

REVIEW ARTICLE

Pathological aspects of lipid peroxidation

ANNE NEGRE-SALVAYRE¹, NATHALIE AUGÉ¹, VICTORIA AYALA²,
HUVEYDA BASAGA³, JORDI BOADA², RAINER BRENKE⁴, SARAH CHAPPLE⁵,
GUY COHEN⁶, JANOS FEHER⁷, TILMAN GRUNE⁸, GABRIELLA LENGYEL⁷,
GIOVANNI E. MANN⁵, REINALD PAMPLONA², GIUSEPPE POLI⁹,
MANUEL PORTERO-OTIN², YAEL RIAHI⁶, ROBERT SALVAYRE¹, SHLOMO SASSON⁶,
JOSE SERRANO², OFER SHAMNI⁶, WERNER SIEMS¹⁰, RICHARD C. M. SIOW⁵,
INGRID WISWEDEL¹¹, KAMELIJA ZARKOVIC¹² & NEVEN ZARKOVIC¹³

¹INSERM I2MR-858, IFR-150, Avenue J Poulhès, BP 84225, Toulouse, France, ²Departament de Medicina Experimental, Facultat de Medicina, Universitat de Lleida, c/ Montserrat Roig-2, E25008 Lleida, Spain, ³Biological Sciences and Bioengineering Program, Faculty of Engineering and Natural Sciences, Sabanci University, Istanbul, Turkey, ⁴Hufeland Hospital, Taunusallee 5, D-56130 Bad Ems, Germany, ⁵Cardiovascular Division, School of Medicine, King's College London, London, UK, ⁶Department of Pharmacology, Institute for Drug Research, School of Pharmacy, The Hebrew University Faculty of Medicine, Jerusalem. P.O. Box 12272, Jerusalem 91120, Israel, ⁷2nd Department of Medicine, Medical Faculty, Semmelweis University, H-1088 Budapest, Szentkiralyi str. 46, Hungary, ⁸Institute of Biological Chemistry and Nutrition, Department of Biofunctionality and Food Safety, University of Hohenheim, 70593 Stuttgart, Germany, ⁹Department of Clinical and Biological Sciences, University of Turin, San Luigi Hospital, 10043 Orbassano, Turin, Italy, ¹⁰Research Institute of Physiotherapy and Gerontology, KortexMed Institute of Medical Education Bad Harzburg, Hindenburgring 12A, D-38667 Bad Harzburg, Germany, ¹¹Department of Pathological Biochemistry, Medical Faculty of the Otto-von-Guericke-University, Leipziger Str. 44, D-39120 Magdeburg, Germany, ¹²University of Zagreb, School of Medicine, Department of Neuropathology, Clinical Hospital Center Zagreb, Kispaticeva 12, 10000 Zagreb, Croatia, and ¹³Rudjer Boskovic Institute, Bijenicka 54, HR-10000 Zagreb, Croatia

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Abstract

Lipid peroxidation (LPO) product accumulation in human tissues is a major cause of tissular and cellular dysfunction that plays a major role in ageing and most age-related and oxidative stress-related diseases. The current evidence for the implication of LPO in pathological processes is discussed in this review. New data and literature review are provided evaluating the role of LPO in the pathophysiology of ageing and classically oxidative stress-linked diseases, such as neurodegenerative diseases, diabetes and atherosclerosis (the main cause of cardiovascular complications). Striking evidences implicating LPO in foetal vascular dysfunction occurring in pre-eclampsia, in renal and liver diseases, as well as their role as cause and consequence to cancer development are addressed.

Keywords: Oxidative stress, oxidative homeostasis, reactive oxygen species, lipid peroxidation, 4-Hydroxynonenal, neurodegenerative diseases, Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, liver disease, fatty liver, atherosclerosis, redox equilibrium, cell signalling, oxidized phospholipids, oxysterol, diabetes, diabetes complications, 4-hydroxydodecadienal, 4-hydroxynonenal, pre-eclampsia, HELLP syndrome, IUGR, glutathione, redox signaling, Nrf2, antioxidant enzymes, foetal endothelium, renal diseases, lymphoedema, hyperglycaemia, advanced glycation end-products, cancer

Correspondence: Anne Negre-Salvayre, INSERM I2MR-858, IFR-150, Avenue J Poulhès, BP 84225, Toulouse Cedex 4, 31432, France.
Tel: (33) 561-32-27-05. Fax: (33) 561-32-20-84. Email: anne.negre-salvayre@inserm.fr

Abbreviations: AD, Alzheimer disease; AGE, advanced-glycation end product; ALT, alanin-aminotransferase; AMA, anti-mitochondrial antibodies; ANA, anti-nuclear antibodies; APP, Amyloid precursor protein; ApoE, apolipoprotein E; AST, aspartate-aminotransferase; BACE1, β -site cleaving enzyme; BAP, amyloid beta protein; BMI, body mass index; CTGF, connective tissue growth factor; CML, carboxymethyl-lysine; CNS, central nervous system; CRA, cardio-renal-anaemia; CRP, C-reactive protein; CSF, colonic stimulating factor; CVD, cardiovascular disease; EGFR, EGF receptor; EGR-1, early growth response factor 1; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; ESRD, End-stage renal disease; EPO, erythropoietin; ET-1, endothelin 1; FALDH, fatty aldehyde dehydrogenase i.e. Aldh3a1; FFA, Free fatty acids; GGT, gammaglutamyl-transpeptidase; GLDH, glutamate dehydrogenase; GPx, glutathione peroxidase; GSH, glutathione (reduced form); GST, glutathione S-transferase; HBV, hepatitis B virus; HCC, hepatocellular cancer; HCV, hepatitis C virus; HD, haemodialysis; 4-HHE, 4-hydroxy-2-hexanal; 4-HDDE, 4-hydroxy-2E,6Z-dodecadienal; HDV, hepatitis D virus; HELLP, v haemolysis elevated liver enzyme low platelet syndrome; 4-HNE, 4-hydroxynonenal, 12-HpETE:12-hydroperoxyeicosatetraenoic, 15-HpETE:15-hydroperoxyeicosatetraenoic acid; 13-HpODE, 13-hydroperoxyoctadecadienoic acid; HSC, hepatic stellate cell; ICAM-1, intercellular adhesion molecule-1; IGF-I, Insulin-like growth factor I; IR, insulin resistance; IRS, Insulin-receptor-substrate; IUGR, intrauterine growth retardation; JNK, Jun N-terminal kinase; LBD, Lewy body disease; LDH, lactate dehydrogenase; LDL, Low density lipoprotein; 12,15 LO, 12,15 lipoxygenase; LPC, lysophosphatidylcholine; LPO, lipid peroxidation; LVMI, Left ventricular mass index; MCP1, monocyte chemoattractant protein-1; MDA, malondialdehyde; MIP-1 β , macrophage inflammatory protein-1 β ; MMPs, matrix metalloproteinases; MRC, Mitochondrial respiratory chain; NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; NFAT, nuclear factor of activated T; NF- κ B, Nuclear factor- κ B; NOD, non-obese diabetic mice; NOSA, Non-organ specific autoantibodies; NRF-1, nuclear respiratory factor-1; NFT, Neurofibrillary tangles; OH \cdot , hydroxyl radical; OHdG, 8-hydroxy-2'-deoxyguanosine; OxPLs, oxidized phospholipids; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; PE, pre-eclampsia; PEIPC, 1-palmitoyl-2-(5,6-epoxyisoprostane E₂)-sn-glycero-3-phosphatidylcholine; PGC1-expression, PPAR- γ co-activator 1- β and 1- α ; PGPC, 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphatidylcholine; PHF, paired helical fragment; PIFKA II, protein induced by vitamin K absence or antagonist II (abnormal plasma prothrombin; PIINP, N-terminal pro-peptide of collagen type III; PON, paraoxonase; POVC, 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphatidylcholine; PPAR γ , peroxisome proliferator-activated receptor gamma; PUFAs, polyunsaturated fatty acids; RAGE, receptor for advanced glycation end-products; SMA, smooth muscle antibodies; SOD, superoxide dismutase; SP, senile plaque; SR-B1, scavenger-receptor-B1; T2DM, Type2 diabetes mellitus; TBARS, thiobarbituric acid-reactive substances; THB, tetrahydrobiopterin; TIMP-1, metalloprotease tissue inhibitor-1; TLRs, toll-like receptors; TNF- α , tumour necrosis factor- α ; UPR, unfolded protein response; VCAM-1, vascular cell adhesion molecule-1; VEC, vascular endothelial cell; VEGFR2, VEGF receptor 2.

Introduction

Accumulating evidence supports the hypothesis that oxidative stress, lipid peroxidation and oxidative damages to tissular and cellular molecules in humans play a major involvement in ageing and most age- and oxidative stress-related diseases. Oxidative stress results from an imbalance between free radicals over-production and a lower degradation rate due to a decreased activity of endogenous defense systems. Reactive oxygen species (ROS) express a variety of molecules and free radicals physiologically generated from the metabolism of molecular oxygen, including superoxide anion, which is the precursor of most ROS and of non-enzymatic reactions with other radicals such as nitric oxide (NO) and a major mediator in oxidative chain reactions. ROS attack/damage all cellular constituents. Lipids (cholesterol, polyunsaturated fatty acids (PUFA) are a main target of oxidative attack and this leads to the formation and the accumulation of lipid oxidation (LPO) products [1,2], in particular oxysterols, hydroperoxides and endoperoxides. The latter undergo fragmentation to produce a broad range of reactive carbonyl intermediates such as α,β -unsaturated aldehydes [4-HNE and acrolein], di-aldehydes [MDA

and glyoxal] and keto-aldehydes [4-oxo-trans-2-nonenal (ONE) and isoketals]. These carbonyl compounds, ubiquitously generated in biological systems, have unique properties contrasted with free radicals. Further, the non-charged structure of aldehydes allows them to migrate with relative ease through hydrophobic membranes and hydrophilic cytosolic media, thereby extending the migration distance far from the production site. Based on these features alone, these carbonyl compounds can be more destructive than ROS and may have far-reaching damaging effects on target sites within or outside membranes, as they react with nucleophilic groups in macromolecules like proteins, DNA and aminophospholipids, among others, resulting in their chemical, non-enzymatic and irreversible modification [3–5].

Tissues and organs in which cell turnover is lower or absent (brain, heart, skeletal tissues) tend to accumulate high quantities of LPO products which first behave as physiological mediators. Further, the progressive accumulation of LPO products is a starting point mechanism of tissular and cellular dysfunction involved in ageing and in well-defined diseases of liver, kidney, neurological and cardiovascular systems,

cancers, endocrine and metabolic disorders, diabetes and its complications and other oxidative stress-related pathologies.

The present review is a critical evaluation of the presence and importance of LPO in initiating and/or mediating some aspects of the pathological processes involved in the installment and further complications of these diseases.

Ageing (T Grune)

Ageing and oxidative stress

The 'free radical theory of ageing' was proposed in 1956 by Harman [6]. Since that time this theory was several-fold revised, changed, interpreted and discussed [7–13]. However, the originally created idea, that free radicals or other metabolic side-products are leading to accumulating damages, which in time cannot be repaired and cause physiological pathways dysfunctions and a reduced ability to adapt to stressors and leading finally to cell death, remains the same in quite a few of the revised versions of the free radical theory of ageing. However, Harman et al's [14] theory that production of free radicals is causing cellular senescence and organism ageing seems to be very simplistic. This is especially true on a background of oxidant requirement for adaptational events and today's concept of oxidative-redox status as a balance between pro- and anti-oxidative processes [15]. However, a number of correlations between oxidant production and lifespan seem to exist in mammals and insects [16,17], indicating a causal relationship. Interestingly, in many cases mitochondria seem to play a major role [16–19] in age-related oxidant production, although the accumulating damage itself might cause enhanced oxidant production [20].

Several theories exist, related either to free radical/oxidant production or to damage-accumulation. This includes the 'network theory of ageing' or 'mitochondrial theory of ageing' favouring the accumulation of oxidatively damaged mitochondria and an increasing disturbance of cellular metabolism [21,22]. Nitric oxide (NO) or accumulating lysosomal iron were also proposed as oxidant sources [23,24]. Interestingly, and most importantly, all these theories are proposing disturbances of the cellular homeostasis as the original reason of cellular senescence and the accumulation of damaged material as the consequence and the final reason for cellular death. However, the proposed correlations do not finally prove the causal role of free radicals in the mechanism of the ageing process.

Historical studies on ageing and oxidative stress

The first discovery of an age-related accumulation of damaged, oxidized material was made in 1842 by Hannover [25], describing for the first time age

pigments in human neuronal cells. However, years later, Konneff [26] realized that this pigment was connected with the ageing process. Since this time the formation of the age pigment (later called lipofuscin) was described and controversially discussed [27–30]. Besides in humans, the formation of this pigment was also described in model organisms as flies and rodents [31–34]. Interestingly, already in 1976 Harman et al. [14] himself studied the effect of antioxidants on the formation of lipofuscin, implying a connection between age pigment accumulation and oxidative processes. Although lipofuscin has now been known for more than 150 years, the exact formation and role in cellular metabolism remains still largely unknown. It becomes more and more clear that this material is not an inert waste product, but has a multitude of cellular effects, as modulating metabolic pathways [35,36], influencing gene expression [37] or inducing apoptosis [20]. In addition to that, the formation of this material remains obscure. However, lipid peroxidation products might play a key role in the cross-linking event of proteins [38–40], leading to the formation of undegradable, insoluble cross-linked proteins, which is today thought to be the starting point of lipofuscin formation [15]. Therefore, an enhanced lipid peroxidation process during ageing seems important for lipofuscin formation.

Lipid peroxidation in ageing

Early studies on the level of lipid peroxidation during ageing gave contradictory results [41–43]. This seems due to the fact that, although lipid peroxidation has been studied for more than 60 years by biochemists, there is still no easy, accurate and reproducible method for the measurement of lipid peroxidation in serum or tissue. In the literature, a wealth of studies exists investigating the steady state concentration of various lipid peroxidation products in a number of different tissues in humans and model organisms. This includes measurements of MDA by thiobarbituric acid-adduct (TBARS) formation, the measurement of conjugated dienes, of 4-HNE, of protein adducts of lipid peroxidation products and of various other products. Although the results are somehow contradictory—in general—an increase of lipid peroxidation products with age is found. Tissue specificity, cellular involvement or linearity of such an accumulation remains obscure. In addition to that (and resulting from the ageing process itself), cells or organs are changing in cell and extracellular matrix composition, cell size and cell-to-extracellular-matrix-ratio, making the standardization and exact comparisons between young and old tissues difficult. For example, the comparison of an accumulated lipid peroxidation product between young and old liver per gram tissue seems to be odd on the background that old liver is often fibrotic and composed of other cells. Since the measurement of lipid peroxidation products reflects a balance between the actual lipid

peroxidation process and the degradation rate of the measured lipid peroxidation products, it is important to note that the metabolic consumption of lipid peroxidation products is cell type specific. Most intensively this was studied for 4-HNE [44]. Therefore, it is hard to quantify the lipid peroxidation in relation to number of cells, wet weight or protein amount. The clinical use of oxidation parameters is reviewed in Voss and Siems [45].

Nevertheless, numerous studies show an increase in lipid peroxidation during ageing, as a change in the membrane transition temperature in human myelin from white matter of patients was found over the age of 50 [46], an increase in lipid peroxidation in rat brain and liver homogenates [47] and individual regions of the brain [48] were found. Lipid peroxidation was investigated by several authors in various brain regions, e.g. Zhang et al. [49] found an age-dependent increase in hydroperoxide level in the striatum of gerbils and not in the hippocampus and cortex, whereas Krisofikova et al. [50] found decreased levels of MDA in rat hippocampus and Hayakawa et al. [48] found increased 4-HNE immunoreactivity in CA1 regions in mice.

More recently, a number of investigations demonstrated not only an age-association of lipid peroxidation, but also an increase of lipid peroxidation in several age-related diseases, such as Alzheimer's [51,52] or in obesity and metabolic syndrome [53]. Due to the existing number of diseases in all cohorts of elderly human beings, therefore, the measurement of pure age-related lipid peroxidation is even more difficult. However, in plasma an age-related increase of MDA, 4-HNE and lipoperoxides was reported [54–56]. The same was also reported for model organisms [57,58]. However, for unknown reasons other groups were unable to demonstrate that [59,60].

In summary, there is good evidence for increased lipid peroxidation product accumulation during the ageing process in humans and other organisms. The development of new more sophisticated, routine detection methods will undoubtedly resolve this issue.

Neurodegenerative diseases (K Zarkovic)

Lipid peroxidation and central nervous system

LPO products accumulate with ageing, in particular in oxidative stress-related neurological disorders such as ischemic, inflammatory, metabolic, developmental and degenerative diseases [61–63]. The central nervous system (CNS) is a very sensitive target for the LPO damage because of a high level of polyunsaturated lipids in neuronal cell membranes, high metabolic rate of transitional metals and poor antioxidative defense [64]. Accordingly, oxidative stress is very pronounced in a number of neurodegenerative disorders by abnormal filament accumulation, deposits of an

abnormal form of specific proteins in affected neurons, inflammation and mitochondrial dysfunction. While the effects of permanent oxidative stress on post-mitotic neuronal cells are cumulative, causes of neuronal death in neurodegenerative diseases are multi-factorial. Advances in molecular genetics increased the knowledge of hereditary pathogenesis of neurodegeneration, while evidences for the importance of metabolic imbalance and oxidative stress in sporadic forms of neurodegenerative diseases are constantly rising [65]. In some familiar cases of amyotrophic lateral sclerosis (ALS), mutation in the gene for Cu/Zn superoxide dismutase (SOD1) can be identified. In other neurodegenerative diseases, reactive oxygen species (ROS) and subsequent LPO products have usually uncertain roles in pathogenesis or implications on neuronal death, thus it is often unclear whether oxidative damage is a cause or consequence of neurodegeneration [66]. A role for permanent oxidative stress in neurodegenerative disorders is particularly important because neurons are post-mitotic cells and gradually accumulate oxidative damage over time.

ROS induce peroxidation of the cellular membrane lipids or circulating lipoprotein molecules generating highly reactive aldehydes [67]. Aldehydes are highly reactive with macromolecules and much more stable than ROS so they can spread from site of origin and act at a more distant site. One of the most important products of LPO generated from polyunsaturated fatty acids (PUFAs) is 4-HNE [68]. This highly reactive α,β -aldehyde is cytotoxic and is generated during various physiological and pathophysiological conditions based on the production of ROS [67,69–71].

