transcription factor) pathway, ensuring the proliferation and the differenciation of osteoblasts [249]. However, an increase of ROS associated with the ageing process favours LPO and HNE production and consecutive activation of FoxO- (Forkhead box O) mediated transcription. Since FoxO-regulated

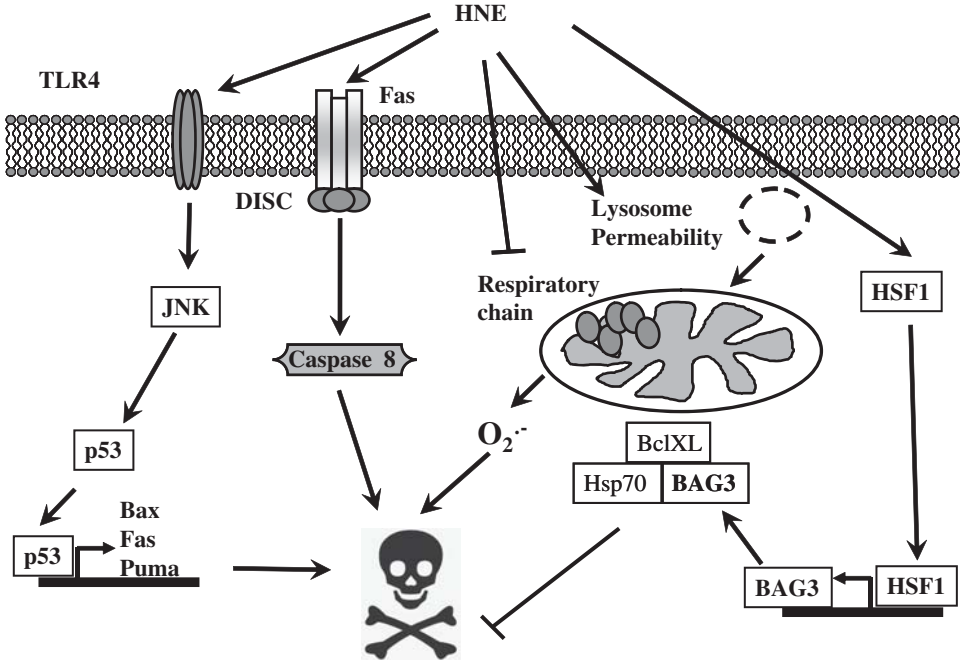

Figure 2. Main apoptotic pathways triggered by HNE.

transcription requires β catenin, the pool of β catenin acting with Tcf decreases and so attenuates Wnt-regulated gene expression [250]. Moreover, at the same time, oxidized lipids bind and activate PPARγ, which promotes β catenin degradation. According to this cascade, LPO is a major process in the decline of osteoblast number, by the extinction of Wnt/β catenin pathway required for their differentiation and survival [251].

Balance survival/cell death. HNE is able to repress the survival process and induce apoptosis by acting on the cell death/survival balance. The TrkA receptor is a high affinity receptor for nerve growth factor (NGF) and is located in lipid rafts [252]. In cholinergic neurons, which express TrkA receptors, HNE favours the oxidation of lipid rafts and, so, impairs internalization and transport of signalling endosomes. Moreover, it prevents retrograde transport, which finally leads to a decrease of TrkA receptors at the plasma membrane and anneals the pro-survival signal. This mechanism could explain the loss of a particular neuron population implicated in memory and attention function in neurodegenerative diseases [253].

HNE also deregulates cell death/survival balance by altering the apoptosis pathway. In the previous part was described the activation of Fas by HNE, via its direct interaction with Fas, mimicking FasL. However, Awasthi et al. [224] have demonstrated an inhibitory loop allowing the self-limitation of the pro-apoptotic signal mediated by HNE. This process involves Daxx, a nuclear protein which is associated to different DNA binding transcription factors and represses their activities. During stress, Daxx translocates to the cytoplasm and acts as a death receptor adaptor at the cell surface [254]. Its role as a pro-survival or pro-apoptotic mediator is still debated. Some studies suggest that Daxx binds Fas and activates apoptosis with the involvement of ASK1 (Apoptosis Signal Regulating Kinase 1) which activates JNK. This process is independent of the DISC formation. In CRL2571 cells, HNE induces Daxx translocation and binding to Fas, with an activation of ASK1 and JNK. However, experiments on Daxx-deficient cells reveal a potentialization of HNE-induced apoptosis, with a higher ASK1/JNK activation and caspase 3 cleavage. So, in this system, Daxx exerts a brake in Fas-dependent apoptosis following HNE treatment. The authors have also demonstrated that this inhibitory action of Daxx was also relevant in the case of classical activation of Fas by Fas antibodies [225]. The protective role of Daxx is extended to the regulation of HSF1. Actually, the release of Daxx from a nuclear compartment allows the upregulation of HSF1, which induces the expression of HSF1-target genes, like Hsp70, involved in cell defence against oxidative stress [219]. This self-limiting signal could have a physiological role, because HNE is highly diffusible. Its spreading among tissue could have detrimental consequences on tissue homeostasis. With this process, apoptosis would stay limited, without affecting neighboured cells.

Balance proliferation/differentiation. The Notch signalling pathway has been implicated in the development of several leukaemia and lymphoma. It is commonly mutated or its ligand Jagged is frequently over-expressed in acute myelogenous leukaemia, leading to a persistent activation [255]. The inhibition of this pathway is a strategy to prevent cell proliferation and promote cell differentiation. In HL60 cells (human promyelocytic leukaemia cells), HNE induces a down-regulation of Notch1. Thus, Notch target genes, like Hes1, are repressed and inhibit cell proliferation [256]. No apoptosis induction was observed but an induction of differentiation: this suggests that HNE acts in that case on the balance proliferation/differentiation. This work also underlines Notch as a molecular target of HNE and knowing the multiple roles of Notch in carcinogenesis [257], development [258], neuronal diseases [259], etc…, it can open new perspectives of studies, in relationship with LPO.

Balance survival/autophagy. Autophagy is a major process regulating catabolic reaction. This pathway is involved in the destruction of dysfunctional organelles and is essential for cell growth and development to balance protein synthesis, organelle biogenesis and degradation. The two major protein degradation and recycling pathways are the Ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway. The UPS is responsible for the degradation of short-lived proteins, whereas autophagy regulates long-lived proteins and organelles. Macroautophagy implies the engulfment of cytoplasmic constituents within cytoplasmic vacuoles and their delivery to lysosomes for degradation. This phenomenon provides substrates for energy metabolism and recycles amino-acids, fatty acids and nucleotides [260]. It is a low level but constitutive process which could be increased upon nutrient deprivation, growth factor withdrawal and other stresses like protein aggregation. mTOR is a negative regulator of autophagy and its inhibition by rapamycin activates autophagy [261]. Autophagy is associated to diseases occurrence, like ageing, cancer and neurodegenerative disorders and can mainly be detected by microscopy analysis of the vacuolization, phagophores and multilamellar vesicles [262]. HNE, as a major cellular stressor, could act on this process; however, the number of studies is quite limited. Relative to cardiovascular diseases,

HNE and acrolein are involved in the activation of autophagy in SMC *in vitro* [263]. HNE modifies proteins that are gradually removed from cells and this process is amplified by cotreatment with rapamycin. HNE, nonenal and acrolein increase the formation of LC3II, a protein located at the membrane of vacuoles. Electron micrographs, with characteristic vacuolization, confirm the induction of autophagy by aldehyde-modified proteins. This process is relative to survival because, when inhibited by 3-methyladenine, it is an apoptotic cell death that is triggered: the accumulation of proteins modified by LPO is deleterious for cell life. The ability for aldehydes to induce autophagy appears dependent on their electrophilicity: acrolein is the more potent inducer, followed by HNE and nonenal. Saturated aldehyde nonanal is inactive, whereas phospholipid aldehyde is barely effective. Finally, it is not the simple protein modification by HNE that promotes deleterious effects, but the capacity to form protein cross-linking that strongly affects cell homeostasis

Autophagy is a primordial process in very specialized cell types, like retinal pigment epithelial cells (RPE). They are non-dividing long-lived cells that require continuous renewal. They are at the interface of photoreceptor layer and choriocapillaris and ensure the photoreceptor outer segment (POS) phagocytosis to maintain the visual cycle. Progressive dysfunction, accelerated in age-related macular degeneration (AMD), leads to accumulation of imperfectly degraded material (called lipofuscin) in acidic vacuoles [264]. This process of lipofuscinogenesis implies HNE and MDA modifications of POS and lysosomal degradation becomes harder in the long-term [265]. In a recent study, Krohne et al. [266] demonstrate that HNE- and MDA-modified POS strongly reduce autophagy in RPE cells. Consequently, they are then more susceptible to cell death regarding any other stresses and the accumulation of this undigested material could contribute to cell ageing and degeneration.

Importance of detoxication/metabolism of lipid oxidation product in cell signalling

We have described non-exhaustively some relevant signalling processes targeted by HNE. Some studies underline the fact that detoxification mechanisms are major in their occurrence. Thus, GSTs, like GSTA4–4 and GST5.8, are key enzymes controlling free HNE concentration. Their over-expressions decrease intracellular free HNE and abrogate HNE-induced effects [225,228,267,268]. When HNE is conjugated to GSH, its reactivity becomes low. In some cells, HNE induces a depletion of GSH. However, some others enzymes like aldehyde dehydrogenase and aldose reductase would also play a role in detoxification. For example, in neurons, ABAD (amyloid β peptide binding-alcohol dehydrogenase) detoxifies HNE when it is over-expressed in SH-SY5Y [269]. This mitochondrial enzyme is inhibited by amyloid β peptide and the absence of HNE catabolism renders it toxic. In the retina, photoreceptor retinol dehydrogenase 12 (RDH12) mutations cause retinal dystrophy. *In vivo* experiments demonstrate that RDH12 prevents light-induced apoptosis of photoreceptors, by limiting the formation of HNE-adducts. The biotransformation of HNE into non-toxic alcohol protects cells from macromolecules modifications [63].

The basal level of intracellular GSH has an importance in HNE toxicity. If GSH is low, it would increase the rate of HNE metabolization. However, basal cellular levels of GSH are physiologically 3-fold higher that HNE concentration and HNE is able to rapidly upregulate glutamate cystein ligase to promote GSH neosynthesis [224,270].

Elimination of HNE as GS-HNE is an efficient mechanism to decrease HNE bioavailability and limit its toxicity. RLIP76 is a Ral binding GTPase-activating protein which transports GS-HNE and metabolite conjugates according to an ATP-dependent manner. Its inhibition strongly triggers HNE accumulation and could lead to apoptosis [271–273], even in the absence of any stressor [184]. Ascorbic acid was shown to promote GS-HNE efflux, via MRP and, hence, prevent apoptosis [88].

Regarding signalling processes mediated by HNE, it appears primordial to consider the molecular events in their whole (metabolism, stress origin, balance survival/proliferation/autophagy/cell death) in a cell type context, to really evaluate the consequences of the products of lipoperoxidation.

Aldehydes as bioactive markers of LPO

The most sensitive cellular target of free radical reactions may represent PUFAs. Lipid peroxidation leads to the formation of a broad array of different products with diverse and powerful biological activities. Among them are a variety of different aldehydes. The primary products of lipid peroxidation, lipid hydroperoxides, can undergo carbon–carbon bond cleavage via alkoxyl radicals in the presence of transition metals, giving rise to the formation of short-chain, unesterified aldehydes of 3–9 carbons in length and a second class of aldehydes still esterified to the parent lipid [35]. The important agents that give rise to the modification of a protein may be represented by reactive aldehydic intermediates, such as 2-alkenals and 4-hydroxy-2-alkenals (Figure 3) [35,39]. These reactive aldehydes are considered important mediators of cell damage due to their ability to covalently modify biomolecules, which can disrupt important

cellular functions and can cause mutations [35]. Furthermore, the adduction of aldehydes to apolipoprotein B in LDL has been strongly implicated in the mechanism by which LDL is converted to an atherogenic form that is taken up by macrophages, leading to the formation of foam cells.