Lipid peroxidation and Alzheimer's disease

Oxidative damage occurs in all human neurodegenerative diseases and seems especially important in Alzheimer's disease (AD). AD is a progressive neurodegeneration characterized clinically by early memory dysfunction, later progression of disorders with dysphasia and dyspraxia to mute and immobility. AD is the commonest cause of dementia and increases in incidence with age. The prevalence of AD is 10% or more after the age of 80, with higher incidence in women. The majority of cases of AD are sporadic and in some patients a familial variant of AD can be recognized. These patients carry mutant genes for membrane spanning amyloid precursor protein (APP). AD is morphologically present by amyloid-containing neuritic (senile) plaques (SP) and neurofibrillary tangles (NFT), predominantly in the hippocampus and frontal cortex. The core of these plaques is composed of amyloid β -protein (BAP), which is derived by proteolysis (β and γ secretase) from a much larger precursor protein APP. In AD the APP molecule is cut at both ends, releasing an intact BAP, which is toxic and accumulates in neuritic plaques as amyloid fibrils

[72]. The increased activity of β -site cleaving enzyme (BACE1) correlates with oxidative stress in Alzheimer's disease [73]. SP formation in AD may be secondary to another pathologic process. NFT are composed of paired helical filaments (PHF) that consist of an abnormal form of normally occurring microtubule-associated protein (MAP), termed tau. Normal tau proteins are microtubule binding proteins that stabilize the microtubular neuronal cytoskeleton and the phosphate groups are rapidly removed by phosphatase in post-mortem tissue. In contrast, tau protein in PHF is phosphorylated on aberrant site and is relatively resistant to post-mortem dephosphorylation [74]. In AD, tau does not associate with microtubules, but instead aggregates in the form of PHF. The link between BAP generation and the formation of NFT is not known. Of possible relevance is the increased neuronal expression of a receptor for advanced glycation end-products (RAGE) and also a receptor for BAP around deposits of amyloid. Soluble oligomers of BAP aggregated with divalent copper bind to cholesterol, adhere to plasma and intracellular membranes and cause radical-initiated LPO. Binding of soluble BAP oligomers with tau results in tau hyperphosphorylation [75]. Another important observation is the accumulation of abnormal ubiquitin-conjugated proteins in affected neurons, suggesting a dysfunction of the proteasome proteolytic system in these cells [76].

Many studies of AD show increased oxidation of brain lipids, carbohydrates, proteins and DNA in NFT and SP. Oxidative modification of macromolecules decreases or eliminates their function and activates inflammatory processes in the brain of patients with AD. In oxidative pathogenesis of AD, a particular role is played by LPO [65], formed from PUFA which build the brain phospholipids. Phosphorus nuclear magnetic resonance of CNS shows a decrease in phosphatidyl-ethanolamine and stearic, oleic, arachidonic and docosahexenoic fatty acids in the hippocampus and inferior parietal lobule of AD patients compared with age-matched controls [77]. On the other hand, isoprostanes (F type prostanoid ring-F₂-IsoP) are formed non-enzymatically by free radical-induced oxidation of arachidonic acid in these patients [78]. Namely, oxidation of docosahexenoic acid leads to the formation of F₂-IsoP-like compounds, so called F₄-neuroprostanes (F₄-NP). Their concentration in CSF of AD patients is significantly elevated if compared with controls [79].

TBARS are elevated in all AD brain regions compared with controls, while the presence of 4-HNE in AD usually shows specific onset of lipid peroxidation, which might reflect the presence of LPO. There are numerous findings supporting an important role of 4-HNE in AD development. 4-HNE causes impaired glucose transport in cultured rat hippocampal neurons and impaired glutamate transport in rat neocortical synaptosomes [80]. Moreover, after administration to

the rat forebrain, 4-HNE damages cholinergic neurons [81]. Exposure of cultured hippocampal neurons to H₂O₂ produces zinc and 4-HNE-mediated lysosomal membrane permeabilization, causing neuronal death [82]. 4-HNE is also capable of inducing apoptosis in PC12 cells and cultured rat hippocampal neurons [83]. A significant increase of free 4-HNE in cerebrospinal fluid [84], amygdala, hippocampus and parahippocampal gyrus was detected in the brain of AD patients when compared with control subjects [85]. Moreover, immunohistochemical studies demonstrated that 4-HNE immunoreactivity is present in NFT in some SP in AD (Figure 1). Oxidatively modified proteins by 4-HNE in AD are an early event in progression of dementia [52,86]. 4-HNE modification of BAP results in an inhibition of degradation of oxidized proteins by 20S proteasome [76], while co-existence of 4-HNE-immunoreactivity in the same cells suggests the essential role of oxidative damage in the altered protein accumulation. 4-HNE is toxic for P19 cells, causing cross-linking of tau into high molecular weight substances that are conjugated with ubiquitin [87]. 4-HNE-immunopositivity seems to be associated with the inheritance of apolipoprotein E 4 (ApoE) alleles. ApoE genotype and advancing ageing are interacting risk factors for late onset and for sporadic AD [88]. 4-HNE is a more potent cross-linker than MDA, for purified ApoE3 and ApoE4 in P19 neuroglial cell culture [89]. A significant decrease of glutathione transferase activity and of other antioxidative enzymes in amygdala, hippocampus and inferior parietal lobule in AD patients [90] lead to more pronounced effects of 4-HNE in these brain regions. However, while 4-HNE seems to be of major importance, this aldehyde cannot be considered as the only causative agent in protein aberrations related to LPO. Namely, acrolein, the most reactive could be rapidly incorporated

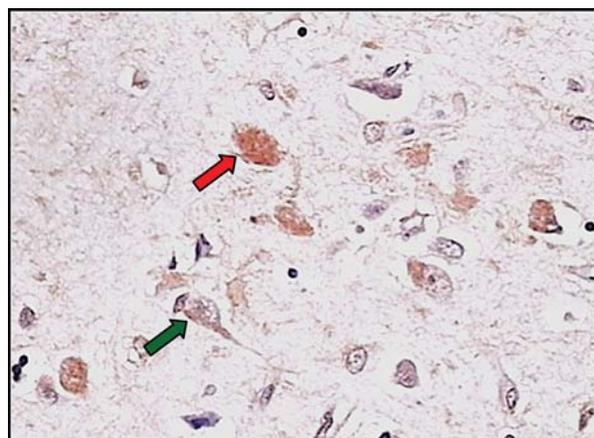


Figure 1. 4-HNE immunopositivity in neurofibrillary tangles. 4-HNE immunopositivity in neurofibrillary tangles within the neuronal cytoplasm (green arrow) and in senile plaques (red arrow) in AD, magnification 400 \times Monoclonal antibodies specific for the HNE-histidine epitope (courtesy of Dr Georg Waeg, IMBBM, KF-University, Graz, Austria).

into proteins generating carbonyls or modifying DNA basis [91]. Acrolein preferentially reacts with lysine residues that are prominent components of tau [92] and are present in NFT and dystrophic neuritis surrounding SP in AD [93]. This aldehyde is neurotoxic in a time- and concentration-dependent manner, even more than 4-HNE for hippocampal tissue cultures [90]. Acrolein might be in part responsible for the reactive carbonyl compounds formed during LPO and sugar glycooxidation that increased in the frontal pole, hippocampus and inferior parietal lobule of AD patients when compared with normal aged control subject [94], producing cell dysfunction, inflammatory response and apoptosis [3,95]. Finally, the oxidative nature of protein alterations in AD is supported by immunohistochemical findings of 2,4-dinitrophenyl-hydrazine (DNP) that demonstrated further presence of protein carbonyls in NFT-bearing neurons and glia, when compared with non-NFT-bearing neurons in AD and with normal brains [96].

Lipid peroxidation and Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disorder clinically characterized by extrapyramidal movement disturbance: rigidity, bradykinesia, resting tremor and sometimes dysphagia, autonomic dysfunction or dementia. Pathologically, PD is characterized by a loss of dopaminergic neurons from the substantia nigra associated with the presence of intraneuronal inclusions denoted as Lewy bodies. PD typically appears in the sixth to eighth decades of life with no gender differences. Mutation in the gene encoding α -synuclein leads to an understanding of the mechanisms underlying selective dopaminergic cell death in familial cases, while such mutation has not been seen in studies of sporadic PD [97]. Thus, aetiology of dopaminergic degeneration remains undefined, especially in the non-familial forms of PD. It has been suggested that aggregates of α -synuclein generate oxidative stress, possibly by interaction with iron [98]. α -synuclein is a pre-synaptic protein that forms the major component of the filament comprising Lewy bodies in PD and in Lewy body disease (LBD). Neurodegenerative disorders characterized by nigral degeneration (such as PD) have high levels of free iron and increase in proteins that store iron in redox-inert forms. H_2O_2 can damage the dopaminergic neurons directly or indirectly through the formation of hydroxyl radicals in the presence of ferrous ions by the Fenton reaction. Possible sources of iron are microglia with ferritin-bound iron and potential increase of iron in astrocytes of substantia nigra [99] combined with an increase in the Fe (III)/Fe (II) ratio and decrease in glutathione (GSH) [31]. Intracellular redox and iron imbalance results in aberrant oxidation of dopamine to 6-hydroxydopamine, which in turn can undergo auto-oxidation to the corresponding

dopamine quinone concomitant with generation of superoxide. Reactive quinone can be neurotoxic more than oxygen free radicals for dopamine-induced protein cross-linking in rat brain membrane fraction [100]. The plasma of PD patients, both sporadic and familial form, has elevated levels of LPO and is furthermore susceptible for lipid peroxidation more than normal healthy human plasma [101]. Oxidative stress cascade based on lipid peroxidation can lead to further generation of ROS, which induces oxidative damage of DNA, particularly in substantia nigra, increase in protein carbonyls [102], protein nitration [98] and viciously increased lipid peroxidation. Inhibition of 26S proteasome due to oxidative protein modifications lead to lower degradation of ubiquitinated oxidized or nitrated proteins [103]. The end-products of lipid peroxidation such as 4-HNE acting as second toxic messengers of free radicals give major impact to this. Namely, 4-HNE is present in Lewy bodies and in mitochondria in PD [104] and in diffuse LBD [105]. Therefore, it is not surprising that high levels of glutathione peroxidase (GPx) conjugate with 4-HNE were found present in cerebrospinal fluid of LBD. This indicates that lipid peroxidation may be causatively related to PD not only appearing as an epiphenomenon of the disease [105]. In favour of this possibility are also some experimental findings. Incubation with ranging concentrations of 4-HNE induces a dose-dependent decrease of dopamine uptake and Na/K ATPase activity and a loss of sulphhydryl (SH) groups. These data suggest that 4-HNE is an important mediator of oxidative stress that alters dopamine uptake after binding to SH groups of the dopamine transporter and Na/K ATPase. Such toxic effects of 4-HNE contribute to the onset and progression of PD.

Lipid peroxidation and amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by weakness and spasticity, which is pronounced by primary loss of lower and upper motor neurons, secondary neurogenic atrophy of striated muscles and can be associated with variable pathology of non-motor systems. ALS is further classified into common sporadic (sALS) and rare familial (fALS) forms. In sALS a specific loss of the glial glutamate transporter protein EAAT2 is observed in spinal cord [106]. The primary pathogenic processes underlying motor neuron injury in sALS are multifactorial and the precise molecular pathways are unknown. Approximately 10% of all ALS cases are fALS inherited by autosomal dominant or recessive transmission. About one quarter of the fALS cases, which are dominantly inherited, are linked with mutation in the gene encoding SOD1 protein [107]. Most of the mutations in SOD1 protein occur outside the active site and produce only a small decrease in enzyme activity as a result of lower stability and

decreased half-life of mutant protein [108]. SOD1 is a Cu/Zn-binding ubiquitous anti-oxidative enzyme that catalyses the conversion of the toxic superoxide radical to H_2O_2 , which in turn is converted to H_2O by the action of glutathione peroxidase or catalase [66]. Similarity between the fALS and sALS suggest that these two sub-types share common pathways of injury by a direct toxic effect of superoxide radicals inadequately scavenged by the mutant enzyme. Weakened affinity for copper in the mutant SOD1 produces competition with other copper and zinc binding proteins, resulting in a diminished amount of active enzyme and insufficient superoxide scavenging [65]. Such changes could allow potential mutant SOD1 to react with various subsidiary activities including peroxidase activity resulting in generation of hydroxyl radicals from H_2O_2 [109] and production of nitrogen ions from tyrosine residues of proteins using peroxynitrite as a substrate [110]. Neurofilament proteins and tyrosine kinase receptors are particularly susceptible to nitrotyrosine damage. Mutant SOD1 A4V protein aggregation and accumulation in the motor neurons of anterior horns in fALS patients was demonstrated in characteristic hyaline inclusions. The same inclusions contain epitopes of ubiquitin and phosphorylated neurofilament protein [111], while a study on autopsy materials indicated that increased level of 4-HNE might be the cause of modification of astrocytic glutamate transporter EAAT2 and impaired glutamate transport in ALS [112]. In patients with sALS, intense immunoreactivity for 4-HNE and 4-hydroxy-2-hexanal (4-HHE) protein adducts, carboxymethyl-lysine (CML), as a lipid peroxidation or protein glycoxidation products, present in the cytoplasm of the degenerated motor neurons, suggest a potential involvement of oxidative stress in the pathomechanisms of this disease. Acrolein protein adducts were not detectable in the spinal cord of sporadic or familial ALS patients [113,114]. Another study demonstrated increased levels of both 8-hydroxy-2-deoxyguanosine (OHdG), as a nucleic acid oxidation product, and protein-bound carbonyl in the motor cortex of sALS cases. Since OHdG immunoreactivity in the cell nuclei was persistent even after RNA digestion, it is believed that the OHdG originated mainly from the nuclear DNA. In fact, protein glycoxidation products imidazolone and pyrroline, formed via reactive intermediates such as 3-deoxyglucosone without oxidation reaction, are present in spinal motor neurons in fALS, imidazolone in cytoplasm and pyrroline in hyaline inclusions [113]. Both oxidative stress with mutant SOD1 and accumulation of specific advanced glycation end products (AGE) occurring in motor neurons needs to be a base for understanding pathomechanisms of ALS.

In conclusion, many experimental and clinical data suggest that oxidative damage could be one important aspect for the onset of neurodegeneration based on

persistent oxidative stress associated with lower enzymatic antioxidant defense and increased production of LPO markers in both CNS and the blood of patients with neurodegenerative disease, defining these diseases also as progressive systemic oxidative stress disorders associated with lipid peroxidation.

Atherosclerosis (N Auge, A Negre-Salvayre, G Poli)

Inflammatory reactions in the arterial wall triggered and sustained mainly by lipid oxidation products underlie the initiation and promotion of atherosclerosis and cardiovascular disease (CVD) [115]. Vascular changes in CVD are afterwards favoured by a wide spectrum of events, such as hyperglycaemia, hypercholesterolemia, smoking, all stimuli that are often, if not always, associated with significant changes of redox equilibrium in blood and vascular cells [116]. Various pathophysiological conditions, therapeutic interventions, blood flow modifications and agents from endogenous or exogenous origin contribute to increase the production of ROS in the vascular wall, by modulating the expression and activity of ROS-producing enzymes, including NAD(P)H oxidase, endothelial NO synthase, xanthine oxidase, myeloperoxidase, SODs, catalase and glutathione peroxidase [117,118]. ROS generate vascular dysfunction and remodelling through oxidative damage, which progressively reduce the NO bioavailability, thereby altering endothelium-dependent vasodilatation. ROS and lipid oxidation (LPO) products contribute to alter the balance survival/apoptosis by their wide range of biological properties that lead to inflammatory reactions, endothelial migration and dysfunction, smooth muscle cell vasoconstriction and apoptosis [117,119,120]. Among the most potent LPO products formed during the LDL oxidation process, oxidized phospholipids (OxPLs), 4-HNE and oxidized cholesterol derivatives (oxysterols) were shown to accumulate in atherosclerotic lesions, implicating these lipids as important factors not only in the initiation but also in the promotion of the monocytic inflammation that underlies atherosclerosis [121,122]. At present, however, the signalling and transcriptional mechanisms mediating pro-inflammatory effects of OxPLs, HNE and oxysterols are only partially understood. The same applies to intracellular signalling and gene transcription pathways sustaining cell-cell and cell-matrix interaction within the arterial wall undergoing damage and subsequent remodelling.

Role of oxidized phospholipids in atherosclerosis

The susceptibility of membrane phospholipids to oxidative alterations is related to two inherent traits, the physico-chemical properties of the membrane bilayer

and the chemical reactivity of the fatty acids composing the membrane, which are extremely sensitive to oxidation. Consequently, the high concentration of unsaturated fatty acids in phospholipids not only makes them prime targets for reaction with oxidizing agents but also enables them to participate in long free radical chain reactions. The formation and the properties of oxPLs have been extensively studied [121,123–125]. OxPLs are present and abundant in oxidized LDL, and are thought to play a major role in oxLDL-induced pro-inflammatory properties. The main oxPLs belong to the oxPAPC series, formed upon oxidation of the 1-palmitoyl-2-arachidonoyl-*sn*-3-glycero-phosphorylcholine. They can be separated by chromatography, which allows one to identify 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphatidylcholine (POVPC), 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphatidylcholine (PGPC) and 1-palmitoyl-2-(5,6-epoxyisoprostane E₂)-*sn*-glycero-3-phosphatidylcholine (PEIPC), among the most potent pro-inflammatory mediators present in atherosclerotic lesions. OxPLs also include platelet-activating factor (PAF)-like lipids and lysophosphatidylcholine (LPC), as well as lipids resulting from the arachidonic acid cascade, which implicates phosphatidylcholine hydrolysis by phospholipase A₂, arachidonic acid release and eicosanoids synthesis (prostaglandins, prostacyclin and thromboxane), via the activation of cyclooxygenases and peroxidases, and the production of leukotrienes through the activation of 5-lipoxygenase. OxPLs exhibit reactive groups that covalently interact with proteins by forming lipid-protein adducts, such as levuglandins, which are present in atherosclerotic lesions [124]. Such adducts are present in human plasma, on the apoA1 of HDL, and could represent suitable biomarkers for oxidative stress [126].

The HDL-associated enzyme paraoxonase (PON) hydrolyses oxidized fatty acids from phospholipids and thus reduces the accumulation of oxPLs in LDL and their pro-inflammatory response [127]. PAF-acetylhydrolase, another enzyme associated to HDL and involved in PAF hydrolysis, participates in the degradation of oxPLs [128].

Interactions with receptors. Most biological effects of oxPLs in macrophages and vascular cells are due to their interactions with the CD36 receptor. The presence on most oxPLs of a terminal γ -hydroxy (or oxo)- α,β -unsaturated carbonyl, allows their interaction with lysines 164 and 166 of the CD36 receptor binding site [124,129]. The presence of oxPLs in highly Cu-oxidized LDL facilitates their binding to CD36 [130]. Interestingly, plasma circulating oxPLs could bind and activate platelets via CD36, thereby linking hyperlipidemia, oxidative stress and prothrombotic events [131]. The interaction of oxPLs with the VEGF

receptor 2 (VEGFR2) stimulates angiogenesis [123], while their binding to the scavenger-receptor-B1 (SR-B1) hinders the binding of HDL and their selective cholesteryl ester uptake from hepatocytes [132]. Berliner and Gharavi [123] reported that the binding of oxPAPC to the prostaglandin receptor EP2 (a G protein-coupled receptor) results in an increased expression of P-selectin in macrophages. Moreover, some oxPLs can bind the PAF receptor, thus mimicking the effects of PAF [133]. Lastly, several reports indicate that some inflammatory properties of oxPLs, such as IL-8 transcription, could result from their interactions with toll-like receptors (TLRs), in particular TLR4 [121,134].