Excellent review papers have been published on the use of HNE-protein adducts as biomarkers of lipid peroxidation [274] and on the methodological aspect of their measurement [275], so we will focus on the saturated aldehydes, such as hexanal, that are most abundantly formed [276,277]. Upon reaction with proteins, these aldehydes react with lysine residues to form an imine or Schiff base adduct [278]. Due to the reversible nature of such unconjugated Schiff bases, these aldehydes have received relatively little attention as the causative agent for modification of nucleophilic biomolecules. However, Ishino et al. [279] have recently established a novel mechanism of irreversible covalent protein modification by aldehydes, in which H_2O_2 and alkyl hydroperoxides mediate the binding of saturated aldehydes to the lysine residues of protein to generate structurally unusual *N*-acylation products.

Here I provide an overview of studies on lipid peroxidation-specific adduction of proteins by the most reactive aldehydes 2-alkenals. In addition, the latest finding on the involvement of H_2O_2 and hydroperoxides in covalent binding of n-alkanals to protein generating *N*-acylation products is also described.

Covalent protein modification by 2-alkenals

2-Alkenals represent a group of highly reactive aldehydes containing two electrophilic reaction centres. A partially positive carbon 1 or 3 in such molecules can attack nucleophiles, such as protein. Acrolein and its methyl derivative, crotonaldehyde, represent the most potent electrophilic 2-alkenals commonly detected in mobile source emissions, cigarette smoke and other products of thermal degradation [280]. Thus, they had been considered as the 'unnatural' environmental pollutants; however, recent studies revealed that these aldehydes were endogenously produced under oxidative stress [281–284].

Among all the α, β-unsaturated aldehydes, acrolein shows the greatest reactivity with proteins. Upon reaction with protein, acrolein selectively reacts with the side-chains of the cysteine, histidine and lysine residues. Of these, lysine generates the most stable product. The β-substituted propanals (R-NH-CH_2-CH_2-CHO) and Schiff's base cross-links (R-NH-CH_2-CH_2-CH=N-R) had been suggested as the predominant adduct; however, the major adduct formed upon the reaction of acrolein with protein was identified as a novel lysine product, N^{ε}-(3-formyl-3,4-dehydropiperidino)lysine (FDP-lysine) (Figure 4), which requires the attachment of two acrolein molecules to one lysine side chain [283, 284]. The formation of a similar FDxP-type adduct (dimethyl-FDP-lysine) has been reported in the lysine modification with the acrolein analogue, crotonaldehyde [281]. In addition, these FDP-type adducts have also been detected in the reaction of other 2-alkenals, such as 2-pentenal and 2-hexenal, with the lysine derivative, suggesting that the condensation reaction via formation of the Michael addition-derived imine derivatives is characteristic of the reaction of 2-alkenals with primary amines. Due to the fact that the core structure of the FDP-lysine is resistant to the conventional acid hydrolysis of proteins even without reduction by pre-treatment with sodium borohydride, the FDP adducts of acrolein and crotonaldehyde have been successfully detected not only in the acrolein-treated LDL but also in LDL exposed to metal-catalysed oxidation [283]. Furthermore, by use of a monoclonal antibody, the detection of the FDP-lysine has so far been reported in

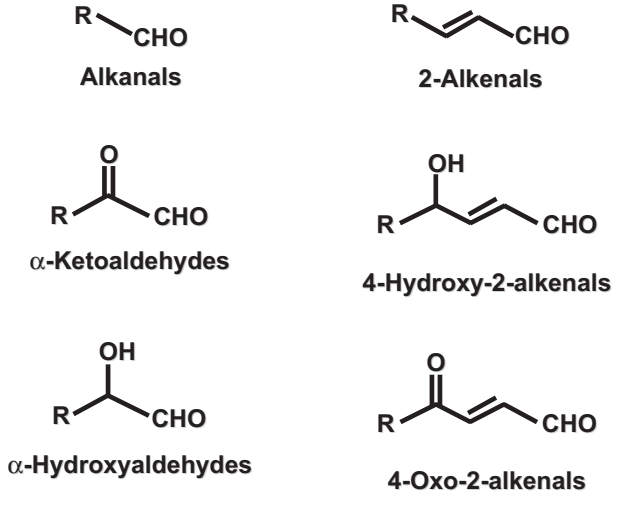

Figure 3. Reactive aldehydic intermediates.

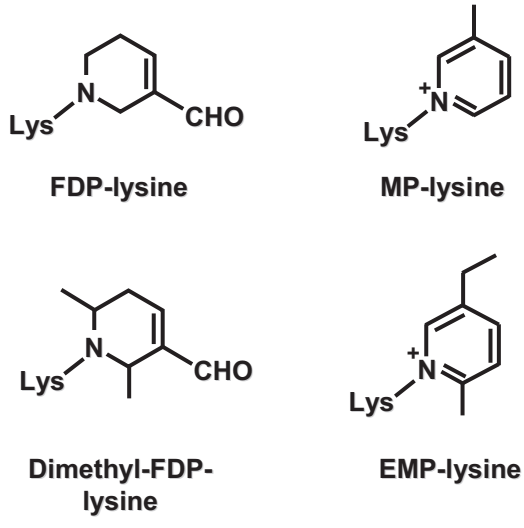

Figure 4. Lysine adducts.

plaque deposits of atherosclerotic lesions [284] and neurofibrillary tangles and plaque neuritic elements in Alzheimer's disease [285]. These observations are in line with the accumulating body of literature supporting the role of oxidative stress in the pathogenesis of these disorders. In a later study, Furuhata et al. [286] also revealed the electrophilic potential of FDP-lysine and established a novel mechanism of protein thiolation in which the FDP-lysine generated in the acrolein-modified protein reacts with sulphydryl groups to form thioether adducts.

On the other hand, it was also shown that acrolein modification of lysine generates an alternative acrolein-lysine adduct, N^{ε}-(3-methylpyridinium)lysine (MP-lysine) [287]. The formation of MP-lysine can be reasonably explained by the mechanism involving the formation of a Schiff base derivative as the first intermediate. The Schiff base further reacts with a second acrolein molecule via a Michael addition to generate an imine derivative. The subsequent conversion of this imine derivative to the final product (MP-lysine) obviously requires two oxidation steps and intramolecular cyclization. The formation of these lysine-pyridinium species in proteins results in the placement of a fixed, positive charge on the e-amino group. Moreover, in contrast to the fact that the FDP-type adducts are unstable intermediates against nucleophilic addition, the pyridinium adducts are highly stable end products. The formation of the pyridinium adducts is also a dominant pathway for the modification of the primary amine with 2-alkenals, such as crotonaldehyde, 2-hexenal and 2-octenal [281]. Based on the identification of a novel acrolein-lysine adduct (MP-lysine), Furuhata et al. [287] re-examined the specificity of the monoclonal antibody raised against acrolein-modified protein and found that the antibody recognized MP-lysine far more efficiently than FDP-lysine. The preferential recognition of the antibody to MP-lysine has been explained by the structural characteristics in the side chain of these adducts. In contrast to FDP-lysine, MP-lysine contains a more fixed, positive charge on the pyridinium side chain, which may represent important immunological epitopes. Indeed, a monoclonal antibody raised against crotonaldehyde-modified proteins recognized a similar pyridinium adduct, N^{ε}-(5-ethyl-2-methylpyridinium) lysine (EMP-lysine), as the major epitope [281]. In addition, Nagai et al. [288] have raised a monoclonal antibody against glycolaldehyde-modified protein and found that a lysine pyridinium adduct constitutes an epitope of the antibody.

Among the 2-alkenals, 2-nonenal is probably the most well recognized substance due to its relevance to ageing. It has a characteristically unpleasant greasy and grassy odour. It is also a major contributor to the unpleasant cardboard flavour in aged beer. It was previously shown that 2-nonenal could be formed through lipid peroxidation as a product in peroxide-mediated oxidation of high concentrations of linoleic acid hydroperoxide or from liver microsomes treated with ADP/iron *in vitro* [35]. Toyokuni et al. [289] also reported the production of C2–C12 saturated and unsaturated aldehydes, including 2-nonenal, in the kidney of rats exposed to ferric nitrilotriacetate (Fe^{3+}-NTA), an iron chelate that induces acute renal proximal tubular necrosis, a consequence of free radical-mediated oxidative tissue damage, eventually leading to a high incidence of renal adenocarcinoma in rodents. More recently, Haze et al. [290] analysed the body odour components that adhered to the subjects' shirts by GC/MS and demonstrated that 2-nonenal is present in increasing amounts in the body odours of persons 40 years or older. They also suggested that *cis*-2-nonenal and *trans*-2-nonenal are formed from the oxidative degradation of polyunsaturated fatty acids, such as palmitoleic acid. A monoclonal antibody against protein-bound 2-nonenal was recently developed and the epitope structure recognized by the antibody was identified to be novel 2-nonenal-lysine adducts possessing a pyridinium structure [279]. The immunohistochemical studies also demonstrated the formation of the immunoreactive materials in the kidney of rats exposed to Fe^{3+}-NTA. Furthermore, using high performance liquid chromatography with on-line electrospray ionization tandem mass spectrometry, the formation of the 2-nonenal-lysine pyridinium adducts during the lipid peroxidation-mediated modification of protein has been confirmed *in vitro* and *in vivo*.

H_2O_2-mediated protein modification by n-alkanals

Among the variety of lipid peroxidation-derived aldehydes, the saturated aldehydes, such as hexanal, are most abundantly formed [276,277]. Hexanal, an aldehyde produced in high quantity during lipid peroxidation, shows metabolic, genotoxic and mutagenic effects, as well as inhibitory effects on cell proliferation [35]. It has also been shown that hexanal is by far the major aldehyde and that its production correlates well with the oxidation of PUFAs in LDL and reflects the degree of LDL oxidation *in vitro* [291–294]. Moreover, a remarkable over-production of this aldehyde was shown in skin fibroblasts from a patient with cardiomyopathy and cataracts both under basal conditions and after menadione or doxorubicin treatment *in vivo* [295]. Other short chain n-alkanals, such as acetaldehyde, have also been detected in micromolar amounts in the effluent perfusates of hearts perfused with a free radical-generating system and are proposed to be useful markers for monitoring oxidative stress during reperfusion of ischemic myocardium [296].

It is well recognized that saturated aldehydes mainly form Schiff bases through the formation of unstable

carbinolamine intermediates. However, due to the reversibility of this reaction, the simple aldehydes have received relatively little attention as protein-modifying reagents. Ishino et al. [279] recently discovered that H_2O_2 and to a lesser extent alkyl hydroperoxides are capable of mediating covalent modification of proteins by saturated aldehydes (Figure 5). This finding suggests the possibility that saturated aldehydes, in combination with H_2O_2 or ROOH, may contribute to the modification of nucleophilic biomolecules and the development of tissue damage under oxidative stress. A probable mechanism for the reaction has been suggested to be the imine analogue of the Baeyer-Villiger reaction of ketones with peroxides to give esters, which also pertains to the mechanism of oxidation of aldehydes to carboxylic acids by ROOH. The reaction would proceed by addition of ROOH (R=H, alkyl) to the Schiff base, followed by 1,2-migration of hydride and expulsion of H_2O or alkyl-OH, respectively. The reaction may be acid-catalysed (to create a better leaving group) and would be more efficient for the latter reason using a peracid rather than ROOH. At the same time, however, it is well known that potent oxene donors like peracids react with imines (usually in organic solvent) directly to generate oxaziridines, semi-stable species that decompose to amides only upon heating or in the presence of transition metal catalysts [297]. Although the Schiff base-derived peroxycarbinolamine could decompose to oxaziridine in competition with 1,2-hydride migration, the distinction of the Baeyer-Villiger as opposed to oxaziridine pathway has been pointed out in the literature [298]. In the reaction using 3-chloroperoxybenzoic acid at low concentration, no oxaziridine product was observed, although it is unclear whether such species would survive the ionization conditions. In any event, since the generation of circulating peracids in physiological oxidative stress is unlikely, it seems unnecessary at this time to invoke a competing oxaziridine pathway for amide formation.

N^{ε}-Hexanoyllysine formed upon the reaction of a lysine derivative with hexanal in the presence of H_2O_2 was previously identified as a product of the reaction of the lysine residue with the oxidized linoleate, 13-hydroperoxyoctadecadienoic acid [299]. The authors speculated the direct interaction between the lipid hydroperoxide and the lysine residues of protein as an underlying mechanism. Metz et al. [300] also isolated a similar *N*-hexanoylated derivative of pyridoxamine and proposed a mechanism in which the conjugated diene hydroperoxides oxidatively decompose to ketoaldehydes, which then react with the primary amine to form the *N*-acylation product through the formation of a hemiacetal derivative. Based on the fact that lipid peroxidation generates a great number of oxidized products, including aldehydes and reactive oxygen species, the combined action of saturated aldehydes and either H_2O_2 or ROOH is likely to occur. Thus, N^{ε}-hexanoyllysine, which has been considered to be one of the earlier and stable markers for lipid peroxidation-derived protein modification compared to the aldehyde-derived protein adducts [301], may actually be the product of the lysine modification by hexanal, originating from the lipid hydroperoxide, and either H_2O_2 or the circulating lipid hydroperoxide directly as the oxidant.

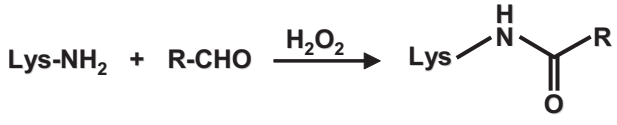

Figure 5. Reaction of saturated aldehydes with lysine.

In conclusion, this part of the review summarized the protein adduction chemistry with volatile aldehydes, such as 2-alkenals and n-alkanals, generated from the peroxidation of polyunsaturated fatty acids. The knowledge of the protein reactivity of these volatile aldehydes provides an under-pinning for the eventual interpretation of various types of biological activities that are being observed for these important exogenously and endogenously formed molecules. Furthermore, it has been shown that the protein bound to aldehydes could be an excellent immunogen that is capable of stimulating an adaptive immune response. Of interest, a monoclonal antibody, showing recognition specificity toward DNA, has been shown to bind protein-bound aldehydes. These findings suggest the connection between modification of proteins by aldehydes and autoimmune response. Further studies are required to understand the biological consequences of the production of aldehydes under oxidative stress.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Gray JI, Monahan FJ. Measurement of lipid oxidation in meat and meat products. Trends Food Sci Technol 1992;3:315–319.

[2] Kubow S. Routes of formation and toxic consequences of lipid oxidation products in foods. Free Radic Biol Med 1992;12:63–81.

[3] Edreva AM, Georgieva ID, Cholakova NI. Pathogenic and non-pathogenic stress effects on peroxidases in leaves of tobacco. Environ Exp Bot 1989;29:365–373, 375–377.

[4] Galliard T, Phillips DR, Matthew JA. Enzymic reactions of fatty acid hydroperoxides in extracts of potato tuber II. Conversion of 9- and 13-hydroperoxy-octadecadienoic acids to monohydroxydienoic acid, epoxyhydroxy- and trihydroxymonoenoic acid derivatives. Biochim Biophys Acta Lipids Lipid Metab 1975;409:157–171.

[5] Hailstones MD, Smith MT. Lipid peroxidation in relation to decilning vigour in seeds of soya (Glycine max L.) and cabbage (Brassica oleraceae L.). J Plant Physiol 1988;133:452–456.

[6] Gardner HW. Sequential enzymes of linoleic acid oxidation in corn germ: lipoxygenase and linoleate hydroperoxide isomerase. J Lipid Res 1970;11:311–321.
[7] Dix TA, Aikens J. Mechanisms and biological relevance of lipid peroxidation initiation. Chem Res Toxicol 1993;6:2–18.
[8] Esterbauer H. Aldehydic products of lipid peroxidation. In: Mc Brien DC, Slater TF, editors. Free radicals, lipid peroxidation and cancer. London, UK: Academic Press; 1982. p. 101–128.
[9] Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nutrition 1993; 57:S715–S724.
[10] Esterbauer H. Cytotoxicity and genotoxicity of lipid-oxidation products. Am J Clin Nutr 1993;57:S779–S785.
[11] Uchida K. Future of Toxicology/Lipid Peroxidation in the Future:From Biomarker to Etiology. Chem Res Toxicol 2006;20:3–5.
[12] Murphy RC, Johnson KM. Cholesterol, Reactive Oxygen Species, and the Formation of Biologically Active Mediators. J Biol Chem 2008;283:15521–15525.
[13] Niki E, Yoshida Y, Saito Y, Noguchi N. Lipid peroxidation: Mechanisms, inhibition, and biological effects. Biochem Biophys Res Commun 2005;338:668–676.
[14] Smith WL, Murphy RC. Oxidized Lipids Formed Nonenzymatically by Reactive Oxygen Species. J Biol Chem 2008;283:15513–15514.
[15] Porter NA, Caldwell SE, Mills KA. Mechanisms of free radical oxidation of unsaturated lipids. Lipids 1995;30:277–292.
[16] Gardner HW. Oxygen radical chemistry of polyunsaturated fatty acids. Free Radic Biol Med 1989;7:65–86.
[17] Spiteller P, Kern W, Reiner J, Spiteller G. Aldehydic lipid peroxidation products derived from linoleic acid. Biochim Biophys Acta Mol Cell Biol L 2001;1531:188–208.
[18] Cheng ZY, Li YZ. What is responsible for the initiating chemistry of iron-mediated lipid peroxidation: An update. Chemical Reviews 2007;107:748–766.
[19] Min B, Ahn DU. Mechanism of lipid peroxidation in meat and meat products - A review. Food Sci Biotechnol 2005; 14:152–163.
[20] Jones CM, Burkitt MJ. EPR Spin-Trapping Evidence for the Direct, One-Electron Reduction of tert-Butylhydroperoxide to the tert-Butoxyl Radical by Copper(II): Paradigm for a Previously Overlooked Reaction in the Initiation of Lipid Peroxidation. J Am Chem Soc 2003;125:6946–6954.
[21] Poli G, Dianzani MU, Cheeseman KH, Slater TF, Lang J, Esterbauer H. Separation and characterization of the aldehydic products of lipid peroxidation stimulated by carbon tetrachloride or ADP-iron in isolated rat hepatocytes and rat liver microsomal suspensions. Biochem J 1985;227:629–638.
[22] Hogg N, Kalyanaraman B. Nitric oxide and lipid peroxidation. BBA Bioenergetics 1999;1411:378–384.
[23] Morita M, Tokita M. The real radical generator other than main-product hydroperoxide in lipid autoxidation. Lipids 2006;41:91–95.
[24] Schneider C, Porter NA, Brash AR. Routes to 4-hydroxynonenal: Fundamental issues in the mechanisms of lipid peroxidation. J Biol Chem 2008;283:15539–15543.
[25] Niki E, Noguchi N. Evaluation of antioxidant capacity. What capacity is being measured by which method? IUBMB Life 2000;50:323–329.
[26] Casalino E, Sblano C, Landriscina C. A possible mechanism for initiation of lipid peroxidation by ascorbate in rat liver microsomes. Int J Biochem Cell Biol 1996;28:137–149.
[27] Neuzil J, Thomas SR, Stocker R. Requirement for, Promotion, or Inhibition by -Tocopherol of Radical-Induced Initiation of Plasma Lipoprotein Lipid Peroxidation. Free Radic Biol Med 1997;22:57–71.
[28] Witting PK, Upston JM, Stocker R. Role of α-Tocopheroxyl Radical in the Initiation of Lipid Peroxidation in Human Low-Density Lipoprotein Exposed to Horse Radish Peroxidase. Biochemistry 1997;36:1251–1258.
[29] Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043–1046.
[30] Joshi M, Billing BH, Hallinan T. Dietary modulation of plasma bilirubin and of hepatic microsomal lipid peroxidation in the Gunn rat. Free Radic Res 1991;11:287–293.
[31] Frankel EN. Volatile lipid oxidation products. Prog Lipid Res 1982;22:1–33.
[32] Nakamura T, Toyomizu M. Lipid degradation products capable of reacting with amino acid. Identification of 4–hydroxy–2–hexenal, 9–formyl methyl-8-nonenoate, and 10-formyl methyl-9-decenoate from autoxidized methyl linolenate. Bull Jp Soc Sci Fisheries 1977;43:1097–1104.
[33] Shibamoto T. Analytical methods for trace levels of reactive carbonyl compounds formed in lipid peroxidation systems. J Pharm Biomed Anal 2006;41:12–25.
[34] Kneepkens CMF, Lepage G, Roy CC. The potential of the hydrocarbon breath test as a measure of lipid peroxidation. Free Radic Biol Med 1994;17:127–160.
[35] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991;11:81–128.
[36] Pryor WA, Stanley JP. Suggested mechanism for the production of malonaldehyde during the autoxidation of polyunsaturated fatty acids. Nonenzymic production of prostaglandin endoperoxides during autoxidation. J Org Chem 1975;40:3615–3617.
[37] Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br J Pharmacol 2004;142:231–255.
[38] Stevens JF, Maier CS. Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease. Mol Nutr Food Res 2008;52:7–25.
[39] Uchida K. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. Prog Lipid Res 2003;42:318–343.
[40] Gasc N, Tache S, Rathahao E, Bertrand-Michel J, Roques V, Gueraud F. 4-hydroxynonenal in foodstuffs: heme concentration, fatty acid composition and freeze-drying are determining factors. Redox Rep 2007;12:40–44.
[41] Esterbauer H, Weger W. Uber die Wirkungen von Aldehyden auf gesunde und maligne Zellen, 3. Mitt. : Synthese von homologen 4-hydroxy-2-alkenalen. Monatsh Chem 1967;98:1994–2000.
[42] Kurangi RF, Tilve SG, Blair IA. Convenient and efficient syntheses of 4-hydroxy-2(E)-nonenal and 4-oxo-2(E)-nonenal. Lipids 2006;41:877–880.
[43] Lee SH, Blair IA. Characterization of 4-oxo-2-nonenal as a novel product of lipid peroxidation. Chem Res Toxicol 2000;13:698–702.
[44] Schneider C, Tallman KA, Porter NA, Brash AR. Two Distinct Pathways of Formation of 4-Hydroxynonenal. J Biol Chem 2001;276:20831–20838.
[45] Gardner HW, Hamberg M. Oxygenation of (3Z)-nonenal to (2E)-4-hydroxy-2-nonenal in the broad bean (Vicia faba L.). J Biol Chem 1993;268:6971–6977.
[46] Van Kuijk FJGM, Holte LL, Dratz EA. 4-Hydroxyhexenal: A lipid peroxidation product derived from oxidized docosahexaenoic acid. Biochim 1 Acta Lipids Lipid Metab 1990;1043:116–118.
[47] Segall H, Wilson D, Dallas J, Haddon W. trans-4-Hydroxy-2-hexenal: a reactive metabolite from the macrocyclic pyrrolizidine alkaloid senecionine. Science 1985;229:472–475.
[48] Rindgen D, Nakajima M, Wehrli S, Xu K, Blair IA. Covalent Modifications to 2'-Deoxyguanosine by 4-Oxo-2-nonenal, a Novel Product of Lipid Peroxidation. Chem Res Toxicol 1999;12:1195–1204.
[49] Kuiper HC, Miranda CL, Sowell JD, Stevens JF. Mercapturic Acid Conjugates of 4-Hydroxy-2-nonenal and 4-Oxo-2-nonenal Metabolites Are in Vivo Markers of Oxidative Stress. J Biol Chem 2008;283:17131–17138.