Biological effects in vascular cells. OxPLs are early generated upon oxidation of LDL and plasma membrane phospholipids and accumulate at all stages of atherosclerosis, in fatty streaks and in more advanced lesions. When injected to animal models for atherosclerosis, oxPLs may initiate pro-atherogenic reactions from monocyte recruitment to thrombus formation. OxPAPC, and particularly POVPC and PGPC, are potent activators of endothelial cells, as they trigger an up-regulation of inflammatory cytokines such as IL-6, IL-8 or the chemokine monocyte chemoattractant protein-1 (MCP-1). The mechanism evoked by POVPC involves protein kinase A and cAMP [123]. Interestingly, oxPLs trigger an up-regulation of P-selectin, but not of ICAM-1, VCAM-1 and E-selectin [123]. Likewise, oxPLs stimulate the recruitment of monocyte to endothelial cells, but not of neutrophils, thus triggering a selective macrophage accumulation which differs from that exerted by LPS, TNF α or IL-1, which recruit both monocytes and neutrophils [135,136]. OxPLs do not activate the classical pro-inflammatory NF- κ B pathway [137]. However, the oxPAPC-mediated inflammatory signalling involves a calcium-dependent activation of the nuclear factor of activated T-cells (NFAT), resulting in over-expression of the prothrombotic tissue factor, via the phosphorylation of extracellular signal-regulated kinase (ERK), and an induction of growth response factor 1 (EGR-1) [137].

OxPLs trigger the over-expression of IL-8 in endothelial cells, via a src/JAK2/STAT3-mediated pathway [138]. SREBP is involved in IL-8 induction through its binding to a SRE element in the IL-8 promoter [123]. The mechanism of IL-8 induction also implicates the phosphatidylinositol-3-kinase/Akt pathway and e-NOS, as assessed by the inhibitory effect of the e-NOS inhibitor L-NAME [123]. OxPLs are potent agonists for PPAR γ and stimulate the formation of lipid vacuoles in macrophages, via a PPAR γ -mediated mechanism [139]. OxPAPC promote angiogenesis by interacting with VEGFR2 and inducing the expression of VEGF via a src-mediated pathway [123].

Some oxPLs may bind the PAF receptor and mimic its inflammatory properties, in particular platelet aggregation, monocyte activation, smooth muscle cell migration and proliferation and increased vascular permeability. The PAF-like effects of oxPLs are not regulated, contrary to PAF whose effects are strictly and tightly controlled [125]. In addition, POVPC trigger a de-differentiation of SMC and alter the secretion and composition of the extracellular matrix, thus could contribute to modify the nature of atherosclerotic lesions in the vascular wall [140]. OxPLs stimulate oxidative stress by activating NADPH oxidase [141], but also induce the expression of antioxidant enzymes such as heme oxygenase [142] and of genes and transcription factors associated with the unfolded protein response (UPR) [125,143]. OxPLs activate the intrinsic mitochondrial apoptotic signalling in smooth muscle cells and macrophages [144,145] and contribute to the removal of apoptotic cells by phagocytic macrophages [146].

OxPLs as biomarkers for cardiovascular diseases. OxPLs exhibit a high affinity for lipoprotein Lp(a), which could represent a detoxification mechanism for low Lp(a) concentrations, since Lp(a) is associated with the PAF acetyl hydrolase that degrades OxPLs [147]. However, high levels of oxPLs in Lp(a) are highly pro-atherogenic as they bind the vessel wall, where they exert pro-inflammatory and pro-thrombotic properties [148]. Such elevated circulating levels of Lp(a), associated with increased oxPLs, are observed in patients affected with cardiovascular diseases [148].

OxPLs plasma levels associated with apoB are elevated in patients with coronary, carotid and femoral artery diseases and could constitute prognostic markers for atherosclerosis and cardiovascular diseases, as well as increased levels in anti-oxPLs antibodies which were reported in patients with hypertension and myocardial infarction [149].

In summary, oxPLs are potent biologically reactive agents, abundantly present in oxidized LDL and in atherosclerotic lesions, where they contribute to modulate inflammation and atherosclerosis development. These agents could also serve as diagnosis markers for the follow-up of coronary diseases and thus represent potential therapeutical targets for cardiovascular diseases.

Role of aldehydes in atherosclerosis

The oxidation of PUFAs generates MDA and highly reactive α,β -unsaturated hydroxyalkenals, such as 4-HNE and 4-HHE [150,151]. Other aldehydes such as α -oxoaldehydes can be generated by lipid oxidation (glyoxal) or through the catabolism of ketone bodies and the fragmentation of triosephosphates (methyl-

glyoxal). The α -oxoaldehyde generation is increased during diabetes and hyperglycaemia and in diabetes-dependent accelerated atherosclerosis [152]. These aldehydes react with amino acids (histidine, cysteine) or lysine residues on proteins to form stable Michael adducts and Schiff-bases, thereby altering progressively the function of circulating, tissular and cellular proteins [4]. Their role in the pathophysiology of cardiovascular diseases has been extensively studied and reviewed [143–147]. Aldehydes are endogenously generated from oxidative attack of PUFA in membranes, lipid-rich tissues and lipoproteins, but high amounts of these compounds are also ingested with fat-rich food [150]. Although many experimental studies in animals point out the atherogenicity and cardiotoxicity of dietary aldehydes present in oxidized fats from deep-fried food, ingested aldehydic LPO are not acutely toxic in humans, contrary to oxysterols [150], this being explained by a low intestinal absorption [153–155]. Moreover, these compounds are rapidly degraded, thus neutralized, by glutathione-dependent enzymes, as reviewed in Esterbauer [150]. It is to note that dietary AGEs may aggravate diabetic complications such as nephropathy and atherosclerosis [156], while fat and carbohydrate-restricted diets ameliorate the antioxidant defenses, decrease age-related cardiovascular complications and increase the lifespan [157].

Aldehydes and lipoprotein modifications. LDL oxidation is a main risk factor in atherogenesis according to the oxidative theory for atherosclerosis [158]. LDL oxidation is a slow process occurring in the sub-intimal space and resulting from the oxidative attack of PUFAs by ROS generated by vascular cells [159]. Aldehydes formed during the oxidation process, and particularly MDA, react with the lysine and arginine residues of apoB, which are necessary for LDL recognition by the apoB/E receptor. The modification of apoB by MDA and hydroxyalkenals alters its recognition by the receptor and increases its affinity for the scavenger receptors expressed by macrophages, that are progressively transformed into foam cells [159]. The accumulation of foam cells leads to the formation of fatty streaks which are characteristic of the early atherosclerotic lesions [160]. Like 4-HNE and MDA, methylglyoxal and glyoxal may directly modify LDLs and deviate their metabolism towards macrophages [161].

Cellular effects of aldehydes. The biological effects of aldehydes are modulated by their local concentration and availability which depends on the presence of cellular detoxifying and conjugating systems (such as cellular GSH catalysed by glutathione-S-transferase or GST) and the cell ability to degrade modified proteins [162]. The functionality of these systems is

progressively modified by aldehydes, which alters cellular responses [162].

Modification of tyrosine kinase receptors. 4-HNE added to cultured smooth muscle cells are mitogenic at low concentration and induce growth arrest and apoptosis at higher doses [70,163]. The mitogenic signalling of low 4-HNE concentrations (< 1–10 μM) could result in part from a direct modification of tyrosine kinase receptors such as EGF receptor (EGFR) and PDGF receptor (PDGFR) and an activation of their downstream signalling pathway, ERK1/2 phosphorylation and cell cycle progression [164,165]. Higher 4-HNE concentrations (> 10 μM) progressively trigger a desensitization of the receptors to their own ligands and impair cellular responses such as cell migration and proliferation mediated by EGF or PDGF [163,166]. 4-HNE- and acrolein-adducts on PDGFR are observed in atherosclerotic lesions from hypercholesterolemic rabbits, apoE^{-/-} mice and human patients [163], which suggests that aldehydes may potentially disturb PDGFR-mediated responses in the fibrous cap. The same results are observed with α -oxoaldehydes, in particular methylglyoxal which inhibits EGFR and PDGFR, with possible implications in accelerated atherosclerosis in diabetes [167,168].

Inflammation. Aldehydes exert a dual effect on inflammatory signalling. 4-HNE activates the I κ B kinase (IKK)/NF- κ B inducing kinase (NIK) pathway via p38 MAPK and ERK1/2 kinase, resulting in NF- κ B activation [169]. At low concentrations, 4-HNE activates PKC β -signalling, which results in MCP-1 overexpression and release from macrophages [170]. In contrast, high 4-HNE and acrolein concentrations inhibit the activation of NF κ B, either via a direct inhibitory effect on proteasome (which is necessary for the degradation of I κ B, the cytosolic inhibitor of NF κ B) [171] or the phosphorylation of I κ B and its subsequent proteolysis [172] or a modification of IKK β -sub-unit by aldehydes [172].

Redox status, endoplasmic reticulum stress and apoptosis
4-HNE triggers intracellular oxidative stress. At low concentrations, this results in increased expression of heme-oxygenase and thioredoxine-1 [173,174], suggesting that 4-HNE induces adaptive responses to oxidative stress. 4-HNE induces the activation of the endoplasmic reticulum (ER) stress and the unfolded protein (UPR) response in endothelial cells [175]. Moreover, ER stress markers co-localize with 4-HNE protein adducts, in advanced human atherosclerotic lesions [175].

Stress kinase pathways such as Jun N-terminal kinase (JNK) and p38 kinase are activated by 4-HNE

and are associated with pro-atherogenic cellular responses including cell proliferation, inflammatory responses and apoptosis [162,176]. Several pathways are involved in cell death triggered by elevated concentrations of 4-HNE or acrolein (> 20 μM), in particular PKC δ and JNK [175]. The phosphorylation of JNK could be mediated by the ER stress sensor pIRE1 α , via a prolonged activation of ER stress and UPR, induced by 4-HNE and oxidized LDL [175]. Methylglyoxal and glyoxal are pro-apoptotic via calcium deregulation, GSH depletion, oxidative stress and the activation of stress kinases p38 and JNK [177,178].

4-HNE alters the metabolism of GSH, which induces mitochondrial oxidative stress and calcium-mediated induction of the mitochondrial transition pore [179]. In addition, 4-HNE could directly modify ANT, thereby impairing its function and activity [180].

Aldehydes and cardiovascular diseases. 4-HNE- and MDA-adducts and oxLDLs are detected in atherosclerotic lesions [181,182], while circulating auto-antibodies recognizing MDA-modified LDLs can be detected in the plasma (their role remains unknown so far) [183]. These anti 4-HNE or anti MDA-modified LDLs could constitute suitable markers for the follow-up of vascular atherosclerotic process [184]. Circulating modified LDLs are considered as suitable prognostic indicators for acute coronary syndromes, vulnerable plaques, pre-clinical or accelerated atherosclerosis in diabetes [185], while increased levels of MDA and 4-HNE-adducts in dialysed patients constitute an additional risk factor for cardiovascular complications [186]. LDL modified by ALE-pre-cursors are found in the plasma of diabetic patients and play a role in the progression of accelerated atherosclerosis.

Role of oxysterols in atherosclerosis

A very efficient way for cholesterol to promote and sustain the progression of atherosclerosis is to undergo oxidation reactions. Cholesterol oxidation leads to the formation of a number of 27-carbon atoms compounds, termed oxysterols, which may either originate in the blood, cells and tissues, through both enzymatic and non-enzymatic reactions, or derive from the diet. These cholesterol metabolites have been consistently demonstrated to be at least one or two orders of magnitude more reactive than unoxidized cholesterol, showing remarkable pro-inflammatory, pro-apoptotic and pro-fibrogenic effects.

Notable is the ability of oxysterols to cause cell injury in a manner analogous to that of oxLDLs [187]. Indeed, oxysterols are abundant in oxLDLs [188] and are consistently detectable in the core region of an unstable atherosclerotic plaque [189].

Apparently their accumulation is directly proportional to the actual cholesterol content of the plaque [190]. It thus seems highly probable that oxysterols significantly contribute to the complex molecular and cellular events leading to fibrotic plaque formation and overall vascular remodelling that characterize atherosclerosis. In fact, they have been shown to be involved in various key events of this multistep disease process, from endothelial cell dysfunction up to fibrotic degeneration of the arterial wall and vulnerable plaque rupture [190].

Biochemical effects on vascular cells. Many studies point at a role for oxysterols in activating and sustaining monocyte/macrophage-driven inflammation, a process that represents a key mechanism of disease progression in atherosclerosis [191]. In addition, they appear to support excessive fibrogenesis, modulate extracellular matrix content and induce apoptotic death of vascular cells, the latter event becoming crucial in the advanced stages of the atherogenic process.

Inflammation. The potentially strong contribution that pro-inflammatory properties of oxysterols might provide to atherosclerosis progression has attracted over the last years the attention of many investigators. As regards the endothelial dysfunction that initiates the process of plaque formation, 7-ketocholesterol (7-K), 7 α -hydroxycholesterol (7 α -OH) and 7 β -hydroxycholesterol (7 β -OH) were shown to over-express vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin in human umbilical vein endothelial cells (HUVECs) [192]. Moreover, incubation with 7-ketocholesterol markedly stimulated expression and synthesis of ICAM-1 and VCAM-1 in human aortic endothelial cells and U937 pro-monocytic cells [193]. Further, oxysterols were consistently proven to up-regulate in human vascular cells the expression of a variety of inflammation-related cytokines and chemokines [190]. In relation to this, multiplex flow cytometry allowed to show 7-K, 7 β -OH and 25-OH as potent inducers of IL-8, TNF- α , macrophage inflammatory protein-1 β (MIP-1 β) and MCP-1 in human monocytic cells [194].

Thus, cholesterol oxidation products should be able to stimulate not only the adhesion of leukocytes to the arterial endothelium but also their transmigration to sub-intimal spaces; in fact, migration, diapedesis and intra-matrix mobility of blood-borne and mesenchymal cells during plaque formation and progression are aspects of a complex process in which at least IL-8 and MCP-1 are considered to play a primary role.

Fibrosis. About a decade ago, a marked up-regulation of both expression and synthesis of the pro-fibrogenic

and pro-inflammatory cytokine TGF β 1 was described in human pro-monocytic cells challenged with an oxysterol mixture compatible with that detectable in human hypercholesterolemic plasma [195]. Subsequently, an increased generation of oxysterols was causatively associated to macrophage infiltration and fibrosis of alveolar septa in the lung of rat poisoned with paraquat [196]. Up-regulation of TGF β 1 in macrophages was very recently confirmed to be induced by a biologically relevant mixture of oxysterols [197].

The potential contribution of cholesterol oxidation products to the extensive changes of extracellular matrix occurring in the arterial wall during atherosclerosis was strengthened by the consistent observation of their ability to increase the expression of metallo-protease tissue inhibitor-1 (TIMP-1) both in macrophages [176] and in smooth muscle cells [198].

Apoptosis. Reliable *in vitro* evidence is available that depicts a potential pro-apoptotic effect of the major oxysterols with regard to smooth muscle cells, endothelial cells and monocyte-macrophages [176]. Investigating 7-ketocholesterol-induced apoptosis in cells of the macrophage lineage, Lizard's group [199,200] showed how complicated this process is, as it simultaneously triggered more than one signalling pathway, namely: (1) increase of cytosolic free calcium and activation of calcineurin with eventual dephosphorylation (i.e. activation) of the pro-apoptotic protein Bad; (2) translocation of the pro-apoptotic protein Bim from the microtubule dynein motor complex to mitochondria, interaction and inhibition of the anti-apoptotic element Bcl-2; and (3) involvement of the proline rich tyrosine kinase-2 (PYK2) which transduces survival signals through the activation of MEK/ERK pathway followed by inactivation of Bad through its phosphorylation. Thus, besides the two, often prevailing, pro-apoptotic pathways, the suitable modulation of the MEK/ERK pathway by oxysterols, also demonstrated by other laboratories [176], could be crucial in providing the cells with a possibly valid anti-apoptotic mechanism.

Oxysterol-induced foam cell formation. The hallmark of the atherosclerotic lesion is represented by the uptake of oxidized lipids by activated macrophages and myofibroblasts with the formation of foam cells. Cholesterol oxidation products markedly induce the expression and synthesis of CD36 in cells of the macrophage lineage [201], as well as other LPO products like 4-HNE [202]. The most efficient oxysterols in CD36 over-expression were 7 α -OH and cholesterol- α -epoxide, actually two of the most represented cholesterol oxidation products in human atheromas [189]. Further, the oxysterol-dependent up-regulation of this specific scavenger receptor was demonstrated to actually stimulate cellular uptake of Cu-oxidized LDL. Of note, the essential role of CD36

in oxysterol-induced foam cell formation was confirmed by the prevention of the latter process when cells were incubated in the presence of an anti-CD36 specific antibody [201].

Oxysterol-mediated cell signalling The investigation of the molecular signalling behind the aforementioned effects of oxysterols allowed one to demonstrate the involvement of defined components of PKC and MAPK enzymatic families, in particular the MEK/ERK pathway, as well as of redox-sensitive transcription factors like NF- κ B and PPAR γ . In fact, when U937 human pro-monocytic cells were incubated with a biologically compatible oxysterol mixture, but in the presence of rottlerin, a selective inhibitor of the isoform δ of PKC, CD36 over-expression was fully prevented [201]. The same event was consistently abrogated when U937 cells were treated with PD98059, a selective MEK/ERK inhibitor. As regards oxysterol-signalling downstream of ERK, monocyte treatment with GW9662, a selective PPAR γ antagonist markedly prevented the oxysterol-dependent up-regulation of CD36 [201]. Up-regulation of MEK/ERK and NF- κ B pathways was shown, underlying the ability of oxysterols to increase expression and synthesis of monocyte chemotactic protein-1 (MCP-1) in macrophagic cells [176].

In conclusion, a very efficient way for LDL lipids to promote and sustain the progression of atherosclerosis is to undergo oxidation reactions. Notably, oxidative stress is a feature that accompanies such a disease process all through its development. Oxidative stress-dependent oxidation of the lipid moiety of LDL generates a large variety of compounds provided with relevant and strong biochemical activities. The main lipid oxidation products that are consistently detectable in atherosclerotic lesions are those stemming from oxidation of LDL phospholipids and cholesterol, but also important appear defined aldehydic end-products of n-6 PUFA peroxidative breakdown, in particular 4-HNE. All these lipid oxidation products exhibit remarkable pro-inflammatory, pro-apoptotic and pro-fibrogenic effects, thus they most likely contribute to the complex molecular and cellular events leading to fibrotic plaque formation and overall vascular changes that characterize atherosclerosis. As regards the way these compounds signal to the nucleus to exert their biochemical effects, they have actually shown a marked ability in modulating the main cell signalling pathways, but with significant specificity of action, among each other. Such a pleiotropic signalling activity of LPO products present in oxLDL and atherosclerotic lesions could represent a real problem for molecular medicine to properly target the mechanisms of progression of atherosclerosis. Monitoring the plasma level of these compounds and/or that of the

inflammatory molecules whose over-production they sustain might significantly help the clinical management of the disease.