[50] Ward JP, van Dorp DA. A stereospecific synthesis of 4-oxo-2trans-hexenal. Recl Trav Chim Pays-Bas 1969;88:989–993.
[51] Maekawa M, Kawai K, Takahashi Y, Nakamura H, Watanabe T, Sawa R, Hachisuka K, Kasai H. Identification of 4-Oxo-2-hexenal and Other Direct Mutagens Formed in Model Lipid Peroxidation Reactions as dGuo Adducts. Chem Res Toxicol 2005;19:130–138.
[52] Kawai K, Matsuno K, Kasai H. Detection of 4-oxo-2-hexenal, a novel mutagenic product of lipid peroxidation, in human diet and cooking vapor. Mutat Res Genet Toxicol Environ Mutagen 2006;603:186–192.
[53] Lee SH, Oe T, Blair IA. Vitamin C-Induced Decomposition of Lipid Hydroperoxides to Endogenous Genotoxins. Science 2001;292:2083–2086.
[54] Lin J, Fay LB, Welti DH, Blank I. Quantification of key odorants formed by autoxidation of arachidonic acid using isotope dilution assay. Lipids 2001;36:749–756.
[55] Jian W, Arora JS, Oe T, Shuvaev VV, Blair IA. Induction of endothelial cell apoptosis by lipid hydroperoxide-derived bifunctional electrophiles. Free Radic Biol Med 2005;39:1162–1176.
[56] Gallasch B, Spiteller G. Synthesis of 9,12-dioxo-10(Z)-dodecenoic acid, a new fatty acid metabolite derived from 9-hydroperoxy-10,12-octadecadienoic acid in lentil seed (*Lens culinaris* Medik.). Lipids 2000;35:953–960.
[57] Alary J, Gueraud F, Cravedi JP. Fate of 4-hydroxynonenal in vivo: disposition and metabolic pathways. Mol Aspects Med 2003;24:177–187.
[58] Siems W, Grune T. Intracellular metabolism of 4-hydroxynonenal. Mol Aspects Med 2003;24:167–175.
[59] Balogh LM, Le Trong I, Kripps KA, Shireman LM, Stenkamp RE, Zhang W, Mannervik B, Atkins WM. Substrate specificity combined with stereopromiscuity in glutathione transferase A4–4-dependent metabolism of 4-hydroxynonenal. Biochemistry 2010;49:1541–1548.
[60] Dick RA, Kwak MK, Sutter TR, Kensler TW. Antioxidative function and substrate specificity of NAD(P)H-dependent alkenal/one oxidoreductase. A new role for leukotriene B4 12-hydroxydehydrogenase/15-oxoprostaglandin 13-reductase. J Biol Chem 2001;276:40803–40810.
[61] Srivastava S, Chandra A, Bhatnagar A, Srivastava SK, Ansari NH. Lipid peroxidation product, 4-hydroxynonenal and its conjugate with GSH are excellent substrates of bovine lens aldose reductase. Biochem Biophys Res Commun 1995; 217:741–746.
[62] Zhong L, Liu Z, Yan R, Johnson S, Zhao Y, Fang X, Cao D. Aldo-keto reductase family 1 B10 protein detoxifies dietary and lipid-derived alpha, beta-unsaturated carbonyls at physiological levels. Biochem Biophys Res Commun 2009;387:245–250.
[63] Marchette LD, Thompson DA, Kravtsova M, Ngansop TN, Mandal MN, Kasus-Jacobi A. Retinol dehydrogenase 12 detoxifies 4-hydroxynonenal in photoreceptor cells. Free Radic Biol Med 2010;48:16–25.
[64] Demozay D, Mas JC, Rocchi S, Van Obberghen E. FALDH reverses the deleterious action of oxidative stress induced by lipid peroxidation product 4-hydroxynonenal on insulin signaling in 3T3-L1 adipocytes. Diabetes 2008;57:1216–1226.
[65] Aldini G, Granata P, Marinello C, Beretta G, Carini M, Facino RM. Effects of UVB radiation on 4-hydroxy-2-trans-nonenal metabolism and toxicity in human keratinocytes. Chem Res Toxicol 2007;20:416–423.
[66] Cheng JZ, Sharma R, Yang Y, Singhal SS, Sharma A, Saini MK, Singh SV, Zimniak P, Awasthi S, Awasthi YC. Accelerated metabolism and exclusion of 4-hydroxynonenal through induction of RLIP76 and hGST5.8 is an early adaptive response of cells to heat and oxidative stress. J Biol Chem 2001;276:41213–41223.
[67] Yang Y, Sharma A, Sharma R, Patrick B, Singhal SS, Zimniak P, Awasthi S, Awasthi YC. Cells preconditioned with mild, transient UVA irradiation acquire resistance to oxidative stress and UVA-induced apoptosis: role of 4-hydroxynonenal in UVA-mediated signaling for apoptosis. J Biol Chem 2003;278:41380–41388.
[68] Alary J, Debrauwer L, Fernandez Y, Paris A, Cravedi JP, Dolo L, Rao D, Bories G. Identification of novel urinary metabolites of the lipid peroxidation product 4-hydroxy-2-nonenal in rats. Chem Res Toxicol 1998;11:1368–1376.
[69] Alary J, Fernandez Y, Debrauwer L, Perdu E, Gueraud F. Identification of intermediate pathways of 4-hydroxynonenal metabolism in the rat. Chem Res Toxicol 2003;16:320–327.
[70] Gueraud F, Alary J, Costet P, Debrauwer L, Dolo L, Pineau T, Paris A. In vivo involvement of cytochrome P450 4A family in the oxidative metabolism of the lipid peroxidation product trans-4-hydroxy-2-nonenal, using PPARalpha-deficient mice. J Lipid Res 1999;40:152–159.
[71] Honzatko A, Brichac J, Picklo MJ. Quantification of trans-4-hydroxy-2-nonenal enantiomers and metabolites by LC-ESI-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 2007;857:115–122.
[72] Alary J, Bravais F, Cravedi JP, Debrauwer L, Rao D, Bories G. Mercapturic acid conjugates as urinary end metabolites of the lipid peroxidation product 4-hydroxy-2-nonenal in the rat. Chem Res Toxicol 1995;8:34–39.
[73] de Zwart LL, Hermanns RC, Meerman JH, Commandeur JN, Vermeulen NP. Disposition in rat of [2–3H]-trans-4-hydroxy-2,3-nonenal, a product of lipid peroxidation. Xenobiotica 1996;26:1087–1100.
[74] Winter CK, Segall HJ, Jones AD. Distribution of trans-4-hydroxy-2-hexenal and tandem mass spectrometric detection of its urinary mercapturic acid in the rat. Drug Metab Dispos 1987;15:608–612.
[75] Alary J, Debrauwer L, Fernandez Y, Cravedi JP, Rao D, Bories G. 1,4-Dihydroxynonene mercapturic acid, the major end metabolite of exogenous 4-hydroxy-2-nonenal, is a physiological component of rat and human urine. Chem Res Toxicol 1998;11:130–135.
[76] Gueraud F, Peiro G, Bernard H, Alary J, Creminon C, Debrauwer L, Rathahao E, Drumare MF, Canlet C, Wal JM, Bories G. Enzyme immunoassay for a urinary metabolite of 4-hydroxynonenal as a marker of lipid peroxidation. Free Radic Biol Med 2006;40:54–62.
[77] Peiro G, Alary J, Cravedi JP, Rathahao E, Steghens JP, Gueraud F. Dihydroxynonene mercapturic acid, a urinary metabolite of 4-hydroxynonenal, as a biomarker of lipid peroxidation. Biofactors 2005;24:89–96.
[78] Pierre F, Peiro G, Tache S, Cross AJ, Bingham SA, Gasc N, Gottardi G, Corpet DE, Gueraud F. New marker of colon cancer risk associated with heme intake: 1,4-dihydroxynonane mercapturic acid. Cancer Epidemiol Biomarkers Prev 2006;15:2274–2279.
[79] Kuiper HC, Langsdorf BL, Miranda CL, Joss J, Jubert C, Mata JE, Stevens JF. Quantitation of mercapturic acid conjugates of 4-hydroxy-2-nonenal and 4-oxo-2-nonenal metabolites in a smoking cessation study. Free Radic Biol Med 2010;48:65–72.
[80] Goicoechea E, Van Twillert K, Duits M, Brandon ED, Kootstra PR, Blokland MH, Guillen MD. Use of an in vitro digestion model to study the bioaccessibility of 4-hydroxy-2-nonenal and related aldehydes present in oxidized oils rich in omega-6 acyl groups. J Agri Food Chem 2008;56:8475–8483.
[81] Enoiu M, Herber R, Wennig R, Marson C, Bodaud H, Leroy P, Mitrea N, Siest G, Wellman M. gamma-Glutamyltranspeptidase-dependent metabolism of 4-hydroxynonenal-glutathione conjugate. Arch Biochem Biophys 2002;397:18–27.
[82] Hashmi M, Vamvakas S, Anders MW. Bioactivation mechanism of S-(3-oxopropyl)-N-acetyl-L-cysteine, the mercapturic acid of acrolein. Chem Res Toxicol 1992;5:360–365.
[83] Yang Y, Trent MB, He N, Lick SD, Zimniak P, Awasthi YC, Boor PJ. Glutathione-S-transferase A4–4 modulates oxidative stress in endothelium: possible role in human atherosclerosis. Atherosclerosis 2004;173:211–221.

[84] Picaud JC, Steghens JP, Auxenfans C, Barbieux A, Laborie S, Claris O. Lipid peroxidation assessment by malondialdehyde measurement in parenteral nutrition solutions for newborn infants: a pilot study. Acta Paediatr 2004;93:241–245.
[85] Steghens JP, van Kappel AL, Denis I, Collombel C. Diaminonaphtalene, a new highly specific reagent for HPLC-UV measurement of total and free malondialdehyde in human plasma or serum. Free Radic Biol Med 2001;31:242–249.
[86] Renes J, de Vries EE, Hooiveld GJ, Krikken I, Jansen PL, Muller M. Multidrug resistance protein MRP1 protects against the toxicity of the major lipid peroxidation product 4-hydroxynonenal. Biochem J 2000;350 Pt 2:555–561.
[87] Reichard JF, Doorn JA, Simon F, Taylor MS, Petersen DR. Characterization of multidrug resistance-associated protein 2 in the hepatocellular disposition of 4-hydroxynonenal. Arch Biochem Biophys 2003;411:243–250.
[88] Miranda CL, Reed RL, Kuiper HC, Alber S, Stevens JF. Ascorbic acid promotes detoxification and elimination of 4-hydroxy-2(E)-nonenal in human monocytic THP-1 cells. Chem Res Toxicol 2009;22:863–874.
[89] Singhal SS, Sehrawat A, Mehta A, Sahu M, Awasthi S. Functional reconstitution of RLIP76 catalyzing ATP-dependent transport of glutathione-conjugates. Int J Oncol 2009;34:191–199.
[90] Singhal SS, Yadav S, Roth C, Singhal J. RLIP76: A novel glutathione-conjugate and multi-drug transporter. Biochem Pharmacol 2009;77:761–769.
[91] Singhal J, Singhal SS, Yadav S, Suzuki S, Warnke MM, Yacoub A, Dent P, Bae S, Sharma R, Awasthi YC, Armstrong DW, Awasthi S. RLIP76 in defense of radiation poisoning. Int J Radiat Oncol Biol Phys 2008;72:553–561.
[92] Warnke MM, Wanigasekara E, Singhal SS, Singhal J, Awasthi S, Armstrong DW. The determination of glutathione-4-hydroxynonenal (GSHNE), E-4-hydroxynonenal (HNE), and E-1-hydroxynon-2-en-4-one (HNO) in mouse liver tissue by LC-ESI-MS. Anal Bioanal Chem 2008;392:1325–1333.
[93] Burcham PC. Genotoxic lipid peroxidation products: their DNA damaging properties and role in formation of endogenous DNA adducts. Mutagenesis 1998;13:287–305.
[94] Benedetti A, Comporti M, Esterbauer H. Identification of 4-hydroxynonenal as a cytotoxic product originating from the peroxidation of liver microsomal lipids. Biochim Biophys Acta 1980;620:281–296.
[95] Nakamura T, Toyomizu M, Nagamoto T. Lipid degradation products capable of reacting with amino acid - identification of 4-hydroxy-2-hexenal, 9-formyl methyl-8-nonenoate, and 10-formyl methyl-9-decenoate from autoxidized methyl linolenate. Bul Japan Soc Sci Fish 1977;43:1097–1104.
[96] Eckl P, Esterbauer H. Genotoxic effects of 4-hydroxyalkenals. Adv Biosci 1989;76:141–157.
[97] Esterbauer H. Cytotoxicity and genotoxicity of lipid-oxidation products. Am J Clin Nutr 1993;57:779S-785S; discussion S785–S786.
[98] Poli G, Schaur RJ, Siems WG, Leonarduzzi G. 4-hydroxynonenal: a membrane lipid oxidation product of medicinal interest. Med Res Rev 2008;28:569–631.
[99] Mukai FH, Goldstein BD. Mutagenicity of malondialdehyde, a decomposition product of peroxidized polyunsaturated fatty acids. Science 1976;191:868–869.
[100] Basu AK, Marnett LJ. Unequivocal demonstration that malondialdehyde is a mutagen. Carcinogenesis 1983;4:331–333.
[101] Marnett LJ, Tuttle MA. Comparison of the mutagenicities of malondialdehyde and the side products formed during its chemical synthesis. Cancer Res 1980;40:276–282.
[102] Marnett LJ, Hurd HK, Hollstein MC, Levin DE, Esterbauer H, Ames BN. Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. Mutat Res 1985;148:25–34.
[103] Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE, Marnett LJ. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. J Biol Chem 2003;278:31426–31433.
[104] Yau TM. Mutagenicity and cytotoxicity of malonaldehyde in mammalian cells. Mech Ageing Dev 1979;11:137–144.
[105] Bird RP, Draper HH, Basrur PK. Effect of malonaldehyde and acetaldehyde on cultured mammalian cells. Production of micronuclei and chromosomal aberrations. Mutat Res 1982;101:237–246.
[106] Brambilla G, Bassi AM, Faggin P, Ferro M, Finollo R, Martelli A, Sciaba L, Marinari UM. Genotoxic effects of lipid peroxidation products. In: Poli G, Cheeseman KH, Dianzani MU, Slater TF, editors. Free Radical in Liver Injury. Oxford: IRL Press; 1985. p. 59–70.
[107] Seto H, Akiyama K, Okuda T, Hashimoto T, Takesue T, Ikemura T. Structure of a New Modified Nucleoside Formed by Guanosine-Malonaldehyde Reaction. Chem Lett + 1981:707–708.
[108] Stone K, Ksebati MB, Marnett LJ. Investigation of the adducts formed by reaction of malondialdehyde with adenosine. Chem Res Toxicol 1990;3:33–38.
[109] Stone K, Uzieblo A, Marnett LJ. Studies of the reaction of malondialdehyde with cytosine nucleosides. Chem Res Toxicol 1990;3:467–472.
[110] Marnett LJ. Oxy radicals, lipid peroxidation and DNA damage. Toxicology 2002;181–182:219–222.
[111] Kadlubar FF, Anderson KE, Haussermann S, Lang NP, Barone GW, Thompson PA, MacLeod SL, Chou MW, Mikhailova M, Plastaras J, Marnett LJ, Nair J, Velic I, Bartsch H. Comparison of DNA adduct levels associated with oxidative stress in human pancreas. Mutat Res 1998;405:125–133.
[112] Marnett LJ. Lipid peroxidation-DNA damage by malondialdehyde. Mutat Res 1999;424:83–95.
[113] Fink SP, Reddy GR, Marnett LJ. Mutagenicity in Escherichia coli of the major DNA adduct derived from the endogenous mutagen malondialdehyde. Proc Natl Acad Sci U S A 1997;94:8652–8657.
[114] Brambilla G, Sciaba L, Faggin P, Maura A, Marinari UM, Ferro M, Esterbauer H. Cytotoxicity, DNA fragmentation and sister-chromatid exchange in Chinese hamster ovary cells exposed to the lipid peroxidation product 4-hydroxynonenal and homologous aldehydes. Mutat Res 1986;171:169–176.
[115] Cajelli E, Ferraris A, Brambilla G. Mutagenicity of 4-hydroxynonenal in V79 Chinese hamster cells. Mutat Res 1987;190:169–171.
[116] Benamira M, Marnett LJ. The lipid peroxidation product 4-hydroxynonenal is a potent inducer of the SOS response. Mutat Res 1992;293:1–10.
[117] Eckl PM, Ortner A, Esterbauer H. Genotoxic properties of 4-hydroxyalkenals and analogous aldehydes. Mutat Res 1993;290:183–192.
[118] Karlhuber GM, Bauer HC, Eckl PM. Cytotoxic and genotoxic effects of 4-hydroxynonenal in cerebral endothelial cells. Mutat Res 1997;381:209–216.
[119] Schaeferhenrich A, Beyer-Sehlmeyer G, Festag G, Kuechler A, Haag N, Weise A, Liehr T, Claussen U, Marian B, Sendt W, Scheele J, Pool-Zobel BL. Human adenoma cells are highly susceptible to the genotoxic action of 4-hydroxy-2-nonenal. Mutat Res 2003;526:19–32.
[120] Honzatko A, Brichac J, Murphy TC, Reberg A, Kubatova A, Smoliakova IP, Picklo MJ, Sr. Enantioselective metabolism of trans-4-hydroxy-2-nonenal by brain mitochondria. Free Radic Biol Med 2005;39:913–924.
[121] Alija A, Picklo M, Bresgen N, Siems W, Ohlenschlager I, Eckl P. Clastogenic effects of 4-hydroxynonenal enantiomers. International COST B35 Meeting „Oxygen, Stress and Lipids". Dubrovnik; 2007.
[122] Chung FL, Nath RG, Ocando J, Nishikawa A, Zhang L. Deoxyguanosine adducts of t-4-hydroxy-2-nonenal are endogenous DNA lesions in rodents and humans: detection and potential sources. Cancer Res 2000;60:1507–1511.

[123] Chen HJ, Chung FL. Epoxidation of trans-4-hydroxy-2-nonenal by fatty acid hydroperoxides and hydrogen peroxide. Chem Res Toxicol 1996;9:306–312.
[124] Chen HJ, Gonzalez FJ, Shou M, Chung FL. 2,3-epoxy-4-hydroxynonanal, a potential lipid peroxidation product for etheno adduct formation, is not a substrate of human epoxide hydrolase. Carcinogenesis 1998;19:939–943.
[125] Wacker M, Wanek P, Eder E. Detection of 1,N2-propanodeoxyguanosine adducts of trans-4-hydroxy-2-nonenal after gavage of trans-4-hydroxy-2-nonenal or induction of lipid peroxidation with carbon tetrachloride in F344 rats. Chem Biol Interact 2001;137:269–283.
[126] Hu W, Feng Z, Eveleigh J, Iyer G, Pan J, Amin S, Chung FL, Tang MS. The major lipid peroxidation product, trans-4-hydroxy-2-nonenal, preferentially forms DNA adducts at codon 249 of human p53 gene, a unique mutational hotspot in hepatocellular carcinoma. Carcinogenesis 2002;23:1781–1789.
[127] Chung FL, Chen HJ, Nath RG. Lipid peroxidation as a potential endogenous source for the formation of exocyclic DNA adducts. Carcinogenesis 1996;17:2105–2111.
[128] Nair J, Vaca CE, Velic I, Mutanen M, Valsta LM, Bartsch H. High dietary omega-6 polyunsaturated fatty acids drastically increase the formation of etheno-DNA base adducts in white blood cells of female subjects. Cancer Epidemiol Biomarkers Prev 1997;6:597–601.
[129] Suzuki YJ, Carini M, Butterfield DA. Protein carbonylation. Antioxid Redox Signal 2010;12:323–325.
[130] Burcham PC, Kuhan YT. Introduction of carbonyl groups into proteins by the lipid peroxidation product, malondialdehyde. Biochem Biophys Res Commun 1996;220:996–1001.
[131] Negre-Salvayre A, Coatrieux C, Ingueneau C, Salvayre R. Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. Br J Pharmacol 2008;153:6–20.
[132] Sayre LM, Lin D, Yuan Q, Zhu X, Tang X. Protein adducts generated from products of lipid oxidation: focus on HNE and one. Drug Metab Rev 2006;38:651–675.
[133] Shimozu Y, Shibata T, Ojika M, Uchida K. Identification of advanced reaction products originating from the initial 4-oxo-2-nonenal-cysteine Michael adducts. Chem Res Toxicol 2009;22:957–964.
[134] Yuan Q, Zhu X, Sayre LM. Chemical nature of stochastic generation of protein-based carbonyls: metal-catalyzed oxidation versus modification by products of lipid oxidation. Chem Res Toxicol 2007;20:129–139.
[135] Grune T, Davies KJ. The proteasomal system and HNE-modified proteins. Mol Aspects Med 2003;24:195–204.
[136] Grune T, Reinheckel T, Davies KJ. Degradation of oxidized proteins in mammalian cells. FASEB J 1997;11:526–534.
[137] Uchida K. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. Prog Lipid Res 2003;42:318–343.
[138] Kurtz AJ, Lloyd RS. 1,N2-deoxyguanosine adducts of acrolein, crotonaldehyde, and trans-4-hydroxynonenal cross-link to peptides via Schiff base linkage. J Biol Chem 2003;278:5970–5976.
[139] Sayre LM, Arora PK, Iyer RS, Salomon RG. Pyrrole formation from 4-hydroxynonenal and primary amines. Chem Res Toxicol 1993;6:19–22.
[140] Nadkarni DV, Sayre LM. Structural definition of early lysine and histidine adduction chemistry of 4-hydroxynonenal. Chem Res Toxicol 1995;8:284–291.
[141] Petersen DR, Doorn JA. Reactions of 4-hydroxynonenal with proteins and cellular targets. Free Radic Biol Med 2004;37:937–945.
[142] Jurgens G, Lang J, Esterbauer H. Modification of human low-density lipoprotein by the lipid peroxidation product 4-hydroxynonenal. Biochim Biophys Acta 1986;875:103–114.
[143] Lin D, Lee HG, Liu Q, Perry G, Smith MA, Sayre LM. 4-Oxo-2-nonenal is both more neurotoxic and more protein reactive than 4-hydroxy-2-nonenal. Chem Res Toxicol 2005;18:1219–1231.
[144] Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem 1997;272:20963–20966.
[145] Chowdhury PK, Halder M, Choudhury PK, Kraus GA, Desai MJ, Armstrong DW, Casey TA, Rasmussen MA, Petrich JW. Generation of fluorescent adducts of malondialdehyde and amino acids: toward an understanding of lipofuscin. Photochem Photobiol 2004;79:21–25.
[146] LoPachin RM, Barber DS, Gavin T. Molecular mechanisms of the conjugated alpha,beta-unsaturated carbonyl derivatives: relevance to neurotoxicity and neurodegenerative diseases. Toxicol Sci 2008;104:235–249.
[147] Hinson JA, Roberts DW. Role of covalent and noncovalent interactions in cell toxicity: effects on proteins. Annu Rev Pharmacol Toxicol 1992;32:471–510.
[148] Schultz TW, Carlson RE, Cronin MT, Hermens JL, Johnson R, O'Brien PJ, Roberts DW, Siraki A, Wallace KB, Veith GD. A conceptual framework for predicting the toxicity of reactive chemicals: modeling soft electrophilicity. SAR QSAR Environ Res 2006;17:413–428.
[149] Kehrer JP, Biswal SS. The molecular effects of acrolein. Toxicol Sci 2000;57:6–15.
[150] Horvat S, Jakas A. Peptide and amino acid glycation: New insights into the Maillard reaction. J Pept Sci 2004;10:119–137.
[151] Thornalley PJ, Yurek-George A, Argirov OK. Kinetics and mechanism of the reaction of aminoguanidine with the alpha-oxoaldehydes glyoxal, methylglyoxal, and 3-deoxyglucosone under physiological conditions. Biochem Pharmacol 2000;60:55–65.
[152] Fink AL. Chaperone-mediated protein folding. Physiol Rev 1999;79:425–449.
[153] Mosser DD, Caron AW, Bourget L, Meriin AB, Sherman MY, Morimoto RI, Massie B. The chaperone function of hsp70 is required for protection against stress-induced apoptosis. Mol Cell Biol 2000;20:7146–7159.
[154] Atalay M, Oksala N, Lappalainen J, Laaksonen DE, Sen CK, Roy S. Heat shock proteins in diabetes and wound healing. Curr Protein Pept Sci 2009;10:85–95.
[155] Nollen EA, Morimoto RI. Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. J Cell Sci 2002;115:2809–2816.
[156] Simon MM, Reikerstorfer A, Schwarz A, Krone C, Luger TA, Jaattela M, Schwarz T. Heat shock protein 70 overexpression affects the response to ultraviolet light in murine fibroblasts. Evidence for increased cell viability and suppression of cytokine release. J Clin Invest 1995;95:926–933.
[157] McKay DB. Structure and mechanism of 70-kDa heat-shock-related proteins. Adv Protein Chem 1993;44:67–98.
[158] Sadis S, Hightower LE. Unfolded proteins stimulate molecular chaperone Hsc70 ATPase by accelerating ADP/ATP exchange. Biochemistry 1992;31:9406–9412.
[159] Schmid D, Baici A, Gehring H, Christen P. Kinetics of molecular chaperone action. Science 1994;263:971–973.
[160] Carbone DL, Doorn JA, Kiebler Z, Sampey BP, Petersen DR. Inhibition of Hsp72-mediated protein refolding by 4-hydroxy-2-nonenal. Chem Res Toxicol 2004;17:1459–1467.
[161] Doorn JA, Petersen DR. Covalent modification of amino acid nucleophiles by the lipid peroxidation products 4-hydroxy-2-nonenal and 4-oxo-2-nonenal. Chem Res Toxicol 2002;15:1445–1450.
[162] Srinivasan G, Post JF, Thompson EB. Optimal ligand binding by the recombinant human glucocorticoid receptor and assembly of the receptor complex with heat shock protein 90 correlate with high intracellular ATP levels in Spodoptera frugiperda cells. J Steroid Biochem Mol Biol 1997;60:1–9.
[163] Carbone DL, Doorn JA, Kiebler Z, Petersen DR. Cysteine modification by lipid peroxidation products inhibits