Foetal vascular dysfunction induced by lipid peroxidation in pre-eclampsia (S Chapple, R CM Siow, GE Mann)

Pre-eclampsia

Pre-eclampsia (PE) is a pregnancy-related disorder associated with systemic oxidative stress in the maternal and foetal vasculature, diagnosed by the onset of hypertension and proteinuria after 20 weeks of gestation in previously normotensive and non-proteinuric pregnant women [203,204]. Worldwide, PE affects between 2–7% of all pregnancies and remains a major contributor towards pregnancy-related morbidity and mortality [203–206]. If left untreated, PE can progress to a convulsive state known as eclampsia and ~ 10% of PE mothers develop secondary haemolysis elevated liver enzyme low platelet (HELLP) syndrome [207]. Foetal health is also compromised with increased incidence of prematurity, intrauterine growth retardation (IUGR) and hypoxemia [208].

The aetiology of PE has been linked to abnormal placental development since the main symptoms of PE are rapidly reversed after parturition. During placentation, insufficient adaptation of the maternal uterine arteries by the invading foetal trophoblasts is thought to result in under-perfusion of the placenta, localized ischemia and the onset of oxidative stress caused by excessive production of ROS [203,204,209]. Accordingly, nitrotyrosine immunoreactivity [210,211] and advanced-glycation end products (AGE) [212] are detected in the placenta in PE, indicative of enhanced ROS generation. ROS in turn induce the formation of lipid peroxidation (LPO) products [213] with both species key mediators of systemic vascular dysfunction and inflammation [201,214,215].

Endothelial dysfunction in the maternal vasculature in pre-eclampsia

Endothelial dysfunction in the maternal circulation is a hallmark of PE [216–219]. An imbalance in anti- and pro-angiogenic factors, such as soluble vascular endothelial growth factor receptor 1 (sFlt-1), soluble endoglin (sEng, CD105 cell surface receptor for TGF- β 1 and TGF- β 3) [220–223], angiotensin II type-1 receptor auto-antibodies, increased sensitivity to endothelin-1 and thromboxane, reduced synthesis of nitric oxide and prostacyclin and enhanced activation of neutrophils and platelets may also contribute to endothelial dysfunction [224]. The foeto-placental vasculature is often grossly abnormal in PE and chronic exposure to ROS may underlie the increased

resistance of the foeto-placental vasculature [225]. Moreover, umbilical artery blood flow can be severely compromised in PE, as evaluated by Doppler waveform analysis [226].

LPO originating from the placenta extend to the maternal circulation

LPO results from the oxidation of biological membranes by hydroxyl radicals (OH•), which can be generated in the presence of free metal ions, and iron overload has previously been reported in PE pregnancies [227]. Compared to uncomplicated normal deliveries, PE mothers have a reduced iron binding capacity due to increased serum iron content and reduced transferrin levels [227]. In agreement with this finding, as recently reviewed [228], numerous markers of LPO are detected in the placenta and maternal circulation. In the placenta, many studies document increased staining of LPO products including 4-HNE and MDA in syncytiotrophoblasts, endothelial cells and macrophages [212,229–232]. There are numerous reports of decreased activities of antioxidant enzymes such as SOD, catalase and GPx, providing further evidence that PE is associated with systemic oxidative stress [233–235]. Glutathione (GSH) utilizing enzymes such as GPx and the glutathione-S-transferases (GST) are critical enzymes for removing lipid hydroperoxides and require selenium for catalytic activity [67,236,237]. Interestingly, selenium deficiency has been reported to increase the risk of developing PE [231,238] and may provide a plausible explanation for increased LPO and reduced GPx activity reported in PE mothers. Propagation of oxidative damage to the maternal and foetal vasculature via lipid peroxides may be exacerbated further by a deficiency in the activity of glucose-6-phosphate dehydrogenase, a key rate-limiting enzyme for NADPH generation required for GSH recycling. While GPx and GST remove LPO end products, the chain-breaking antioxidant α -tocopherol plays a key role in preventing the propagation of LPO within membranes [239,240]. A reduction in α -tocopherol availability or its recycling via ascorbate could therefore potentiate maternal LPO reported in PE pregnancies. Currently, the status of α -tocopherol concentrations (normalized for lipid profile) in the maternal circulation remains somewhat controversial, with the majority of studies reporting decreased α -tocopherol content [241,242] or others negligible changes [243]. It is worth highlighting that other studies appear not to have adjusted α -tocopherol concentrations for differences in lipid profile [232,233,244–247].

Importantly, placental LPO products such as 4-HNE and MDA [67] are capable not only of entering the maternal circulation to induce systemic oxidative stress, but can also cross the maternal-foetal interface and enter the foetal circulation. Although less well characterized, markers of oxidative stress

have been detected in the foetal vasculature [248] and an increasing number of studies document that maternal plasma from PE patients induces phenotypic alterations in endothelial cells cultured from the foetal vasculature, e.g. human umbilical vein and artery.

Effects of maternal PE plasma on foetal endothelial cells

As summarized in Table I, a limited number of studies have reported that exposure of healthy foetal endothelial cells (derived from human umbilical vein, HUVEC) to plasma from PE mothers induces the accumulation of intracellular lipids and increases the formation of LPO end products 4-HNE and MDA [249,250]. Interestingly, as may be anticipated from findings in the maternal circulation, increased LPO was not accompanied by a compensatory increase in antioxidant capacity as measured by cellular GSH content [251]. Moreover, maternal serum obtained from normal pregnancies appears to decrease the LPO product content in HUVEC, in line with reports that normal pregnancy is associated with increased cellular antioxidant capacity. Increased LPO product formation will result in dysregulation, perturbation or activation of a number of intracellular signalling pathways involved in modulating inflammation, mitochondrial function, GSH depletion, cell cycle arrest and apoptosis. Although DNA adducts have not been measured directly in foetal endothelium in PE pregnancies, exposure of normal HUVEC to maternal plasma from PE pregnancies increases apoptosis [240], which can be mimicked by treatment of cells with 4-HNE [252]. 4-HNE is known to decrease mitochondrial ATP synthesis and ROS production as a consequence of depolarization of the inner mitochondrial membrane [240]. In addition, 4-HNE has pro-inflammatory actions, and studies by Takacs et al. [250] demonstrate that 4-HNE stimulates intracellular ROS production, induces NF- κ B nuclear translocation and transcriptional activation of ICAM-1.

Evidence of LPO in the foetal vasculature of PE pregnancies

Several studies have suggested that treatment of foetal endothelial cells with PE plasma or serum may mimic the effects of LPO products *in utero*, potentially accounting for altered redox regulation in the endothelium of PE offspring. As summarized in Table II, the majority of studies examining LPO products in the foetal circulation report increased plasma concentrations of MDA [231,235,238,247,253]. It should, however, be noted that a limited number of studies, measuring 8-isoprostane concentrations (a more specific marker of LPO), report no change in isoprostane levels [254]. Most plasma/serum samples will have been collected from a relatively small cohort of

Table I. Effects of maternal PE plasma on foetal endothelial LPO and antioxidant status.

Source	LPO marker	ROS production	Antioxidant status	Other	Reference
<i>Study design:</i> Pooled maternal plasma from 23 normal or 23 PE women matched for GA. HUVEC (Cellworks, P1-10) cultured for 24 h before addition of 2% plasma or fatty acid mixtures (μM) found in normal or PE pregnancies for a further 24 h. Triplicate measurements in a minimum of two passages.					[249]
HUVEC treated with PE vs N plasma or fatty acids	\uparrow lipid accumulation	\downarrow mitochondrial redox capacity $\downarrow \Delta\Psi_m$		\uparrow apoptosis	
<i>Study design:</i> Maternal serum from 18 non-pregnant, 18 normal pregnant and 18 PE women matched for MA, GA and BMI (pregnant groups). Primary isolated HUVEC (P2) were cultured for 72 h before being exposed to serum for a further 24 h.					[251]
HUVEC treated with N vs NP serum	\downarrow		\leftrightarrow [GSH]		
HUVEC treated with PE vs NP serum	\leftrightarrow		\leftrightarrow [GSH]		
HUVEC treated with PE vs N serum	\uparrow		\leftrightarrow [GSH]		
<i>Study design:</i> Maternal plasma from normal or severe [§] PE women matched for MA and GA. Primary isolated HUVEC (P6-7) were exposed to plasma for 48 h.					[250]
HUVEC treated with PE vs N plasma	\uparrow	NF κ B activation inhibited by α -tocopherol or NAC		\uparrow NF κ B activation \uparrow ICAM-1 expression	

NP, non-pregnant; N, normal pregnant; PE, pre-eclamptic; HELLP, haemolysis elevated liver enzyme low platelet syndrome; LPO, lipid peroxidation; MDA, malondialdehyde; HNE, 4-hydroxynonenal; ROS, reactive oxygen species; GSH, glutathione; MA, maternal age; GA, gestational age; BMI, body mass index; BP, blood pressure; ICAM-1, intracellular adhesion molecule-1; $\Delta\Psi_m$, mitochondrial membrane potential; NAC, N-acetyl cysteine.

PE defined as BP >140/90 mmHg on two occasions 6 h apart and proteinuria 0.3 g/24 h or +1 protein after 20 weeks gestation. [§]Severe PE defined as BP >60/110 mmHg, proteinuria >5 g/24 h or HELLP.

patients and possibly following different modes of delivery. Thus, there is a need for further studies using larger populations and measuring multiple markers for oxidative stress and LPO in age- and gestation-matched maternal cohorts.

Intracellular ROS production and antioxidant status have been determined as indices of foetal LPO and/or oxidative stress [255,256]. Many studies report that PE is associated with increased cellular ROS production, with uncoupled eNOS and mitochondria the two most likely intracellular sources. In contrast, antioxidant capacity is thought to be reduced in foetal plasma from PE pregnancies [235,247,256]. As in the maternal circulation of PE mothers, the status of ascorbate and α -tocopherol levels in the foetus remains poorly characterized. Decreased plasma ascorbate levels with negligible changes in α -tocopherol (uncorrected for foetal lipid profiles) have been reported in cord blood from PE pregnancies [232,247]. The majority of studies also report reduced selenium content and GPx activity; however, the molecular mechanisms underlying alterations in GPx expression require further characterization [231,235]. Moreover, reduced activity of catalase and reduced, unaltered or increased SOD activity in foetal plasma from PE pregnancies have been reported [234,235,253]. Taken together, these findings suggest that, similar to the maternal circulation, foetal endothelial cells will be subjected to increased ROS production. We have shown that foetal endothelial cells from PE

pregnancies are unable to up-regulate antioxidant defences when challenged (data not shown), conferring a pro-oxidant phenotype [257].

PE is also associated with alterations in maternal and foetal fatty acid profiles, which may prime the foetal vasculature for enhanced LPO. While arachidonic acid and linoleic acid fatty acid oxidation result principally in the formation of 4-HNE, the production of MDA can be achieved by the oxidation of arachidonic acid or docosahexaenoic acid [67]. In the maternal circulation, fatty acid profiling suggests that, although arachidonic acid content is unaltered, PE is associated with a marked increase in membrane linoleic acid and docosahexaenoic acid content, known to induce intracellular HNE formation [232,249,252]. In comparison, foetal fatty acid profiling from the umbilical cord reveals no change in arachidonic acid or docosahexaenoic acid content, although γ -linoleic acid concentrations are enhanced, suggesting an increased disposition towards increased endogenous 4-HNE production in the foetal vasculature [232]. Another study, measuring fatty acid composition in umbilical arteries from PE pregnancies, reported a lower long chain polyunsaturated fatty acids (PUFA) content, which the authors suggested could lead to impaired foetal blood flow due to a reduction in prostaglandin synthesis [258].

In studies documenting increased LPO in foetal plasma, the severity of maternal symptoms is positively correlated with foetal MDA levels [235]. These findings imply that the maternal circulation and placenta

Table II. Alterations in fetal LPO and redox status reported in PE

Source	LPO marker						Antioxidant activity				Other	Reference
	MDA	8-IP	PAC	GPx	SOD	Catalase	[GSH]	[α -tocopherol]	[ascorbate]	[Selenium]		
<i>Study design:</i> 30 normal, 19 PE and 25 severe [§] PE women												
PE plasma	↑		↓	↓	↓			↔*		↓		[235]
Severe PE plasma	↑↑			↓↓	↓↓							
<i>Study design:</i> 55 normal, 60 PE women matched for MA and GA												
Plasma	↑							↔*		↓		[232]
RBCs											↑ γ -linoleic acid ↔ Arachidonic acid ↔ Arachidonic acid ↑ Omega-6:omega-3 ratio	
<i>Study design:</i> 13 normal and 13 PE women matched for MA and GA												
Plasma	↑			↓	↔	↓						[224]
<i>Study design:</i> 33 normal and 19 PE women delivered by fasting c-section. Matched for MA and BMI (before and during pregnancy)												
Venous plasma		↔	↑					↑*				[254]
Arterial plasma		↔	↑					↔*				
<i>Study design:</i> 9 normal and 18 PE [†] women matched for MA and GA (all pre-term < 36 weeks)												
Plasma	↔			↔	↑		↔					[253]
<i>Study design:</i> 23 normal and 18 PE women matched for MA and GA												
Plasma	↑		↓					↔*		↓		[247]
<i>Study design:</i> 22 non-pregnant, 27 normal pregnant and 25 PE women, matched for MA. BMI significantly ↑ in pregnant vs non-pregnant women at booking. GA significantly ↓ in PE vs normal pregnancies.												
Plasma	↔			↓						↓		[231]
<i>Study design:</i> 33 normal and 29 PE women matched for MA and GA												
Plasma	↑										↑ XO activity ↑ ADA activity	
<i>Study design:</i> 16 normal and 20 PE women												
Plasma	↑										↔ [PO ₂]mmHg ↑ [PCO ₂]mmHg ↓ [pH]	

PE, pre-eclampsia; LPO, lipid peroxidation; MDA, malondialdehyde; 8-IP, 8-isoprostane; PAC, plasma antioxidant capacity; GPx, glutathione peroxidase; SOD, superoxide dismutase; GSH, glutathione; RBC, Red blood cell/erythrocyte; MA, maternal age; GA, gestational age; BMI, body mass index; BP, blood pressure; ADA, adenosine deaminase; XO, xanthine oxidase.

PE defined as BP > 140/90 mmHg on two occasions 6 h apart and proteinuria 0.3 g/24h or +1 protein after 20 weeks gestation. [†]PE defined as BP > 40/90 mmHg on two occasions 6 h apart with proteinuria > 0.3 h/24 h OR oedema. [§]Severe PE defined as BP > 160/110 mmHg. * [α -tocopherol] not adjusted for lipid content.

may be major contributors to foetal vascular dysfunction and notably foetal plasma LPO levels are higher in the umbilical vein compared to the umbilical artery [254]. Thus, increased ROS generation and impaired antioxidant defences in the foetal vasculature may arise primarily due to increased LPO products crossing the placenta and forming DNA and protein adducts, leading to foetal endothelial dysfunction.

Long-term impact of LPO on PE infants and adolescents

Accumulating evidence suggests that an adverse environment during development *in utero* is associated with an increased susceptibility to cardiovascular disease and diabetes in adulthood [259–262]. In

the case of PE, abnormal placentation and placental hypo-perfusion and reperfusion injury may result in long-term ‘foetal programming’ of impaired vascular function in adulthood [263]. LPO is well known to enhance ROS production, NF- κ B activation, protein nitrosylation, apoptosis and to alter the thiol redox status leading to an increased demand on cellular antioxidant systems [67]. In cultured PE HUVEC and umbilical artery smooth muscle cells, which have been removed from the pro-oxidant environment of the placenta and cultured *in vitro* for several passages, we and others have identified alterations in Ca²⁺-handling, NO production, cGMP formation, ROS production and arachidonic acid metabolism [257,264–266]. As these changes persist in culture, we hypothesize that sustained oxidative stress during

the course of PE leads to *in utero* programming of impaired redox signalling and vascular function in adulthood. Under these circumstances, increased LPO may further exacerbate a weakened redox balance.

Studies monitoring adolescents from PE pregnancies also indicate impaired vascular function. Following exposure to PE *in utero*, children aged 12–17 years old show a consistent elevation in blood pressure compared to their peers. When accounting for body mass index (BMI), which largely appears unaltered, systolic pressure was always found to be significantly elevated [267–271]. Recently a longer-term study of PE offspring concluded that *in utero* exposure to PE increases the risk of developing hypertension and incidence of stroke in later life [272]. While these data suggest that PE is associated with ‘programming’ of impaired vascular function in adulthood, this may not be the only explanation. As recently reviewed by Ferreira et al. [273] and Oglaend et al. [271], several factors can confound recorded blood pressure measurements in PE adolescents, such as sudden weight gain in underweight PE neonates, socio-economic variables and increased maternal BMI post-delivery. It may in fact be difficult to dissect out potential confounding variables, particularly in relation to maternal parameters which may be altered after the development of PE [274].

In conclusion, although the primary symptoms of PE resolve upon delivery, increased maternal LPO and impaired antioxidant defences persist in the months following parturition [235], enhancing their risk of cardiovascular disease in later life [274]. The foeto-placental vasculature is often abnormal in PE and Doppler waveform analysis reveals that umbilical artery blood flow is severely compromised [226]. There is increasing evidence that PE and other pregnancy-associated diseases cause alterations in foetal endothelial function. Increased LPO products in PE can activate NF- κ B and ICAM-1 expression in foetal umbilical vein endothelial cells. As phenotypic changes persist in culture [257,265,275–278], this may have implications for long-term ‘programming’ of the foetal cardiovascular system [260], as implied by a study in which men whose mothers suffered from PE were found to be at increased risk of developing hypertension in adulthood [279].

Diabetes

Lipid peroxidation in human diabetes: From the beginning to the end (R Pamplona, J Serrano, J Boada, V Ayala, A Negre-Salvayre, R Salvayre, M Portero-Otin)

The prevalence of diabetes mellitus (DM) will reach 300 million in 20 years, in a close relationship with over-weight. This is mainly due to consumption of high-energy diets unbalanced with physical activity. Both chronic hyperglycaemia and hyperglycaemic peaks during post-prandial periods constitute a factor for increased oxidative stress in diabetes. In this short

review we will depict the evidences in human studies of the importance of lipid oxidation in the development of diabetes from impaired fasting glucose up to DM chronic complications.