protein disulfide isomerase. Chem Res Toxicol 2005;18: 1324–1331.

[164] Atalay M, Oksala NK, Laaksonen DE, Khanna S, Nakao C, Lappalainen J, Roy S, Hanninen O, Sen CK. Exercise training modulates heat shock protein response in diabetic rats. J Appl Physiol 2004;97:605–611.

[165] Oksala NK, Laaksonen DE, Lappalainen J, Khanna S, Nakao C, Hanninen O, Sen CK, Atalay M. Heat shock protein 60 response to exercise in diabetes: effects of alpha-lipoic acid supplementation. J Diabetes Complications 2006;20:257–261.

[166] Uchida K, Stadtman ER. Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase. A possible involvement of intra- and intermolecular cross-linking reaction. J Biol Chem 1993;268:6388–6393.

[167] Luckey SW, Tjalkens RB, Petersen DR. Mechanism of inhibition of rat liver class 2 ALDH by 4-hydroxynonenal. Adv Exp Med Biol 1999;463:71–77.

[168] Carbone DL, Doorn JA, Kiebler Z, Ickes BR, Petersen DR. Modification of heat shock protein 90 by 4-hydroxynonenal in a rat model of chronic alcoholic liver disease. J Pharmacol Exp Ther 2005;315:8–15.

[169] Suh SK, Hood BL, Kim BJ, Conrads TP, Veenstra TD, Song BJ. Identification of oxidized mitochondrial proteins in alcohol-exposed human hepatoma cells and mouse liver. Proteomics 2004;4:3401–3412.

[170] Walker KW, Gilbert HF. Scanning and escape during protein-disulfide isomerase-assisted protein folding. J Biol Chem 1997;272:8845–8848.

[171] Liu XW, Sok DE. Inactivation of protein disulfide isomerase by alkylators including alpha,beta-unsaturated aldehydes at low physiological pHs. Biol Chem 2004;385:633–637.

[172] West JD, Ji C, Duncan ST, Amarnath V, Schneider C, Rizzo CJ, Brash AR, Marnett LJ. Induction of apoptosis in colorectal carcinoma cells treated with 4-hydroxy-2-nonenal and structurally related aldehydic products of lipid peroxidation. Chem Res Toxicol 2004;17:453–462.

[173] Forman HJ, Maiorino M, Ursini F. Signaling functions of reactive oxygen species. Biochemistry 2010;49:835–842.

[174] Roede JR, Carbone DL, Doorn JA, Kirichenko OV, Reigan P, Petersen DR. In vitro and in silico characterization of peroxiredoxin 6 modified by 4-hydroxynonenal and 4-oxononenal. Chem Res Toxicol 2008;21:2289–2299.

[175] Doorn JA, Hurley TD, Petersen DR. Inhibition of human mitochondrial aldehyde dehydrogenase by 4-hydroxynon-2-enal and 4-oxonon-2-enal. Chem Res Toxicol 2006;19:102–110.

[176] Meister A. Selective modification of glutathione metabolism. Science 1983;220:472–477.

[177] Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. Mol Aspects Med 2009;30:1–12.

[178] Sen CK. Cellular thiols and redox-regulated signal transduction. Curr Top Cell Regul 2000;36:1–30.

[179] Jones DP. Redefining oxidative stress. Antioxid Redox Signal 2006;8:1865–1879.

[180] Evans DC, Watt AP, Nicoll-Griffith DA, Baillie TA. Drug-protein adducts: an industry perspective on minimizing the potential for drug bioactivation in drug discovery and development. Chem Res Toxicol 2004;17:3–16.

[181] Blair IA. Endogenous glutathione adducts. Curr Drug Metab 2006;7:853–872.

[182] Jian W, Lee SH, Mesaros C, Oe T, Elipe MV, Blair IA. A novel 4-oxo-2(E)-nonenal-derived endogenous thiadiazabicyclo glutathione adduct formed during cellular oxidative stress. Chem Res Toxicol 2007;20:1008–1018.

[183] Zhu P, Jian W, Blair IA. A 4-oxo-2(E)-nonenal-derived glutathione adduct from 15-lipoxygenase-1-mediated oxidation of cytosolic and esterified arachidonic acid. Free Radic Biol Med 2009;47:953–961.

[184] Yadav S, Zajac E, Singhal SS, Singhal J, Drake K, Awasthi YC, Awasthi S. POB1 over-expression inhibits RLIP76-mediated transport of glutathione-conjugates, drugs and promotes apoptosis. Biochem Biophys Res Commun 2005;328:1003–1009.

[185] Tammali R, Ramana KV, Singhal SS, Awasthi S, Srivastava SK. Aldose reductase regulates growth factor-induced cyclooxygenase-2 expression and prostaglandin E2 production in human colon cancer cells. Cancer Res 2006;66:9705–9713.

[186] Volkel W, Alvarez-Sanchez R, Weick I, Mally A, Dekant W, Pahler A. Glutathione conjugates of 4-hydroxy-2(E)-nonenal as biomarkers of hepatic oxidative stress-induced lipid peroxidation in rats. Free Radic Biol Med 2005;38:1526–1536.

[187] Catala A. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. Chem Phys Lipids 2009;157:1–11.

[188] Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. Science 1972;175:720–731.

[189] Engelman DM. Membranes are more mosaic than fluid. Nature 2005;438:578–580.

[190] Fan J, Sammalkorpi M, Haataja M. Formation and regulation of lipid microdomains in cell membranes: Theory, modeling, and speculation. FEBS Lett 2010;584:1678–1684.

[191] Awasthi YC, Sharma R, Cheng JZ, Yang Y, Sharma A, Singhal SS, Awasthi S. Role of 4-hydroxynonenal in stress-mediated apoptosis signaling. Mol Aspects Med 2003;24:219–230.

[192] Zarkovic N, Ilic Z, Jurin M, Schaur RJ, Puhl H, Esterbauer H. Stimulation of HeLa cell growth by physiological concentrations of 4-hydroxynonenal. Cell Biochem Funct 1993;11:279–286.

[193] Bacot S, Bernoud-Hubac N, Chantegrel B, Deshayes C, Doutheau A, Ponsin G, Lagarde M, Guichardant M. Evidence for in situ ethanolamine phospholipid adducts with hydroxy-alkenals. J Lipid Res 2007;48:816–825.

[194] Schaur RJ. Basic aspects of the biochemical reactivity of 4-hydroxynonenal. Mol Aspects Med 2003;24:149–159.

[195] Guichardant M, Taibi-Tronche P, Fay LB, Lagarde M. Covalent modifications of aminophospholipids by 4-hydroxynonenal. Free Radic Biol Med 1998;25:1049–1056.

[196] Guichardant M, Bernoud-Hubac N, Chantegrel B, Deshayes C, Lagarde M. Aldehydes from n-6 fatty acid peroxidation. Effects on aminophospholipids. Prostaglandins Leukot Essent Fatty Acids 2002;67:147–149.

[197] Irvine RF. How is the level of free arachidonic acid controlled in mammalian cells? Biochem J 1982;204:3–16.

[198] Selley ML, McGuiness JA, Jenkin LA, Bartlett MR, Ardlie NG. Effect of 4-hydroxy-2,3-trans-nonenal on platelet function. Thromb Haemost 1988;59:143–146.

[199] Akaishi T, Nakazawa K, Sato K, Saito H, Ohno Y, Ito Y. Modulation of voltage-gated Ca^{2+} current by 4-hydroxynonenal in dentate granule cells. Biol Pharm Bull 2004;27:174–179.

[200] de Jongh R, Haenen GR, van Koeveringe GA, Dambros M, van Kerrebroeck PE. Lipid peroxidation product 4-hydroxynonenal contributes to bladder smooth muscle damage. Urology 2008;71:974–978.

[201] Lekehal M, Pessayre D, Lereau JM, Moulis C, Fouraste I, Fau D. Hepatotoxicity of the herbal medicine germander: metabolic activation of its furano diterpenoids by cytochrome P450 3A Depletes cytoskeleton-associated protein thiols and forms plasma membrane blebs in rat hepatocytes. Hepatology 1996;24:212–218.

[202] Sastry PS, Rao KS. Apoptosis and the nervous system. J Neurochem 2000;74:1–20.