Extracellular evidences of lipid peroxidation in human diabetes. MDA levels are increased in T2DM patients with complications compared with those non-complicated [280], a fact also present in comparison with non-DM individuals [281–284]. Besides, other aldehydes directly related to hyperglycaemia [285] as glyoxal and methylglyoxal, also increase in T1DM patients as a very early event after hyperglycaemia [286,287]. Interaction between hyperglycaemia and lipid peroxidation also includes the fact that MDA increases *in vivo* modification by glycation, as recently shown in chronic renal failure patients [288]. Other lipoxidation markers, as the isoketal F₂-isoprostanes [289], oxidized cholesterol [290] and increased conjugated linolenic acid [291] also increase in DM patients. It has also been shown that TBARS increase with the degree of metabolic impairment, from healthy patients to T2DM in serum [292]. However, this later marker has been criticized in the context of human diabetes, due to lack of specificity [287]. Furthermore, hyperglycaemia, before insulinization, leads to increased lipid peroxidation in humans [293–295]. Recent data show that HDL from T2DM patients is modified by lipid oxidation, in a niacine-preventable fashion [296]. Similarly, T1DM patients exhibit elevated concentration of spin-trapped alpha-phenyl-tert-butyl nitron adducts, measured by electron paramagnetic resonance spectroscopic detection, as well as lipid-derived oxygen-centred alkoxy. This was accompanied by increased levels of lipid hydroperoxides, with diminished levels of lycopene and retinol [297]. These findings are in accordance with those present in T2DM, where the same adducts were increased, in the form of alkoxy free radicals derived from the peroxidation of lipid membranes [298]. Since direct spectroscopic evidences of lipid peroxidation are correlated with lipid hydroperoxides, it is suggested that metal-induced decomposition of those species are the precursors of alkoxy radicals that are thermodynamically able to further propagate oxidative damage to proteins.

In plasma, those lipids may arise from phospholipid moieties belonging to either endothelial cells or erythrocytes, even from circulating lipids. The implication of erythrocytes is sustained by increased MDA levels in erythrocyte ghost membranes of T1DM, when compared with euglycemic individuals [283]. In line with this, pioneering work of Jain and colleagues [299,300] led to the identification of lipid peroxidation as an important factor increasing erythrocyte fragility in human diabetes with a good correlation with glycaemic control.

The initiation agent in the absence of mitochondrial activities (i.e. in plasma) should be the superoxide

resulting from glucose and glycation products decomposition [301], in accordance to pioneering work by Hunt et al. [302]. This work evidenced an increase in hydroxyl radicals in the presence of glucose (at concentrations present in human diabetes), in a process termed glucose auto-oxidation. This process, even in non-diabetic states, may contribute to the increased lipoxidative stress evidenced during oral glucose tolerance tests [303]. In accordance with this, T1DM patients show increases in plasma MDA levels after standardized meals [304]. Not only do diabetes patients show increased lipoxidation: it has been demonstrated that MDA and F2-isoprostanes in plasma correlate with glycaemic indexes of meals, suggesting that even in euglycaemia post-pandrial increases in oxidative stress lead to increased lipid peroxidation [298,305].

Besides nutritional supplementation, also correcting in some instances lipid peroxidation, treatment aimed at tackling basic mechanisms of chronic diabetes complications, such as aldose reductase pathway, also show some effect in lipid peroxidation. Thus, the use of an inhibitor of aldose reductase could specifically reduce lipid hydroperoxides in erythrocytes in T2DM patients after 3 months of treatment. This reinforces the concept of an adequate choice for an indicator of lipid peroxidation damage, as other markers, such as plasma TBARS, MDA-modified LDL or even vitamin E were not affected by this treatments [306].

In all these evidences reported for human subjects, it is demonstrated that in a close relationship with glycaemic control, both T1DM and T2DM leads to increased extracellular lipid peroxidation, potentially contributing to their development and complications. Besides obvious solutions, as insulinization, nutritional and advanced pharmacological therapies could also improve lipoxidative status, aimed at preventing long-term complications of DM.

Intracellular evidences of lipid peroxidation in diabetes: The skeletal muscle case and involvement in insulin resistance. Since the key works led by Brownlee and colleagues [307–309] in which oxidative stress was identified as an unifying mechanism behind other recognized pathways between hyperglycaemia and chronic complications in endothelial cells, several other works have extended this concept to other cells and tissues. High glucose levels, in T2DM high fatty acid content in muscle cells, are associated both as a cause and/or a consequence of mitochondrial function [310]. As a cause, in humans, even a short consumption of high-fat diets led to increases in intracellular lipids in skeletal muscles in healthy individuals [311]. Also, in individuals with relatives suffering of T2DM, a small reduction in mitochondrial function is accompanied by an unexpectedly high accumulation in intracellular lipid contents [312,313]. As a consequence, lipids would induce impairment of

mitochondrial function. In T2DM patients, plasma FFA levels are significantly and negatively correlated with mitochondrial function, leading to impaired ATP production [314]. Thus, diminished PGC1 α levels—a key regulator of energy metabolism—in insulin resistant individuals could be induced by accumulation of intramuscular lipids [315,316].

Mitochondrial ROS (mitROS) can rapidly react with mitochondrial DNA, protein and lipids, thereby leading to oxidative damage. As an example, in skeletal muscle, the fatty acids present in excessive amounts in T2DM patients can be very prone to ROS-induced oxidative damage, resulting in the formation of lipid peroxides. Especially accumulation of fatty acids in the inner mitochondrial membrane of mitochondria, at the site where ROS are formed, would be susceptible to peroxidation, subsequently inducing oxidative damage to the mitochondrial machinery. In this line, it should be remarked that the percentage of total electron flow directed to free radical generation in mitochondria is not constant in different tissues and different conditions inside a given tissue, which suggests that mitROS generation is more than a simple byproduct of mitochondrial respiration, as frequently assumed, and should be better viewed as a homeostatically controlled variable. Oxygen radical generation at the respiratory chain has been classically attributed to complex III semiquinone [317] and complex I flavin mononucleotide [318,319] or complex I FeS clusters near the rotenone-binding site [320]. Besides those structural characteristics, adaptable variables such as entry of substrates could also play a key role in determining mitROS production. Thus, β -oxidation controls entry of fatty acids into mitochondria, although this system does not prevent completely the interaction of fats with mitochondria and hence its contact with mitROS sources. This is due to the fact that physical interaction, via ‘flip-flop’ mechanism, could allow matrix membrane entry of excess of fatty acids [321]. Since in this location they cannot enter energy production due to the absence of acyl-CoA synthetase, they remain into the inner mitochondrial membrane, exposed to mitROS. Alternatively, and analogously with the mitochondrial changes induced by high glucose concentrations, increased fatty acid efflux to mitochondria could directly increase mitROS production, more glucose being oxidized in the TCA cycle. This situation drives one to push more electron donors (NADH and FADH₂) into the electron transport chain, thus leading to an increase in ROS generation [307]. In this situation, there is a higher degree of reduction of complexes I and III, increasing their rate of ROS production. Likewise, in the insulin resistance syndrome, there is an increased free fatty acids flux from adipocytes into arterial endothelial cells that would result in increased fatty acid oxidation by mitochondria [322,323]. Since both β -oxidation of fatty acids and oxidation of fatty

acid-derived acetyl CoA by the TCA cycle generate the same electron donors (NADH and FADH₂) generated by glucose oxidation, increased FFA oxidation may cause mitochondrial over-production of ROS by the mechanism above described for hyperglycaemia.

The mechanism notwithstanding, muscle cells from obese pre-diabetic patients show increased intramyocellular lipid peroxidation [324]. As in ageing, where high lipid peroxidation is associated to lower lifespan by increased oxidation-derived damage to proteins and nucleic acids [325], it is suggested that lipid peroxidation in mitochondria from insulin resistant individuals could lead to mitochondrial function impairment [310], thereby generating a vicious circle. In accordance with this, high fat diets induced increased mitROS production [326].

Losing to win: Uncoupling mechanisms for mitROS control in DM. Mitochondrial uncoupling through proton leakage has been proposed as a mechanism to lower mitROS. This is especially interesting as the relationship between proton gradient and mitROS is exponential, so a small loss in ATP production could have a high trade-off value [327]. In human ageing, this mild mitochondrial uncoupling could explain why some muscles show more age-related losses than others [328].

As known since the early 1960s, fatty acids are capable to induce mitochondrial uncoupling [329]. Our previous data show that a homeostatic mechanism depending on lipid peroxidation controls mitROS production through mild uncoupling. Thus, increased mitROS production generates 4-HNE, which, besides a signalling role, shows also both a propagation role and also an uncoupling role, leading to diminished mitROS production [330]. In T2DM, though speculatively, this mechanism would be overwhelmed, as increased lipid peroxidation derived protein damage has been evidenced in samples from animal models of this disease [331]. Furthermore, expression of uncoupling proteins leads to both diminished lipid peroxidation [332] and lipid peroxidation-derived damage: in mitochondria from UCP3-under-expressing models significantly higher levels of lipoxidative damage than wild-type controls were found, suggesting that UCP3 functions *in vivo* as part of the antioxidant defences of the cell [333], a finding also observed by other authors [334]. Noteworthy, T2DM patients and insulin-resistant individuals show a diminished value of UCP3 protein levels in muscle, suggesting that an impairment in the expression of this protein and, possibly, other mitochondrial mechanisms for mitROS normalization could underlie mitochondrial dysfunction in insulin-resistant and diabetic states [316,335].

In conclusion, these data reveal that not only extracellular lipid peroxidation is important for chronic complications, but also intracellular lipid

peroxidation could be a key factor in the relationship between unbalanced energy homeostasis and T2 DM rising incidence.

Protective and detrimental effects of 4-hydroxyalkenals in diabetes (Y. Riahi, G Cohen, O Shammi, S Sasson)

The peroxidation of polyunsaturated fatty acids is intensified in diabetes. The peroxidation of n-3 and n-6 polyunsaturated fatty acids and the production of 4-hydroxyalkenals are intensified in diabetes due to the pro-oxidative environment induced by prolonged hyperglycaemia [336]. Of particular interest are the peroxidation products of n-6 poly-unsaturated fatty acids, namely 4-HNE and 4-HDDE [67,70,337]. The former is the peroxidation product of 15-hydroperoxyeicosatetraenoic acid (15-HpETE) and 13-hydroperoxyoctadecadienoic acid (13-HpODE), which are 15-lipoxygenase (15-LO) metabolites of arachidonic- and linoleic acid, respectively. 4-HDDE is the peroxidation product of 12-hydroperoxyeicosatetraenoic (12-HpETE), the 12-LO metabolite of arachidonic acid [337,338].

The physiological, pathophysiological or cytotoxic effects of 4-hydroxyalkenals depend on their absolute concentrations. The best example is 4-HNE: Esterbauer et al. [67] showed that at concentrations higher than 20 $\mu\text{mol/L}$ it exhibited cytotoxic effects that result from its chemical reactivity and the formation of stable adducts with proteins and nucleic acids, which alter their functions [70]. Bacot et al. [337,339] demonstrated that 4-hydroxyalkenal-induced membrane disorders resulted from the formation of adducts with ethanolamine phospholipids. They also reported that the potency of 4-HDDE in forming such ethanolamine phospholipids-adducts was ~ 3 -fold higher than that of 4-HNE [337,338]. Likewise, we found that 4-HDDE induced vascular endothelial cell (VEC) death at significantly lower concentrations than 4-HNE (1 vs 25 $\mu\text{mol/L}$, respectively) [340]. The higher chemical reactivity of 4-HDDE is attributed to the presence of two double bonds and its higher hydrophobicity in comparison with 4-HNE (Log *p*-values 3.48 and 2.45, respectively) [339].

Normally, the cellular levels of 4-hydroxyalkenals are well-controlled because their precursors, 12- and 15-HpETEs, are effectively reduced by GPx to the corresponding hydroxyl-derivatives 12- or 15-hydroxyeicosatetraenoic acid (12-HETE and 15-HETE) [336]. However, free radicals can slow and even block this pathway by inactivating GPx [337]. This leads to the accumulation of HpETEs, which are then subjected to the free radical-initiated peroxidation process that ends in the generation of 4-HDDE and 4-HNE. Similarly, 13-HpODE is peroxidized to generate 4-HNE. SEVERAL alternative mechanisms for the peroxidation process and the generation of these reactive

aldehydes have been proposed [4,341–346]. Common to these models is the primary hydroxyl radical-induced abstraction of hydrogen atom from 12- and 15-HpETE. The resulting radicals interact with free oxygen and further undergo a chain-breaking reaction to generate the corresponding 4-hydroxyalkenals.

Figure 2 depicts the two main routes of arachidonic acid transformation: the common pathway that occurs in metabolically undisturbed cells and the aberrant peroxidation pathway that is induced by an excessive oxidative stress. This model suggests that tissue-specific production of 4-hydroxyalkenals correlates with the specific pattern of expression of lipoxygenases and the biosynthesis of the corresponding HpETEs. This has been demonstrated by Guichardant's group [337,347] in diabetic rat's retina that expresses 15-LO and generate 4-HNE, but not 4-HDDE. Similarly, Coleman et al. [348] have found that 4-HNE, generated from 15-HpETE, is the major peroxidation product in 3T3-L1 pre-adipocytes. We have found that bovine aortic endothelial cells that express 12-LO generate under hyperglycaemic conditions excessive levels of 4-HDDE, but not 4-HNE [340,349,350]. It is reasonable to suggest that human VEC that also predominantly over-express 12-LO under high glucose conditions preferentially generate 4-HDDE under such conditions [351]. A similar relationship between 12-HpETE and 15-HpETE and the generation of the corresponding 4-HDDE and 4-HNE has been reported by Bacot et al. [337]. We speculate that 4-HDDE is the putative lipid peroxidation product that Bleich et al. [352] have implicated in β -cell dysfunction following cytokine-mediated increased expression of 12-LO. This model also shows that oxidative stress induced by hyperglycaemia diverts the flux of HpETEs from the enzymatic pathway towards the peroxidative arm. This role of free radicals in lipid

peroxidation has been demonstrated in several systems [347,353–355].

In addition, hyperglycaemia increases the expression and cellular level of 12- and 15-LO in cells and subsequently the availability of HpETEs to peroxidation. Such induction of 12-LO was discovered in islets of Langerhans from Zucker diabetic fatty rats (a rodent model of T2DM) in comparison with the non-diabetic controls [356] and in islets isolated from others types of diabetic rats [357]. We investigated the pattern of expression of 12-LO in primary cultures of aortic endothelial and smooth muscle cells and discovered that high glucose levels increased the cell content of 12-LO (but not 15-LO) [340,349,350]. Similarly, Natarajan et al. [358] reported that elevated glucose levels increased the expression of 12-LO in porcine aortic SMC.

These findings suggest that the impact of hyperglycaemia on lipid peroxidation is synergetic: the expression of lipoxygenases is elevated in cells and the products of these enzymes are channelled to peroxidation and an augmented formation of 4-hydroxyalkenals. This model predicts an increased abundance of 4-hydroxyalkenals in diabetic individuals. Indeed, Toyokuni et al. [359] found a marked increase in the level of 4-HNE-modified albumin in sera of T2DM patients in comparison with normoglycaemic subjects. These elevated levels were attributed to an increased generation of 4-HNE in peripheral tissues. Similar observations were made in Zucker obese rats [360]. Hitherto, no such determinations of 4-HDDE and 4-HDDE-protein adducts in diabetic patients have been reported. Nevertheless, Guichardant et al. [347] measured significant amounts of 4-HDDE metabolites along with 4-HNE and 4-HHE metabolites in urine samples of healthy individuals.

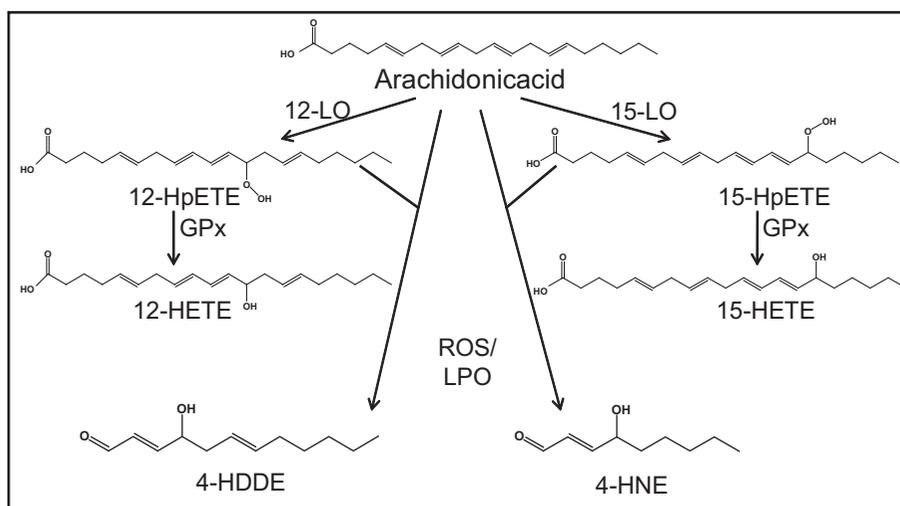


Figure 2. Mechanism of 4-hydroxyalkenal generation. The enzymes 12- and 15-lipoxygenase (12- and 15-LO) metabolize arachidonic acid to the corresponding hydroperoxyeicosatetraenoic acids (12- and 15-HpETE), which are further converted by glutathione peroxidase (GPx) to 12- or 15-hydroxyeicosatetraenoic acid (12- and 15-HETE). Radical induced inactivation of GPx diverts 12- and 15-HpETE to the peroxidation pathway to ultimately generate the respective 4-hydroxydodecadienal (4-HDDE) and 4-hydroxynonenal (4-HNE).

Roles of 4-hydroxyalkenals in diabetes. Products of lipid peroxidation can affect cells in diabetic subjects in two major ways, depending on their concentrations in cells and organs. First, high levels of 4-hydroxyalkenals induce detrimental effects and substantial cell damage that lead to severe complications of diabetes. Second, intermediate non-toxic concentrations of these compounds may attenuate cellular signalling and metabolic pathways, which may be either unfavourable or protective.

Role of 4-hydroxyalkenals in β -cell dysfunction. The tendency of 4-hydroxyalkenals to bind covalently to amino acid moieties (i.e. histidine, lysine, cysteine) in proteins, guanine nucleotides in DNA and phospholipids underlies their significant contribution to the development of complications of diabetes. Foremost, the failure of pancreatic β -cells to adequately increase insulin secretion in response to hyperglycaemia is considered, together with peripheral insulin resistance, a critical factor in the development of T2DM. Hyperglycaemia-induced free radical formation is a major contributing factor to the deterioration of β -cell in T2DM. Various studies have alluded to a detrimental role of 4-hydroxyalkenals in this process: Bleich et al. [361] linked the increased expression of 12-LO in islets of diabetic rats to impaired insulin secretory function. Prasad et al. [362] found that over-expression of 12-LO in cultured INS-1E β -cells halved their glucose-induced insulin secretory capacity. Similarly, the incubation of isolated rat islets with a high level of 4-HNE (100 μ M) significantly lowered their insulin secretory capacity [363]. Conversely, 12-LO knockout mice did not develop glucose intolerance and β -cell dysfunction when fed a diabetogenic high fat diet [364]. In addition, such 12-LO knockout mice were highly resistant to streptozotocin-induced diabetes and their isolated islets maintained good insulin secretory capacity following treatment with inflammatory cytokines. Collectively, these findings suggest that 12-HpETE, the immediate product of 12-LO, and its peroxidation products adversely affect β -cell function [352]. This idea was confirmed when over-expression of 12-LO in cultured INS-1E β -cells abrogated glucose-induced insulin secretion [362]. Further support comes from the finding that β -cell death in rodent and human islets exposed *in vitro* to inflammatory cytokines (i.e. IL-1 β , INF γ , TNF α) was prevented in the presence of baicalin, a specific inhibitor of 12-LO, whereas incubation of the cells with 12-LO metabolites reduced basal insulin secretion and increased cell death [365]. It has been suggested that 4-HNE at non-toxic concentrations inhibits cell growth and induces accumulation of cells in the G0/G1 phase of the cell cycle [366]. Plausibly, a similar inhibitory effect of 4-HNE on β -cell replications may reduce their mass in islets, a phenomenon characteristic to diabetic islets.

The autoimmune and cytokine-mediated destruction of β -cells in Type-1 diabetes has also been associated with over-production of reactive aldehydes, such as 4-HNE. The non-obese diabetic (NOD) mice usually develop severe autoimmune insulinitis and diabetes. However, the expression of an inactive 12/15-LO in these mice rendered them resistant to the development of diabetes [367]. Similarly, Bleich et al. [352] found that islets isolated from 12-LO knockout mice were resistant to cytokine-induced damage. Suarez-Pinzon et al. [368] also identified a key role for 4-HNE and other cytotoxic aldehydes in the destruction of rat pancreatic islet β -cells by cytokines. Other β -cell lines (RINm5F and HIT-T15) were particularly susceptible to 4-HNE, which caused 75–100% cell death at 50 μ M [369]. It was suggested that 4-HNE and other lipid peroxidation products induce apoptosis in RINm5F cells [370]. Noteworthy, not all cells are equally susceptible to 4-hydroxyalkenal-induced damage due to a variable expression of the major detoxifying enzymes glutathione S-transferase (GST, i.e. Gst- μ 3 and Gst- ω 1) and fatty aldehyde dehydrogenase (FALDH, i.e. Aldh3a1) [348]. GSTs detoxify 4-hydroxyalkenals through conjugative reactions, while FALDH transforms the aldehyde group into a carboxylic moiety to produce 4-hydroxy-2E,6Z-dodecadienoic acid (4-HDDA) from 4-HDDE and 4-hydroxy-2E-nonenoic acid (4-HNA) from 4-HNE. Both 4-HNA and 4-HDDA were identified in human urine [347] and in various cells and tissues [67,371]. Only few studies addressed the possibility that these metabolites are biologically active. Murphy et al. [372] reported that 4-HNA bound to the γ -butyrate receptor in the CNS, while Echtay et al. [330] suggested that it uncoupled proton conductance in mitochondria. The susceptibility of β -cells to oxidative stress- and ROS-induced damages has been attributed to their inherent poor intrinsic antioxidant defence [373–376]. This, coupled to a progressive 4-hydroxyalkenal-induced inactivation of FALDH and GST, may exaggerate β -cell dysfunction. Of interest is also the report on an impaired hepatic disposal of 4-HNE in diabetic rats [377]. Consequently, the slow clearance may extend the exposure of β -cells to 4-hydroxyalkenals and further intensify β -cell dysfunction.

Role of 4-hydroxyalkenals in macrovascular diseases. Hyperglycaemia is an established major and independent risk factor in the development of cardiovascular disease in diabetes [378–381]. Endothelial cell dysfunction is the earliest event in the development of atherosclerotic lesions in blood vessels. This condition may result from a direct oxidative damage and over-production of 4-hydroxyalkenals that compromise the integrity and functions of the endothelial cell monolayer. For instance, Minekura et al. [382] found that 4-HNE significantly reduced the expression of adhesion molecules

(i.e. ICAM-1, VCAM-1) induced by TNF- α and NF- κ B activation in primary cultures of human aortic endothelial cells. Tetrahydrobiopterin (THB), the cofactor of endothelial nitric oxide synthase (eNOS), is particularly susceptible to inactivating interactions with 4-HNE. The inactivation and lack of THB induce uncoupling of the enzymatic reaction of eNOS, which leads to the generation of superoxide radicals that further exacerbate the oxidative damage [383]. Interestingly, 4-HNE has been implicated in macrophage activation and their transformation to foam cells in diabetic blood vessels [5]. This is partly due to the 4-HNE capacity to increase the expression of class A scavenger receptors, which enhances macrophage foam cell formation [384]. Moreover, oxLDL has a considerable capacity to covalently bind 4-HNE [385].

The smooth muscle cell layer in blood vessels is also a target for 4-HNE, which induces a marked mitogenic response, characteristic to the early event in atherosclerosis [386–388]. High levels of 4-HNE were associated with other effects in smooth muscle cells, such as apoptosis [389], autophagy [390], cytotoxicity [391], production of TGF- β [392], impaired PDGF receptor activity [393] and enhanced matrix metalloproteinase-2 production [394,395].

The physiological relevance of these *in vitro* studies has been corroborated by Yamanouchi et al. [396], who studied streptozotocin-diabetic APA Syrian hamsters that developed atheromatous lesions. Immunohistochemical analysis of foam cells in the fatty streaks in blood vessels showed a significant increased abundance of 4-HNE-protein adducts. Similarly, Meng et al. [397] found a high content of 4-HNE-protein adducts in aortae of streptozotocin (STZ)-diabetic rats. Recently, Mattson [53] suggested in a comprehensive review a central role of 4-HNE in obesity, in the metabolic syndrome and in vascular and neurodegenerative disorders. Of particular interest in this review is the suggestion to use 4-HNE as a target to prevent and treat the metabolic syndrome and associated diseases. Two main strategies were put forward: to reduce 4-HNE production by suppressing lipid peroxidation or by its detoxification.

Other complications

The aetiology of other peripheral complications in diabetes has also been linked to aberrant interactions of 4-HNE. For example, Polak and Zagorski [398] found significantly higher levels of 4-HNE and MDA in diabetic patients with retinopathy in comparison with patients that did not develop this complication. The presence of AGEs and 4-HNE-protein adducts was evidenced immunohistochemically in the glomerulus in diabetic nephropathy in humans [399,400]. Others found that 4-HNE mimicked features of diabetic neuropathy by inducing mitochondrial dysfunction and aberrant axonal outgrowth in adult sensory

neurons isolated from rats [401,402]. It has been shown that effective quenching of 4-HNE can greatly improve the wound healing process, which is a serious complication in diabetes [403].

Non-toxic interactions of 4-hydroxyalkenals in diabetes.

Unfavourable interactions: By their tendency to form covalent bonds with various amino acid moieties, 4-hydroxyalkenals can modify structures of proteins even at non-toxic levels and alter their function. Several reports have addressed such interactions of 4-HNE that affect signalling pathways in cells [404–406]. Some interactions have been associated with cell differentiation, transcription factors regulation, activation of stress kinases, mitogenic response, stimulation of heat shock proteins or modulation of transduction mechanisms. Demozay et al. [407] have demonstrated the role of 4-HNE in attenuating the insulin transduction mechanism and inducing insulin resistance in 3T3-L1 adipocytes. Incubation of these cells with non-toxic concentrations of HNE significantly decreased the level of the insulin receptor substrate (IRS)-1/2 proteins. This led to a down-regulated response of downstream targets of the insulin transduction pathway, leading to insulin resistance. The underlying mechanism was the formation of 4-HNE-IRS protein adducts and their rapid degradation. Grimsrud et al. [408] performed a thorough LC-ESI MS/MS analysis of proteins from epididymal adipose tissue of diabetic mice and identified a number of regulatory proteins that bound 4-HNE that were linked to oxidative stress responses and the development of insulin resistance.

Glucose transporters are also targets for 4-HNE. Reagan et al. [409] found an increased level of 4-HNE conjugation with glucose transporter-3 (GLUT-3) in the hippocampus of diabetic rats and linked it to decreased hippocampal neural glucose utilization. It is not clear whether 4-HNE similarly interacts with the ubiquitous GLUT-1, the hepatic and β -cell specific GLUT-2 or the insulin-sensitive GLUT-4.

Protective interactions: We have discovered that VEC exposed to high glucose levels can reduce the rate of glucose uptake and prevent adverse effects associated with an increased influx of glucose. Specifically, these cells reduced the cellular level of their principal glucose transporter GLUT-1 mRNA and protein, as well as its plasma membrane content [349,350,410]. High glucose also increased the expression of 12-LO and the production of 12-HpETE and 12-HETE. Pharmacological inhibition of 12-LO completely blocked these high glucose-induced down-regulatory effects [349–351]. The reduced expression of GLUT-1 in VEC exposed to high glucose resulted from post-transcriptional destabilization and degradation of GLUT-1 mRNA. The protein calreticulin, which was also over-expressed under high glucose conditions, bound to a specific 10-nucleotide sequence in the

3'-UTR of GLUT-1 mRNA, destabilized the molecule and rendered it susceptible to nuclease digestion [411]. We also discovered that the expression of calreticulin was augmented in blood vessels of diabetic animals [411]. Collectively, our studies suggest a functional link between 12-LO and its metabolites to GLUT-1 mRNA destabilization by calreticulin.

We have recently found [340] that the augmented expression of 12-LO in VEC under high glucose conditions was accompanied with a marked increase in the generation and secretion of 4-HDDE, but not 4-HNE. Moreover, using pharmacological and molecular approaches we proved that 4-HDDE was an endogenous ligand of the Peroxisome Proliferator-Activated Receptor (PPAR)- δ in these cells. When added to cells at non-toxic concentrations (50 nM) 4-HDDE activated PPAR δ , which specifically interacted with a PPAR-response element in the calreticulin gene and augmented its expression. Previous studies have shown that PPAR δ preferentially interacts with unsaturated fatty acids and eicosanoids [406,412]. X-ray analyses of crystal structures of the PPAR δ ligand binding domain (LBD) identified a network of hydrogen bonds with His⁴¹³, Tyr⁴²⁷, His²⁸⁷ and Thr²⁵³ involved in the interaction with eicosapentanoic acid [413]. Interestingly, His⁴¹³ has also been implicated in a hydrogen-bonding interaction with the 4-hydroxy group of 4-HNE [348]. The sites involved in 4-HDDE interaction with the LBD of PPAR δ have not been identified yet. This natural protective mechanism against the deleterious effects of hyperglycaemia in vascular cells may explain why some diabetic patients never develop cardiovascular complications, as was found in the Adult Treatment Panel III trial [414]. Figure 3 depicts our working

hypothesis: patients who moderately increase the generation of 4-HDDE in VEC under hyperglycaemic conditions benefit from its protective interaction with PPAR δ and the subsequent reduction in GLUT-1 levels. When 4-HDDE production is not controlled, it adversely interacts with proteins, DNA and phospholipid and causes substantial damage to the cells. Theoretically, low levels of 4-hydroxyalkenals in VEC may result from a moderate increase in 12/15-LO expression and/or an efficient neutralization of 4-hydroxyalkenals.

In summary, lipid peroxidation is a prominent feature of diabetes. Both 4-HNE and 4-HDDE contribute to the development of β -cell dysfunction and the development of peripheral complications of diabetes. Yet, when produced at low and non-toxic levels these molecules may have beneficial roles, such as the protection of VEC against deleterious effects of increased flux of glucose. In addition, less is known and reported on 4-HDDE in comparison with 4-HNE. We strongly suggest considering the former as a potent and equally important product of lipid peroxidation in general, and in diabetes in particular.

Renal diseases (W Siems, I Wiswedel)

It is generally accepted that renal failure is associated with drastic lipid peroxidation [415–417]. Lipid peroxidation contributes to pathogenesis and progression of renal failure. Cardiovascular (CV) injury has been shown to be the most critical factor affecting quality-of-life and mortality in patients suffering from end-stage renal disease (ESRD) undergoing haemodialysis (HD). Lipid peroxidation and oxidative stress

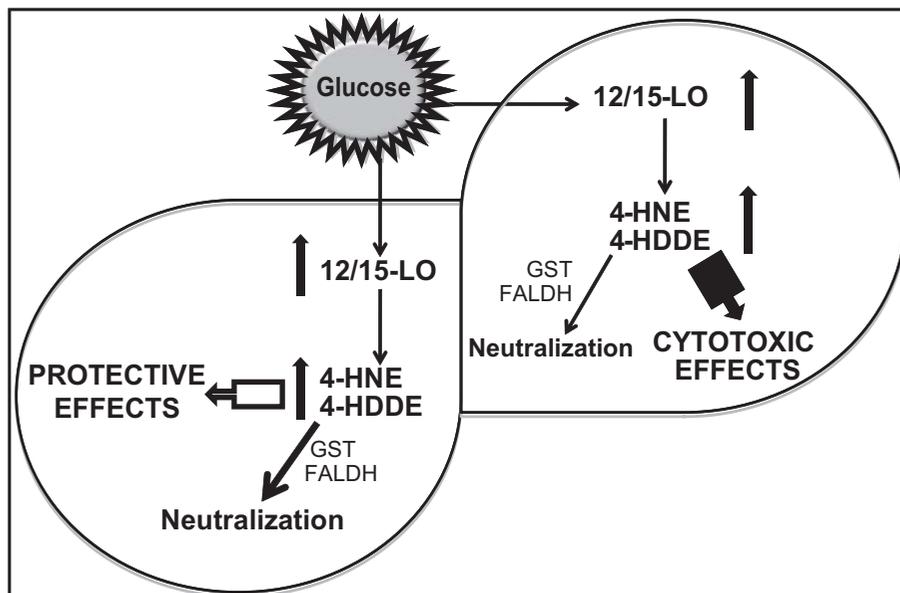


Figure 3. Adverse and beneficial effects of 4-hydroxyalkenals in cells exposed to high glucose levels. The right-hand panel shows a cell that overly increases the expression of lipoxygenases and the downstream generation of 4-hydroxyalkenal, which exerts cytotoxic effects. The left-hand panel depicts a cell that moderately increases lipoxygenase expression and generates non-cytotoxic levels of 4-hydroxyalkenal that may attenuate cell function and even mediate protective interactions.

have been thought to be important risk factors for cardiovascular disorders in renal patients.

Excessive CV morbidity and mortality in kidney insufficiency and uremia can be explained in part by the classical risk factors. However, the pathophysiology of CV lesions is a combination of classical and new emerging risk factors, some of them being strictly related to ESRD [418–421]. Among the new emerging risk factors, anaemia and lipid peroxidation/oxidative stress are two of the most mentioned and studied. These two pathophysiological conditions were already described in the first decline of glomerular filtration rate and several studies have proposed their active role in deterioration of kidney function, especially in diabetic nephropathy [422–429]. We, as others, detected in previous studies dramatic oxidative stress status in ESRD and in patients undergoing haemodialysis [415,427,430–434].

HNE, MDA, protein carbonyls, F2-isoprostanes and cholesterol oxidation products (COP) are increased in plasma of patients with renal failure. The HNE and MDA values were unusually high in the serum of patients. The highest values, e.g. for HNE, in the serum of ESRD patients were around $0.65 \mu\text{M}$ [430]—comparing with normal physiological values of 0.072 ± 0.020 (40–49 years), 0.083 ± 0.020 (50–59 years), 0.096 ± 0.022 (60–69 years) or $0.107 \pm 0.027 \mu\text{M}$, respectively (oldest control group with 70–84 years age) [54]. The HNE and MDA values in patients suffering from renal insufficiency were lower after dialysis compared with the values before dialysis. For serum protein carbonyls there was no difference between values before and following dialysis. For HNE, MDA and protein carbonyls there exists a strong direct correlation with the degree of renal anaemia, i.e. the lower the haemoglobin level, the higher HNE, MDA and protein carbonyl levels were [435]. This is shown in Figure 4.

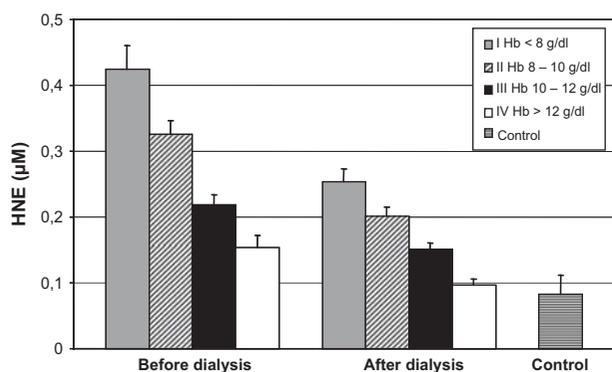


Figure 4. Serum 4-HNE levels in patients with end-stage renal disease. Serum 4-HNE levels in patients with end-stage renal disease before and after dialysis in relation to the degree of renal anaemia (four groups: group I with Hb < 8 g/dl; group II with HB 8–10 g/dl; group III with Hb 10–12 g/dl; group IV Hb > 12 g/dl). Total number of HD patients in this investigation was 107, total number of control probands was 80. Data were taken from Siems et al. [435].

F2-isoprostane levels in patients with chronic renal failure related directly to the degree of inflammation, e.g. to C-reactive protein [436] (Figure 5). Besides lipid peroxidation, an increased serum homocysteine level was typical for 80% of patients. Obviously, serum homocysteine levels represent an independent risk factor in ESRD. We found a correlation between serum homocysteine and plasma albumin as a parameter for nutritional status.

Moreover, parameters of lipid peroxidation are a strong indicator for clinical worsening or improvement. There was an almost linear correlation between HNE plasma level and left ventricular mass index (LVMI) of the heart (Figure 6). Both parameters—the CV parameter LVMI and the lipid peroxidation parameter HNE—showed an inverse relationship with haemoglobin concentration. Inter-relationships between renal disease, anaemia and CV injury are called cardio-renal-anaemia (CRA) syndrome [430,437]. Within the CRA syndrome, oxidative stress including lipid peroxidation is a central pathogenetic and pathophysiological event. If lipid peroxidation and oxidative stress exert a central point in the CRA syndrome, the therapeutic strategy has to take into account the minimization of oxidative attack and/or the strengthening of antioxidative defence mechanisms. The treatment of renal anaemia by means of erythropoietin (EPO) in patients suffering from ESRD undergoing HD, therefore, was controlled concerning its effects on the degree of oxidative stress parameters in relation to clinical improvements of the patients.