[203] Huber J, Vales A, Mitulovic G, Blumer M, Schmid R, Witztum JL, Binder BR, Leitinger N. Oxidized membrane vesicles and blebs from apoptotic cells contain biologically active oxidized phospholipids that induce monocyte-endothelial

interactions. Arterioscler Thromb Vasc Biol 2002;22: 101–107.
[204] Davies SS, Amarnath V, Roberts LJ, 2nd. Isoketals: highly reactive gamma-ketoaldehydes formed from the H2-isoprostane pathway. Chem Phys Lipids 2004;128:85–99.
[205] Michel P, Eggert W, Albrecht-Nebe H, Grune T. Increased lipid peroxidation in children with autoimmune diseases. Acta Paediatr 1997;86:609–612.
[206] Ito Y, Kosuge Y, Sakikubo T, Horie K, Ishikawa N, Obokata N, Yokoyama E, Yamashina K, Yamamoto M, Saito H, Arakawa M, Ishige K. Protective effect of S-allyl-L-cysteine, a garlic compound, on amyloid beta-protein-induced cell death in nerve growth factor-differentiated PC12 cells. Neurosci Res 2003;46:119–125.
[207] Glei M, Hofmann T, Kuster K, Hollmann J, Lindhauer MG, Pool-Zobel BL. Both wheat (Triticum aestivum) bran arabinoxylans and gut flora-mediated fermentation products protect human colon cells from genotoxic activities of 4-hydroxynonenal and hydrogen peroxide. J Agric Food Chem 2006;54:2088–2095.
[208] Siems WG, Brenke R, Beier A, Grune T. Oxidative stress in chronic lymphoedema. QJM 2002;95:803–809.
[209] Esterbauer H, Zollner H, Lang J. Metabolism of the lipid peroxidation product 4-hydroxynonenal by isolated hepatocytes and by liver cytosolic fractions. Biochem J 1985; 228:363–373.
[210] Subramaniam R, Roediger F, Jordan B, Mattson MP, Keller JN, Waeg G, Butterfield DA. The lipid peroxidation product, 4-hydroxy-2-trans-nonenal, alters the conformation of cortical synaptosomal membrane proteins. J Neurochem 1997;69:1161–1169.
[211] Nair J, Barbin A, Velic I, Bartsch H. Etheno DNA-base adducts from endogenous reactive species. Mutat Res 1999;424:59–69.
[212] Nath RG, Chung FL. Detection of exocyclic 1,N2-propanodeoxyguanosine adducts as common DNA lesions in rodents and humans. Proc Natl Acad Sci U S A 1994; 91:7491–7495.
[213] Singh SP, Chen T, Chen L, Mei N, McLain E, Samokyszyn V, Thaden JJ, Moore MM, Zimniak P. Mutagenic effects of 4-hydroxynonenal triacetate, a chemically protected form of the lipid peroxidation product 4-hydroxynonenal, as assayed in L5178Y/Tk+/- mouse lymphoma cells. J Pharmacol Exp Ther 2005;313:855–861.
[214] Drake J, Petroze R, Castegna A, Ding Q, Keller JN, Markesbery WR, Lovell MA, Butterfield DA. 4-Hydroxynonenal oxidatively modifies histones: implications for Alzheimer's disease. Neurosci Lett 2004;356:155–158.
[215] Raza H, John A, Brown EM, Benedict S, Kambal A. Alterations in mitochondrial respiratory functions, redox metabolism and apoptosis by oxidant 4-hydroxynonenal and antioxidants curcumin and melatonin in PC12 cells. Toxicol Appl Pharmacol 2008;226:161–168.
[216] Chen JJ, Bertrand H, Yu BP. Inhibition of adenine nucleotide translocator by lipid peroxidation products. Free Radic Biol Med 1995;19:583–590.
[217] Bertram KM, Baglole CJ, Phipps RP, Libby RT. Molecular regulation of cigarette smoke induced-oxidative stress in human retinal pigment epithelial cells: implications for age-related macular degeneration. Am J Physiol Cell Physiol 2009;297:C1200–1210.
[218] Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. Genes Dev 1999;13:1899–1911.
[219] Jacobs AT, Marnett LJ. Heat shock factor 1 attenuates 4-Hydroxynonenal-mediated apoptosis: critical role for heat shock protein 70 induction and stabilization of Bcl-XL. J Biol Chem 2007;282:33412–33420.
[220] Jacobs AT, Marnett LJ. HSF1-mediated BAG3 expression attenuates apoptosis in 4-hydroxynonenal-treated colon cancer cells via stabilization of anti-apoptotic Bcl-2 proteins. J Biol Chem 2009;284:9176–9183.
[221] Helton ES, Chen X. p53 modulation of the DNA damage response. J Cell Biochem 2007;100:883–896.
[222] Riley T, Sontag E, Chen P, Levine A. Transcriptional control of human p53-regulated genes. Nat Rev Mol Cell Biol 2008;9:402–412.
[223] Fridman JS, Lowe SW. Control of apoptosis by p53. Oncogene 2003;22:9030–9040.
[224] Awasthi YC, Sharma R, Sharma A, Yadav S, Singhal SS, Chaudhary P, Awasthi S. Self-regulatory role of 4-hydroxynonenal in signaling for stress-induced programmed cell death. Free Radic Biol Med 2008;45:111–118.
[225] Sharma R, Sharma A, Dwivedi S, Zimniak P, Awasthi S, Awasthi YC. 4-Hydroxynonenal self-limits fas-mediated DISC-independent apoptosis by promoting export of Daxx from the nucleus to the cytosol and its binding to Fas. Biochemistry 2008;47:143–156.
[226] Lee HP, Zhu X, Skidmore SC, Perry G, Sayre LM, Smith MA, Lee HG. The essential role of ERK in 4-oxo-2-nonenal-mediated cytotoxicity in SH-SY5Y human neuroblastoma cells. J Neurochem 2009;108:1434–1441.
[227] Jang YJ, Kim JE, Kang NJ, Lee KW, Lee HJ. Piceatannol attenuates 4-hydroxynonenal-induced apoptosis of PC12 cells by blocking activation of c-Jun N-terminal kinase. Ann N Y Acad Sci 2009;1171:176–182.
[228] Vaillancourt F, Fahmi H, Shi Q, Lavigne P, Ranger P, Fernandes JC, Benderdour M. 4-Hydroxynonenal induces apoptosis in human osteoarthritic chondrocytes: the protective role of glutathione-S-transferase. Arthritis Res Ther 2008;10:R107.
[229] Sharma A, Sharma R, Chaudhary P, Vatsyayan R, Pearce V, Jeyabal PV, Zimniak P, Awasthi S, Awasthi YC. 4-Hydroxynonenal induces p53-mediated apoptosis in retinal pigment epithelial cells. Arch Biochem Biophys 2008;480:85–94.
[230] Zarkovic K. 4-hydroxynonenal and neurodegenerative diseases. Mol Aspects Med 2003;24:293–303.
[231] Cenini G, Sultana R, Memo M, Butterfield DA. Elevated levels of pro-apoptotic p53 and its oxidative modification by the lipid peroxidation product, HNE, in brain from subjects with amnestic mild cognitive impairment and Alzheimer's disease. J Cell Mol Med 2008;12:987–994.
[232] Braga M, Sinha Hikim AP, Datta S, Ferrini MG, Brown D, Kovacheva EL, Gonzalez-Cadavid NF, Sinha-Hikim I. Involvement of oxidative stress and caspase 2-mediated intrinsic pathway signaling in age-related increase in muscle cell apoptosis in mice. Apoptosis 2008;13:822–832.
[233] Cho ES, Jang YJ, Kang NJ, Hwang MK, Kim YT, Lee KW, Lee HJ. Cocoa procyanidins attenuate 4-hydroxynonenal-induced apoptosis of PC12 cells by directly inhibiting mitogen-activated protein kinase kinase 4 activity. Free Radic Biol Med 2009;46:1319–1327.
[234] Bresgen N, Jaksch H, Lacher H, Ohlenschlager I, Uchida K, Eckl PM. Iron mediated oxidative stress plays an essential role in ferritin induced cell death. Free Radic Biol Med 2010;48:1347–1357.
[235] Terman A, Kurz T, Navratil M, Arriaga EA, Brunk UT. Mitochondrial turnover and aging of long-lived postmitotic cells: the mitochondrial-lysosomal axis theory of aging. Antioxid Redox Signal 2010;12:503–535.
[236] Wallach D, Kang TB, Kovalenko A. The extrinsic cell death pathway and the elan mortel. Cell Death Differ 2008;15: 1533–1541.
[237] Li J, Sharma R, Patrick B, Sharma A, Jeyabal PV, Reddy PM, Saini MK, Dwivedi S, Dhanani S, Ansari NH, Zimniak P, Awasthi S, Awasthi YC. Regulation of CD95 (Fas) expression and Fas-mediated apoptotic signaling in HLE B-3 cells by 4-hydroxynonenal. Biochemistry 2006;45:12253–12264.
[238] Salaun B, Romero P, Lebecque S. Toll-like receptors' two-edged sword: when immunity meets apoptosis. Eur J Immunol 2007;37:3311–3318.

[239] Tang SC, Lathia JD, Selvaraj PK, Jo DG, Mughal MR, Cheng A, Siler DA, Markesbery WR, Arumugam TV, Mattson MP. Toll-like receptor-4 mediates neuronal apoptosis induced by amyloid beta-peptide and the membrane lipid peroxidation product 4-hydroxynonenal. Exp Neurol 2008;213:114–121.

[240] Zhang W, He Q, Chan LL, Zhou F, El Naghy M, Thompson EB, Ansari NH. Involvement of caspases in 4-hydroxy-alkenal-induced apoptosis in human leukemic cells. Free Radic Biol Med 2001;30:699–706.

[241] Kumar S. Caspase 2 in apoptosis, the DNA damage response and tumour suppression: enigma no more? Nat Rev Cancer 2009;9:897–903.

[242] Uchida K, Stadtman ER. Selective cleavage of thioether linkage in proteins modified with 4-hydroxynonenal. Proc Natl Acad Sci U S A 1992;89:5611–5615.

[243] Negre-Salvayre A, Vieira O, Escargueil-Blanc I, Salvayre R. Oxidized LDL and 4-hydroxynonenal modulate tyrosine kinase receptor activity. Mol Aspects Med 2003;24:251–261.

[244] Auge N, Garcia V, Maupas-Schwalm F, Levade T, Salvayre R, Negre-Salvayre A. Oxidized LDL-induced smooth muscle cell proliferation involves the EGF receptor/PI-3 kinase/Akt and the sphingolipid signaling pathways. Arterioscler Thromb Vasc Biol 2002;22:1990–1995.

[245] Hannan RD, Jenkins A, Jenkins AK, Brandenburger Y. Cardiac hypertrophy: a matter of translation. Clin Exp Pharmacol Physiol 2003;30:517–527.

[246] Shaw RJ, Cantley LC. Ras, PI(3)K and mTOR signalling controls tumour cell growth. Nature 2006;441:424–430.

[247] Dolinsky VW, Chan AY, Robillard Frayne I, Light PE, Des Rosiers C, Dyck JR. Resveratrol prevents the prohypertrophic effects of oxidative stress on LKB1. Circulation 2009;119:1643–1652.

[248] Wagner TM, Mullally JE, Fitzpatrick FA. Reactive lipid species from cyclooxygenase-2 inactivate tumor suppressor LKB1/STK11: cyclopentenone prostaglandins and 4-hydroxy-2-nonenal covalently modify and inhibit the AMP-kinase kinase that modulates cellular energy homeostasis and protein translation. J Biol Chem 2006;281:2598–2604.

[249] Krishnan V, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. J Clin Invest 2006;116:1202–1209.