The correction of renal anaemia by means of EPO led to an efficient strengthening of antioxidative defence. The improvement of antioxidative capacity by means

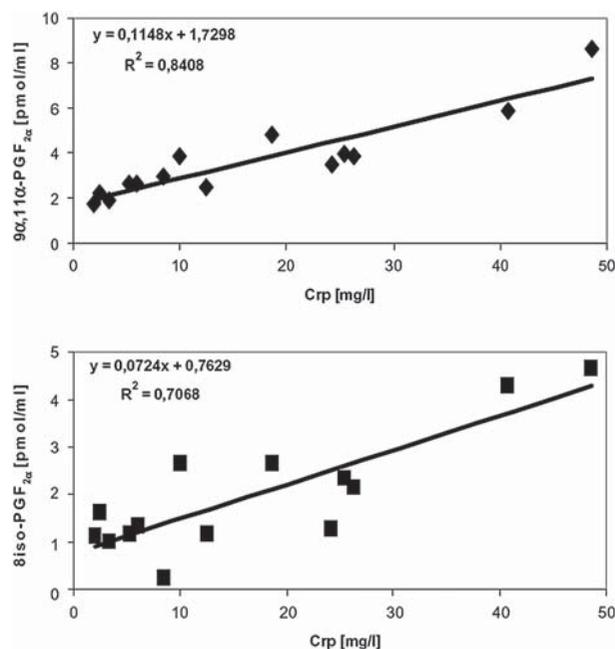


Figure 5. Correlation between serum levels of F2-isoprostanes 8-iso-PGF_{2α} and 9α,11α-PGF_{2α} and C-reactive protein; $n = 14$ patients [436].

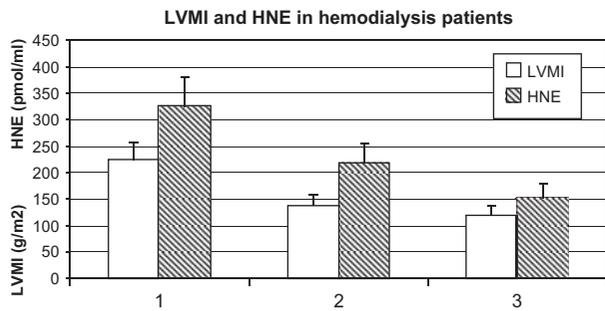


Figure 6. Left ventricular mass index and HNE in relation to the degree of renal anaemia. Left ventricular mass index (LVMI)—as g/m²—and HNE as pmol/ml serum in relation to the degree of renal anaemia. The values are given for three groups of patients concerning the degree of anaemia: 1 Hb < 10 g/dl; 2 Hb between 10–12 g/dl; 3 Hb > 12 g/dl.

of EPO therapy is of complex nature comprising both enzymatic pathways of erythrocytes as mobile radical scavengers and low molecular antioxidants. The therapy of renal anaemia—even the final target Hb is under discussion—with its reduction both of HNE and other LPO products and of the CV risk is regarded as a complex therapeutical strategy. The correction of renal anaemia as antioxidative therapy leads to significant biochemical and clinical advantages in patients with ESRD [430] (see Figure 7). That the treatment with EPO really leads to improvements in lipid peroxidation was shown in two observational studies [435]: in a 2-year investigation with patients from Scorrano/Lecce (Italy) (56 patients and 80 controls) the decrease of plasma HNE was observed; in a smaller and shorter (1 year) observation with 20 patients vs 10 controls from University Nis (Serbia) the MDA decrease was demonstrated (Figure 8). A further therapeutic application of antioxidative therapy in chronic renal failure was shown by Tepel et al. [438] by using acetylcysteine.

Lymphoedema (W Siems, R Brenke)

Chronic lymphoedema is one of the most frequent and debilitating complications after surgical and

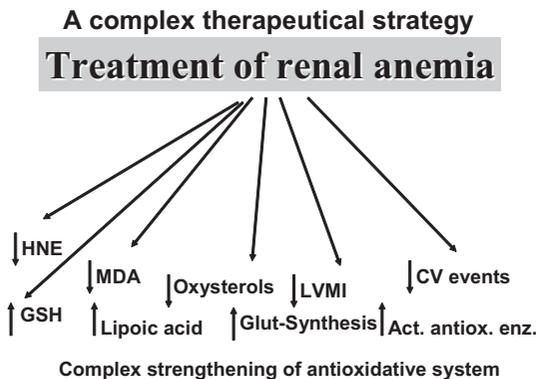


Figure 7. The therapy of renal anaemia is seen as complex therapeutical strategy improving the biochemical and clinical parameters in ESRD.

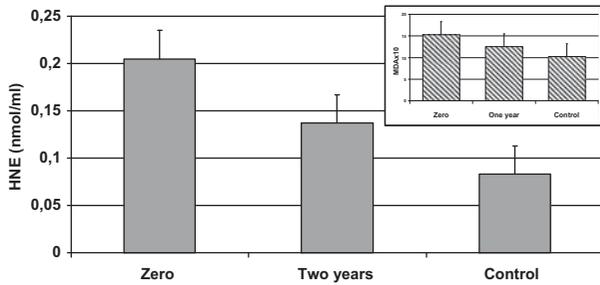


Figure 8. Changes in HNE (main figure) and MDA (inserted figure) serum levels during epoetin treatment of ESRD patients. Average epoetin dose was 60–90 units/kg body weight/week. The data are mean ± SE. Main part of the figure gives the result of Scorrano/Lecce investigation with 56 patients and 80 controls for HNE. Inserted small figure presents the result of Nis study with 20 patients compared to 10 controls, where MDA values were measured (data taken from [434]).

radiological tumour treatment. Prevention and therapy of lymphoedema is therefore an important problem of the rehabilitation of those patients. In total—together with the filariasis-induced lymphoedema—there are more than 200 million patients suffering from lymphoedema worldwide. Increased interstitial volume, pressure and oncotic pressure lead to a variety of structural and functional deteriorations both of the lymphatic vessels and the interstitium itself. These include dilatation of lymphatic capillaries, damage of endothelial cells, sclerosis of collector vessels and the reduction of smooth muscle cells. Furthermore, one finds stimulation of fibroblasts, activation of PMNL, an increased and partially abnormal formation of collagen, the sclerosis of connective tissue and secondary inflammatory processes [427,439, 440] (see also Figure 9). First findings on accelerated lipid peroxidation in lymphoedema were from Ohkuma in 1989 and 1993. In the last 15 years, aldehydes, oxysterols and antioxidative compounds were analysed in more detail in this disease.

The blood of patients with chronic lymphoedema contains lower concentrations of GSH and higher levels of disulphides and of MDA and HNE, compared to the control group. MDA was even increased by ~3-fold in the serum of the lymphoedema patients [427]. Accelerated free radical formation and lipid peroxidation processes were further demonstrated by the liberation of MDA and HNE into the blood serum after manual lymph drainage [427]. These data demonstrate accelerated lipid peroxidation processes in chronic lymphoedematous tissue. Therefore, it is suggested that the strengthening of antioxidative defence mechanisms could be useful in the adjuvant therapy of chronic lymphoedema [440].

Previously it was found that the concentrations of oxysterols in lymphoedematous tissue and blood of patients suffering from lymphoedema are increased compared to healthy control subjects [441]. Figure 10. A shows differences between 7-keto-cholesterol, α-epoxy-cholesterol and for β-epoxy-cholesterol in

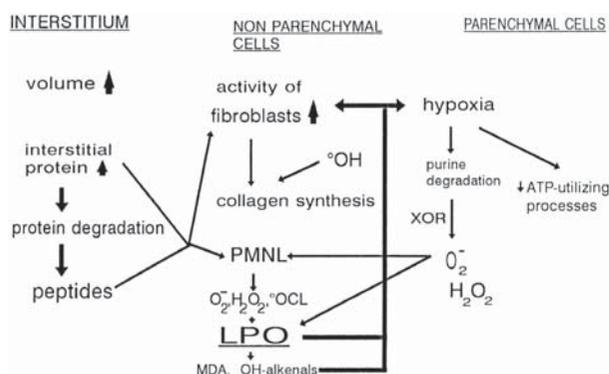


Figure 9. Changes in lymphoedema. Changes in lymphoedema in interstitial matrix and in the cells: activated PMNL and purine degradation as important sources of reactive oxygen species initiating lipid peroxidation in this disease.

patients compared with healthy control subjects. Obviously, increased oxysterol concentration plays a pathogenetic role during tissue remodelling in fibrosis and sclerosis. Those suggestions are in agreement with the role of oxysterols in fibrosis of other organs such as liver [190,442–444]. Additionally, it should be mentioned that the MDA concentration of blood plasma of patients correlates to the volume of the oedema (Figure 10B) and to the reduction of compressibility of lymphoedematous tissue. The higher the MDA, the lower was the reduction of the oedema.

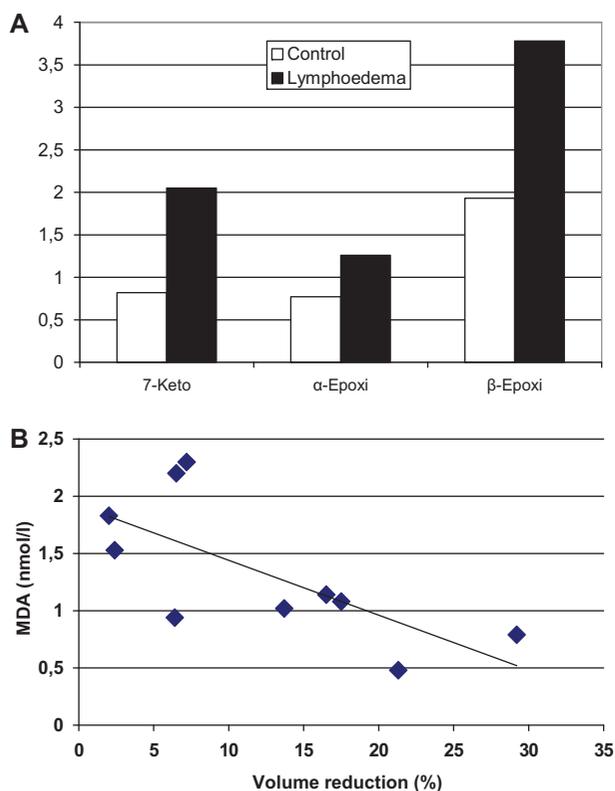


Figure 10. Oxysterols and MDA in patients with lymphoedema. (A) Oxysterols are increased in plasma of patients with lymphoedema compared with controls. Values as mean from 30 patients and 26 controls. (B) As higher MDA as lower was the reduction of the oedema by manual lymph drainage.

Hepatic diseases (J Feher, G Lengyel)

The role of oxidative stress is very important in different liver diseases, mainly in alcoholic and non-alcoholic steatohepatitis and liver cirrhosis, furthermore in chronic viral hepatitis, especially in chronic hepatitis C as well as in primary hepatocellular cancer (HCC). The main processes producing free radicals in mammals are the following: mitochondrial oxidative metabolism providing energy supply, microsomal drug-metabolizing enzyme system, biosynthesis of prostaglandins, constitutive and inducible NO-synthase activity, free radical-producing reactions of phagocytes, monocytes, macrophages and Kupffer's cells and auto-oxidation of H_2O_2 produced in peroxisomes. Another consequence of free radical activity is lipid peroxidation [445]. In the mechanism of oxidative stress and also in its biological consequences, free radicals can attack small molecules containing thiol and amine groups as well as macromolecules building up the cells. Due to peroxidative damage of the lipids in the cell membrane, permeability changes may occur. Nucleic acid injury can lead to mutations and neoplastic processes of the cells.

The healthy organism is able to prevent the overproduction of free radicals. Low oxygen tension of the tissues is a basic condition. Its value is ~ 26 mmHg or less. The primary line of antioxidants consists of representatives of the enzymatic defence. It is supplemented by antioxidant vitamins with scavenger property (vitamins C, A, E, K), the cofactors, compounds containing thiol, phosphor, amine, polyamine, phenols, quinolines, ubiquinone (coenzyme Q), flavonoids, polyenes, glucose, urate, bilirubin, etc.

Non-alcoholic steatohepatitis and liver cirrhosis

Non-alcoholic fatty liver (NAFLD) is a multi-factorial liver disease. It includes a wide spectrum of liver damage characterized by histological changes of alcoholic origin (ranging from uncomplicated fatty liver to steatohepatitis, fibrosis and cirrhosis) in non-alcoholics (< 20 g/day ethanol consumption). The *non-alcoholic steatohepatitis* (NASH) is part of this disease spectrum, which can progress to *hepatic cirrhosis* and liver failure. Its primary forms have been proposed to be a manifestation or consequence of the metabolic syndrome, closely related to insulin resistance. It can be caused by several other metabolic factors and viral infections, like hepatitis C. The history of non-alcoholic fatty liver disease is not always benign, sometimes it can be accompanied by hepatocellular carcinoma [446].

Researches have identified the factors that can play a causative role: oxidative stress, lipid peroxidation, abnormal cytokine production, fatty acid metabolic disturbance and insulin resistance. The pathophysiology of non-alcoholic fatty liver involves insulin

resistance and production of reactive oxygen species, which stimulate the synthesis of several cytokines (Figure 11) through the up-regulation of their transcription by nuclear factor- κ B (NF- κ B). The combination of these events causes hepatocyte injury via direct oxidative injury, TNF- α induced apoptosis or inflammation [447].

Role of free fatty acids and insulin resistance. Free fatty acids (FFA) have a decisive role in the development of insulin resistance by being transformed into acyl-CoA intracellularly. In addition to free fatty acids, TNF α and hyperinsulinaemia also play a role in the activation of one of the mechanisms responsible for the negative control of signal transduction of insulin—namely the Ser/Thr-phosphorylation of insulin receptor and insulin-receptor-substrate (IRS) protein—that blocks the signal transduction processes of insulin. In this way muscle cells, hepatocytes and adipocytes all become resistant to insulin. The activity of mitochondrial respiratory chain complexes is reduced in patients with NASH, showing a positive correlation with TNF α levels, insulin resistance (IR) and body mass index (BMI) values.

Inherited defects in the mitochondrial oxidative phosphorylation are supposed to exist in the offspring (diagnosed with IR) of patients with T2DM, leading to disorders in the intramyocellular fatty acid metabolism. The genes dependent on nuclear respiratory factor-1 (NRF-1) that code enzymes, that play crucial roles in the oxidative metabolism and mitochondrial function, are expressed to a lesser extent in diabetes

mellitus and IR, possibly due to the reduction of PGC1-expression (PPAR- γ co-activator 1- β and 1- α). In the metabolic syndrome, FFA are released from the abnormal mesenteric tissue to a certain extent depending on the ratio of the β -3- and α -2 receptors in the given fat tissue, as β -3-adrenoreceptors and α -2-receptors are responsible for the induction and the inhibition of the lipolytic process, respectively. Polymorphism of the β -3-receptor has been connected with IR and visceral obesity for a long time, most recently with NAFLD. IR is of decisive importance in how NASH is expressed in a patient, especially in the presence of a reduced mitochondrial oxidation. According to recent research, this involves increased lipolysis in the peripheral tissues, an increase in fatty acid uptake by the hepatocytes, the β -oxidation of the fatty acids taken up by the hepatocytes increases, and the uptake of lipoprotein by the tissues is reduced, due to insufficient lipoprotein lipase. IR is much more severe in patients with NASH than in those diagnosed with 'simple' fatty liver.

Mitochondrial dysfunction in NAFLD. Mitochondrial abnormalities are closely related to the pathogenesis of NAFLD, which raised the possibility that NAFLD is a mitochondrial disease [448]. Many genes encoding mitochondrial proteins in skeletal muscle and fat are negatively correlated with body mass, mtDNA depletion in hepatocytes impairs mitochondrial function and causes hepatic steatosis and other liver injuries. Multiple enzymes are involved in mitochondrial β -oxidation and their deficiency may lead to the development of hepatic steatosis.

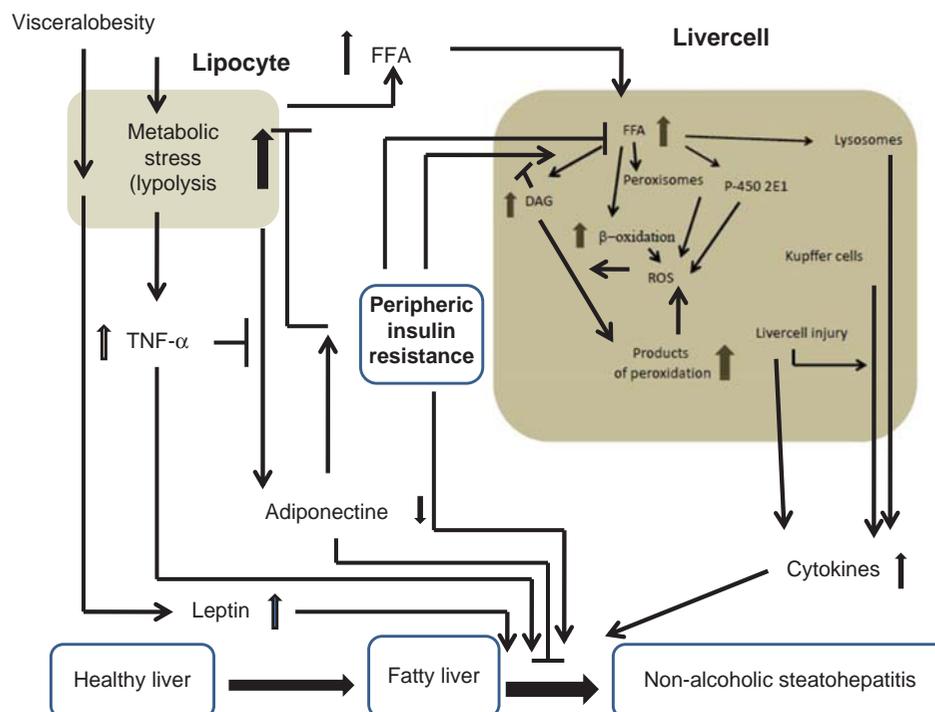


Figure 11. Progression process of non-alcoholic fatty liver. FFA = free fatty acid; DAG = diacylglycerol; ROS = reactive oxygen species (modified from [512]).

A number of mechanisms can be considered to explain the mitochondrial dysfunction found in NAFLD patients and animal models. Possible mechanisms include (a) excessive ROS production, (b) increased TNF α expression and (c) altered PGC-1 expression. MRC dysfunction can directly lead to the production of ROS. If electron flow is interrupted at any point in the respiratory chain, the preceding respiratory intermediates can transfer electrons to molecular oxygen to produce superoxide anion and H₂O₂. MDA and 4-HNE can inhibit mitochondrial cytochrome *c* oxidase by forming adducts with this enzyme. ROS-induced depletion in mtDNA can severely lower mitochondrial number and function, leading to steatosis and liver lesions. Mitochondrial dysfunction may not only cause fat accumulation, but also may generate ROS and cytokines, contributing to the progression of NAFLD by inducing hepatic inflammation and fibrosis [448].