[250] Almeida M, Han L, Martin-Millan M, O'Brien CA, Manolagas SC. Oxidative stress antagonizes Wnt signaling in osteoblast precursors by diverting beta-catenin from T cell factor- to forkhead box O-mediated transcription. J Biol Chem 2007;282:27298–27305.

[251] Almeida M, Ambrogini E, Han L, Manolagas SC, Jilka RL. Increased lipid oxidation causes oxidative stress, increased peroxisome proliferator-activated receptor-gamma expression, and diminished pro-osteogenic Wnt signaling in the skeleton. J Biol Chem 2009;284:27438–27448.

[252] Limpert AS, Karlo JC, Landreth GE. Nerve growth factor stimulates the concentration of TrkA within lipid rafts and extracellular signal-regulated kinase activation through c-Cbl-associated protein. Mol Cell Biol 2007;27:5686–5698.

[253] Zarate J, Goicoechea E, Pascual J, Echevarria E, Guillen MD. A study of the toxic effect of oxidized sunflower oil containing 4-hydroperoxy-2-nonenal and 4-hydroxy-2-nonenal on cortical TrkA receptor expression in rats. Nutr Neurosci 2009;12:249–259.

[254] Salomoni P, Khelifi AF. Daxx: death or survival protein? Trends Cell Biol 2006;16:97–104.

[255] Tohda S, Nara N. Expression of Notch1 and Jagged1 proteins in acute myeloid leukemia cells. Leuk Lymphoma 2001;42:467–472.

[256] Pizzimenti S, Barrera G, Calzavara E, Mirandola L, Toaldo C, Dianzani MU, Comi P, Chiaramonte R. Down-regulation of Notch1 expression is involved in HL-60 cell growth inhibition induced by 4-hydroxynonenal, a product of lipid peroxidation. Med Chem 2008;4:551–557.

[257] Qiao L, Wong BC. Role of Notch signaling in colorectal cancer. Carcinogenesis 2009;30:1979–1986.

[258] Zanotti S, Canalis E. Notch and the skeleton. Mol Cell Biol 2010;30:886–896.

[259] Woo HN, Park JS, Gwon AR, Arumugam TV, Jo DG. Alzheimer's disease and Notch signaling. Biochem Biophys Res Commun 2009;390:1093–1097.

[260] Mizushima N. Autophagy: process and function. Genes Dev 2007;21:2861–2873.

[261] Noda T, Ohsumi Y. Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. J Biol Chem 1998;273:3963–3966.

[262] Martinet W, Agostinis P, Vanhoecke B, Dewaele M, De Meyer GR. Autophagy in disease: a double-edged sword with therapeutic potential. Clin Sci (Lond) 2009;116:697–712.

[263] Hill BG, Haberzettl P, Ahmed Y, Srivastava S, Bhatnagar A. Unsaturated lipid peroxidation-derived aldehydes activate autophagy in vascular smooth-muscle cells. Biochem J 2008;410:525–534.

[264] Sparrow JR, Boulton M. RPE lipofuscin and its role in retinal pathobiology. Exp Eye Res 2005;80:595–606.

[265] Kaemmerer E, Schutt F, Krohne TU, Holz FG, Kopitz J. Effects of lipid peroxidation-related protein modifications on RPE lysosomal functions and POS phagocytosis. Invest Ophthalmol Vis Sci 2007;48:1342–1347.

[266] Krohne TU, Stratmann NK, Kopitz J, Holz FG. Effects of lipid peroxidation products on lipofuscinogenesis and autophagy in human retinal pigment epithelial cells. Exp Eye Res 2010;90:465–471.

[267] Sharma R, Brown D, Awasthi S, Yang Y, Sharma A, Patrick B, Saini MK, Singh SP, Zimniak P, Singh SV, Awasthi YC. Transfection with 4-hydroxynonenal-metabolizing glutathione S-transferase isozymes leads to phenotypic transformation and immortalization of adherent cells. Eur J Biochem 2004;271:1690–1701.

[268] Yang Y, Xu Y, Lick SD, Awasthi YC, Boor PJ. Endothelial glutathione-S-transferase A4–4 protects against oxidative stress and modulates iNOS expression through NF-kappaB translocation. Toxicol Appl Pharmacol 2008;230:187–196.

[269] Murakami Y, Ohsawa I, Kasahara T, Ohta S. Cytoprotective role of mitochondrial amyloid beta peptide-binding alcohol dehydrogenase against a cytotoxic aldehyde. Neurobiol Aging 2009;30:325–329.

[270] Forman HJ, Dickinson DA, Iles KE. HNE—signaling pathways leading to its elimination. Mol Aspects Med 2003;24:189–194.

[271] Singhal SS, Awasthi YC, Awasthi S. Regression of melanoma in a murine model by RLIP76 depletion. Cancer Res 2006;66:2354–2360.

[272] Singhal SS, Roth C, Leake K, Singhal J, Yadav S, Awasthi S. Regression of prostate cancer xenografts by RLIP76 depletion. Biochem Pharmacol 2009;77:1074–1083.

[273] Singhal SS, Singhal J, Yadav S, Dwivedi S, Boor PJ, Awasthi YC, Awasthi S. Regression of lung and colon cancer xenografts by depleting or inhibiting RLIP76 (Ral-binding protein 1). Cancer Res 2007;67:4382–4389.

[274] Poli G, Biasi F, Leonarduzzi G. 4-Hydroxynonenal-protein adducts: A reliable biomarker of lipid oxidation in liver diseases. Mol Aspects Med 2008;29:67–71.

[275] Carini M, Aldini G, Facino RM. Mass spectrometry for detection of 4-hydroxy-trans-2-nonenal (HNE) adducts with peptides and proteins. Mass Spectrom Rev 2004;23:281–305.

[276] Esterbauer H, Zollner H. Methods for determination of aldehydic lipid peroxidation products. Free Radic Biol Med 1989;7:197–203.

[277] Spiteller P, Kern W, Reiner J, Spiteller G. Measurement of n-alkanals and hydroxyalkenals in biological samples. Biochim Biophys Acta 2001;1531:188–208.
[278] Fenaille F, Guy PA, Tabet JC. Study of protein modification by 4-hydroxy-2-nonenal and other short chain aldehydes analyzed by electrospray ionization tandem mass spectrometry. J Am Soc Mass Spectrom 2003;14:215–226.
[279] Ishino K, Shibata T, Ishii T, Liu YT, Toyokuni S, Zhu X, Sayre LM, Uchida K. Protein N-acylation: H2O2-mediated covalent modification of protein by lipid peroxidation-derived saturated aldehydes. Chem Res Toxicol 2008; 21:1261–1270.
[280] Dry cleaning, some chlorinated solvents and other industrial chemicals. Lyon, France, 7–14 February 1995. IARC Monogr Eval Carcinog Risks Hum 1995;63:33–477.
[281] Ichihashi K, Osawa T, Toyokuni S, Uchida K. Endogenous formation of protein adducts with carcinogenic aldehydes: implications for oxidative stress. J Biol Chem 2001;276: 23903–23913.
[282] Kondo M, Oya-Ito T, Kumagai T, Osawa T, Uchida K. Cyclopentenone prostaglandins as potential inducers of intracellular oxidative stress. J Biol Chem 2001;276: 12076–12083.
[283] Uchida K, Kanematsu M, Morimitsu Y, Osawa T, Noguchi N, Niki E. Acrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins. J Biol Chem 1998;273:16058–16066.
[284] Uchida K, Kanematsu M, Sakai K, Matsuda T, Hattori N, Mizuno Y, Suzuki D, Miyata T, Noguchi N, Niki E, Osawa T. Protein-bound acrolein: potential markers for oxidative stress. Proc Natl Acad Sci U S A 1998;95:4882–4887.
[285] Calingasan NY, Uchida K, Gibson GE. Protein-bound acrolein: a novel marker of oxidative stress in Alzheimer's disease. J Neurochem 1999;72:751–756.
[286] Furuhata A, Nakamura M, Osawa T, Uchida K. Thiolation of protein-bound carcinogenic aldehyde. An electrophilic acrolein-lysine adduct that covalently binds to thiols. J Biol Chem 2002;277:27919–27926.
[287] Furuhata A, Ishii T, Kumazawa S, Yamada T, Nakayama T, Uchida K. N(epsilon)-(3-methylpyridinium)lysine, a major antigenic adduct generated in acrolein-modified protein. J Biol Chem 2003;278:48658–48665.
[288] Nagai R, Hayashi CM, Xia L, Takeya M, Horiuchi S. Identification in human atherosclerotic lesions of GA-pyridine, a novel structure derived from glycolaldehyde-modified proteins. J Biol Chem 2002;277:48905–48912.
[289] Toyokuni S, Luo XP, Tanaka T, Uchida K, Hiai H, Lehotay DC. Induction of a wide range of C(2–12) aldehydes and C(7–12) acyloins in the kidney of Wistar rats after treatment with a renal carcinogen, ferric nitrilotriacetate. Free Radic Biol Med 1997;22:1019–1027.
[290] Haze S, Gozu Y, Nakamura S, Kohno Y, Sawano K, Ohta H, Yamazaki K. 2-Nonenal newly found in human body odor tends to increase with aging. J Invest Dermatol 2001;116:520–524.
[291] Esterbauer H, Jurgens G, Quehenberger O, Koller E. Autoxidation of human low density lipoprotein: loss of polyunsaturated fatty acids and vitamin E and generation of aldehydes. J Lipid Res 1987;28:495–509.
[292] Frankel EN, German JB, Davis PA. Headspace gas chromatography to determine human low density lipoprotein oxidation. Lipids 1992;27:1047–1051.
[293] Frankel EN, Hu ML, Tappel AL. Rapid headspace gas chromatography of hexanal as a measure of lipid peroxidation in biological samples. Lipids 1989;24:976–981.
[294] Frankel EN, Kanner J, German JB, Parks E, Kinsella JE. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet 1993;341:454–457.
[295] Luo X, Pitkanen S, Kassovska-Bratinova S, Robinson BH, Lehotay DC. Excessive formation of hydroxyl radicals and aldehydic lipid peroxidation products in cultured skin fibroblasts from patients with complex I deficiency. J Clin Invest 1997;99:2877–2882.
[296] Cordis GA, Bagchi D, Maulik N, Das DK. High-performance liquid chromatographic method for the simultaneous detection of malonaldehyde, acetaldehyde, formaldehyde, acetone and propionaldehyde to monitor the oxidative stress in heart. J Chromatogr A 1994;661:181–191.
[297] Leung CH, Voutchkova AM, Crabtree RH, Balcells D, Eisenstein O. Atom economic synthesis of amides via transition metal catalyzed rearrangement of oxaziridines. Green Chemistry 2007;9:976–979.
[298] Ceruti M, Viola F, Grosa G, Balliano G, Delprino L, Cattel L. Synthesis of Squalenoid Acetylenes and Allenes, as Inhibitors of Squalene Epoxidase. J Chem Res-S 1988:18–19.
[299] Kato Y, Mori Y, Makino Y, Morimitsu Y, Hiroi S, Ishikawa T, Osawa T. Formation of Nepsilon-(hexanonyl)lysine in protein exposed to lipid hydroperoxide. A plausible marker for lipid hydroperoxide-derived protein modification. J Biol Chem 1999;274:20406–20414.
[300] Metz TO, Alderson NL, Chachich ME, Thorpe SR, Baynes JW. Pyridoxamine traps intermediates in lipid peroxidation reactions in vivo: evidence on the role of lipids in chemical modification of protein and development of diabetic complications. J Biol Chem 2003;278:42012–42019.
[301] Kato Y, Yoshida A, Naito M, Kawai Y, Tsuji K, Kitamura M, Kitamoto N, Osawa T. Identification and quantification of N(epsilon)-(Hexanoyl)lysine in human urine by liquid chromatography/tandem mass spectrometry. Free Radic Biol Med 2004;37:1864–1874.