Non-organ-specific autoantibodies (NOSA); positive fatty liver. Non-organ specific autoantibodies (NOSA) include anti-nuclear antibodies (ANA), smooth muscle antibodies (SMA) and anti-mitochondrial antibodies (AMA). They occur in a variety of non-autoimmune chronic liver disease. Their production could be secondary to or triggered by hepatocellular inflammation and necrosis. NOSA positivity in NAFLD has been found to be more prevalent compared to the general population. We have studied the prevalence of non-organ-specific autoantibodies and their correlates with cytokine release and free radical-antioxidant balance in non-alcoholic fatty liver disease [449]. Prevalence of NOSA positivity was found to be 55% in our NAFLD patients. Autoantibody positive patients showed lower antioxidant capacity: lower SH-group concentration, decreased total antioxidant status and elevated chemiluminescence intensity. We suppose that, similar to alcoholic liver disease, the aldehyde products of lipid peroxidation, such as MDA, can react with proteins and form stable protein adducts, which are very immunogenic and capable of inducing immune response, resulting in generation of antibodies. NAFLD with autoantibody positivity may have a worse prognosis because of the impaired antioxidant status. It can be supposed that the appearance of autoantibodies in NAFLD is triggered by free radical reaction, but further investigations are needed to understand the significance, role and the exact mechanisms of NOSA production in NAFLD [449].

Fatty liver in childhood

According to studies, the idiopathic steatohepatitis is characteristic mostly of obese children of peripubertal age. The development of fatty liver can be attributed to several factors. Pathogenetic causes of lipid

accumulation in the hepatocytes—likely in adults—may include (1) reduced oxidation and expenditure of fatty acids; (2) increased synthesis of fatty acids; (3) reduced release of triglycerides from the liver; and (4) increased release of fatty acids from the peripheral fat depots [6]. The pathomechanism has a characteristic two-step form: at first fat is accumulated in the liver cells and then this induces a fibrous reaction. Accumulation of ROS has an important role in both steps. Probably the FFA reaching mitochondria cause saturation of mitochondrial beta-oxidation, creating ROS and lipid peroxides, as well as they activate the CYP2E1 isoform of cytochrome P450 enzyme in hepatocytes and Kupffer cells. Children with NAFLD or NASH have an increased disposition to cardiovascular complications, hyperlipidemia and insulin resistance—or, in the case of alcohol consumption, to alcoholic liver disease—in adulthood. The principal treatment in obese children consists of a diet which is low in fat and poor in refined carbohydrates, as well as physical exercise of medium intensity. Blood glucose and lipid levels should be monitored during the treatment [450].

Fatty liver and hepatitis C viral infection

The significance of fatty liver alteration in the hepatitis C virus (HCV) infection [451] is important because of the decreased viral response to the antiviral drug therapy in these cases. Among patients with HCV genotype 1 infection, the grade of steatosis was correlated with host-related factors, mainly with the presence of the metabolic syndrome. In genotype 3, steatosis degree correlates with liver HCV quantification and serum viral load. In genotype 1, steatosis depends on leptin levels and insulin resistance.

Insulin resistance and hyperinsulinaemia have been found in association with liver fibrosis in hepatitis C [451]. The mechanisms through which insulin resistance promotes fibrosis progression include: oxidative stress, lipid peroxidation, fat accumulation in the hepatocytes, hyperleptinaemia, increased TNF α production and impaired expression of PPAR γ receptors. Liver cell-related steatosis induces fibrosis progression also through ROS accumulation. Hyperleptinaemia with insulin resistance may play a role in the activation of hepatic stellate cells and fibrosis progression. In insulin resistance the TNF α production is enhanced in patients with hepatitis C and the increased level of TNF α initiates the fibrosis progression, owing to their ability to activate HSC and promote collagen deposits. Moreover, TNF α may inhibit PPAR γ activity. An impaired expression of PPAR γ receptors was found in patients with hepatitis C. PPAR γ agonists inhibit inflammation and fibrosis progression by blocking the activation of the redox-sensitive transcription factor NF- κ B and TGF β 1 [452].

The presence of steatosis not only significantly influences the natural history of patients with chronic hepatitis C, but the anti-viral response in these cases is highly decreased. While the sustained virological response is ~ 60% of patients with hepatitis C infection without liver steatosis after 1 year peginterferon-alpha plus ribavirin treatment, this effect is only 30% with liver steatosis in HCV infection with genotype 1. Hepatic steatosis is a high risk factor for reduced response to anti-viral treatment and for evolution towards fibrosis. Obesity and fibrosis represent major therapeutic targets, in association with standard anti-viral regimes, like insulin-sensitizing drugs and free radical scavengers (silymarin, ursodeoxycholic acid, metadoxine); furthermore, the life mode with weight loss can help in therapy efficiency [447,453]. Body-weight reduction and hepatoprotective drugs might have increased the effectivity of combined peginterferon and ribavirin treatment, and thus the sustained virological response.

Alcoholic liver disease

Both acute and chronic alcohol consumption enhances the production of ROS, with the peroxidation of lipids, proteins and DNA. The mechanism by which alcohol causes cell injury is very similar to those observed in non-alcoholic steatohepatitis. Many pathways have been demonstrated in the literature for the alcohol effect, like the oxidative stress, acetaldehyde production, mitochondrial alterations, membrane

injury, apoptosis, ethanol-induced hypoxia, effects on the immune system and altered cytokine production, increased endotoxin levels and activation of Kupffer cells, mobilization of iron, changes in the antioxidant defence, particularly mitochondrial glutathione (GSH), one electron oxidation of ethanol to 1-hydroxy-ethyl radical and induction of CYP2E1. These pathways are not exclusive of one another and it is likely that many systems contribute to the ability of ethanol to induce a state of oxidative stress [447,453,454].

Hepatocellular carcinoma

Hepatocellular carcinoma can develop in all kind of chronic liver diseases. The connections of the different factors are shown in Figure 12. In NAFLD the possible follow-up of the pathogenetic trends is: increased fatty acid fluxus, the increased fatty acid content in the liver (VLDL over-production, dyslipidaemia), advanced oxidation and peroxidation in fatty acids, highly increased free radical production (insufficient antioxidant capacity), flow out of inflammatory and immune reactive mediators (the changes in transcription and translation helping the progression of fibrosis) and finally the carcinogenesis.

In conclusion, oxidative stress plays a very important role in most chronic liver diseases, mainly in alcoholic and non-alcoholic steatohepatitis and liver cirrhosis, furthermore in chronic viral hepatitis, especially in chronic hepatitis C as well as in primary

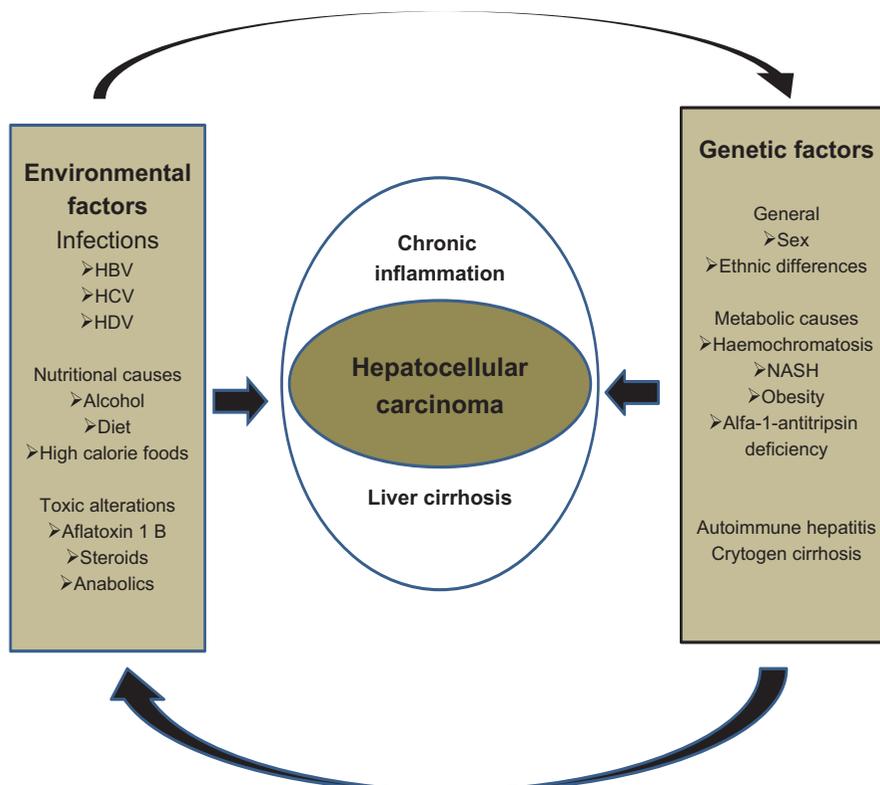


Figure 12. Factors influencing the progression of chronic liver disease to liver cirrhosis and hepatocellular carcinoma.

hepatocellular cancer (HCC). The diagnostic biomarkers to the clinical praxis can be seen in Table III.

Lipid peroxidation and cancer (H Basaga, N Zarkovic)

Cancer is an abnormal mass of tissue the growth of which exceeds, and is uncoordinated with that of the normal tissue, continuing its destructive spread even after the stimuli that initiated it have ceased. Although cancer is not generally considered to be a typical LPO-associated disease, as are neurodegenerative or cardiovascular disorders, not only carcinogenesis but also cancer therapies are strongly influenced by oxidative stress and by LPO. Namely, harmful carcinogenic/mutagenic effects of free radicals play important roles in malignant transformation, while cytotoxicity of oxygen free radicals is important for various anti-cancer therapies, such as radiotherapy and doxorubicin chemotherapy. On the other hand, intensive lipid metabolism is necessary for dynamic proliferation of cancer cells, while lipid peroxidation products, in particular 4-HNE, are known to act not only as second toxic messengers of free radicals, but also as signalling molecules and growth regulating factors influencing proliferation, differentiation and apoptosis of cancer cells. Therefore, some fundamental aspects of cancer association to LPO will be given in this paper.

Oxidative stress and cancer. Carcinogenesis is thought to occur in stages which could be in general denoted as initiation stage and promotion stage followed usually, but not necessarily, with cancer progression. In the initiation stage, physical, chemical or biologic agents cause an alteration in the molecular structure of the cellular DNA. This molecular injury is followed by a promotion stage, in which the expression of the genes that regulate cell growth (proliferation and differentiation) is altered. While initiating agents usually react directly with the genetic material, promoting agents usually do not act this way, but alter gene

expression by indirect mechanisms. Some compounds are 'complete' carcinogens since they possess both initiating and promoting activity, thus by themselves could cause cancer. Among the complete carcinogens are various agents (tumourigenic viruses, carcinogenic chemicals and ionizing radiation) that often cause oxidative stress. ROS play a role mostly in the promotion phase of carcinogenesis [455]. Polycyclic hydrocarbons are complete chemical carcinogens whose harmful activities are correlated with their ability to form free radicals [456]. At least four different radicals might be formed from benzo(a)pyrene (BaP): the benzo(a)pyrene anion radical (BaP⁻), benzo(a)pyrene cation radical (BaP⁺), radical species formed by heating BaP (related to the radicals formed in cigarette smoke) and oxygenated BaP radicals. The most relevant from the last group are 6-oxyBaP radical and radicals formed from BaP quinones [457]. The production of BaP⁺ and oxygenated BaP radicals can be considered as an ubiquitous and frequent process since they can be formed by the activity of the enzyme cytochrome P450 or peroxidase-hydrogen peroxide. Based on the activity of these radicals the binding of BaP to DNA has been implicated as the first step of carcinogenesis caused by BaP. The chromosome breaking (clastogenic) effect of the 'tumour promoter' phorbol myristate acetate (PMA) is mediated by the production of ROS [458]. Additionally, carcinogenic activities of tumour promoters are associated with a decrease of the cellular antioxidant defences, such as the key enzymes defending from the oxidative stress, catalase and superoxide dismutase (SOD).

Antioxidants are often acting as anti-promoters and anti-carcinogens [459], in agreement with their fundamental role of biological response modifiers of oxidative homeostasis [460]. Thus, suppression of the cancer development by antioxidants on one side and, on the other, carcinogenesis based on oxidative stress should not only cause structural changes in the genome DNA, but also functional changes, i.e. changes of the gene expression. That should be particularly relevant if genes encoding for the enzymes

Table III. Biomarkers of liver cell injury, inflammation, fibrogenesis, fibrosis and hepatocellular carcinoma.

Liver cell injury	Inflammation	Fibrogenesis	Fibrosis and ECM turnover	Hepatocellular carcinoma
ALT	CRP	fibrogenic cytokines	hyaluronan	alfa-fetoprotein
AST	alfa-2-macroglobulin	CTGF	PIII NP	PIVKA II
GGT	haptoglobin	circulating fibrocytes	MMPs	
GLDH	chemokines	CSF	TIMPs	
LDH5		chemokines	laminin	
Liver cell injury caused by: alcohol; drugs, toxin; chemicals; NAFLD; metabolic diseases; HBV; HCV; HDV; autoimmune reaction; cryptogenic; venous obstruction; cholestasis	inflammatory cells	activation of hepatic stellate cells expansion of myofibroblast pool mediators: IGF-I; PDGF; TGF-beta; ET-1; ROS	characterizing in the tissue: collagen; elastin; glycoproteins; proteoglycans; hyaluronan	produced by the liver: tumour cells

involved in detoxification of ROS, DNA repair and in cell growth regulation (proto-oncogenes) would be affected. Hence, the same or similar mechanisms of oxidative stress could lead on one side to depressed expression of the genes involved in defence against ROS and on the other to enhanced expression of the genes stimulating cell growth. This should, although pathologically altered, be relatively balanced to allow not only survival of the cancer cells but also its further growth and progression of cancer. Unfortunately, the mechanisms that guide the carcinogenesis in such a 'controlled' way are not understood [461].

However, fortunately, some recent discoveries on Ral-binding protein 1 (RALBP1), a stress-responsive and stress-protective multi-specific transporter of glutathione conjugates (which is often over-expressed in malignant cells and plays a prominent anti-apoptotic role selectively in cancer cells through its ability to control cellular concentration of pro-apoptotic LPO products such as HNE), not only increase our understanding on how tumour cells escape for growth regulating and anti-cancer effects of LPO but also open novel approaches to cancer therapies. Namely, even in the absence of chemotherapy, inhibition of RALBP1 by specific antibodies causes regression of murine melanoma B16 [462], while inhibition of RALBP1 combined with appropriate chemotherapies could cause regression of human solid tumours that over-express RALBP1 such as non-small-cell lung cancer (NSCLC H358 and H520) and colon SW480 cell lines [463].

Lowered activities of primary antioxidant enzymes, such as the cytoplasmic CuZnSOD (SOD1), are often, but not always, seen in tumours, suggesting that decreased antioxidant protection accompanied by increased ROS production might explain not only essential steps in carcinogenesis, but, more generally, many cancers cell properties. Among the hereditary factors of cancer development there are examples of genetic alterations related to the lack of defence against oxidative stress and harmful effects of ROS. An example are patients with triple chromosome 21 (trisomy 21 or Down's syndrome) in which the tissue content of cytoplasmic SOD1 is increased for ~ 50%, since the gene encoding for the enzyme is located on chromosome 21. Erythrocyte GSH-peroxidase activity is also increased in patients with Down's syndrome, probably due to the higher H₂O₂ production resulting from the increased SOD activity. However, in patients with trisomy 21, only cytoplasmic SOD1 is increased while mitochondrial MnSOD (the gene for the mitochondrial SOD2 is located on chromosome 6) is reduced by one third [464]. This suggests the possibility of regulation of mitochondrial SOD2 activity by cytosolic feedback mechanisms of SOD1 and/or superoxide ions. Thus, altered SOD activity pattern may be significant in carcinogenesis because trisomy 21 patients have a 10–30% increased risk of developing acute leukaemia. Hence, a decrease of SOD2

activity may be associated with the acquisition of the cancer. In agreement with this are findings of higher incidence of cancer in patients with Dubin-Johnson-Sprinz syndrome, an inherited disease which shows deficiency of SOD2 [465].

The relevance of persistent oxidative stress in cancer could be in continuous activation of transcription factors leading to intensive proliferation of the cells through altered expression of c-fos, c-jun and c-myc genes [466]. Additionally, ROS can specifically damage proteinase inhibitors (alpha-1-proteinase inhibitors, alpha-1-macroglobulin, alpha-1-plasmin, etc.) by oxidizing methionine residues of the active sites, which would consequently allow further progression of cancer [467]. Thus, it is obvious that ROS play a relevant role not only in cancer induction and promotion but also in its progression; hence, cancer itself represents a condition of oxidative stress, which is furthermore often associated with another oxidative stress and LPO associated disorder—inflammation.

Chronic inflammation, oxidative stress and lipid peroxidation in the genesis and perpetuation of cancer

A number of chronic inflammatory conditions induced by biological, chemical and physical factors pre-dispose susceptible cells to malignant transformation and cancer progression. Chronic inflammatory processes induce oxidative/nitrosative stress and LPO, thereby generating excessive ROS, reactive nitrogen species (RNS) and DNA-reactive aldehydes. In general, the longer the inflammation persists, the higher the risk of cancer. It has been estimated that chronic infection and associated inflammation contribute to about one in four of all cancer cases worldwide [468]. For example, ulcerative colitis has long been linked with high incidence of colorectal cancer; and chronic gastritis, such as from infection with *H. pylori*, has been associated with a high incidence of gastric cancer [469,470].

Targets of free radicals in inflammation include DNA, RNA proteins and lipids. Among these, oxidatively modified DNA is a major source of the mutation in living organisms and more than 100 oxidative DNA adducts with purine, pyrimidine and the deoxyribose backbone have been identified [391,471–473]. ROS induced DNA damage can result in single- or double-strand breakage, base modifications, deoxyribose modification and DNA cross-linking. Moreover, cell death, replication errors and genomic instability can occur if the oxidative DNA damage is not repaired prior to DNA replication [474–477]. The most extensively studied and most abundant oxidative DNA lesion produced is 8-hydroxydeoxy guanosine (OHdG) and many studies have demonstrated that OHdG levels are elevated in various human cancers [478–481]. In addition, RNS are produced during the chronic inflammation process, which causes nitritative DNA damage to

form 8-nitroguanine. The formation of 8-nitroguanine has been observed in human samples and experimental evidence has suggested that 8-nitroguanine is also mutagenic to form DNA lesions [482].

It has been shown that mutations in cancer-related genes or post-translational modification of proteins by nitration, nitrosation, phosphorylation, acetylation or poly ADP-ribosylation by free radicals or lipid peroxidation derived reactive aldehyde species such as 4-HNE are some of the events that increase the risk of cancer. For example, oxidative damage to DNA has been linked to aflatoxin B-induced *p53* and *ras* gene mutations in hepatocarcinogenesis [483] and in UV-induced mouse and human skin cancers [484,485]. Most notable among pre-cancerous mutations are those that result in modulated signal transduction pathways, thereby increased expression of oncogenes (e.g. *myc*, *ras*, *abl*, *bcl-2*) or decreased activity of tumour-suppressor genes (e.g. *p53*, *Rb*), conferring a selective growth or survival advantage to the cell. Under circumstances of prolonged stress, such as chronic inflammation, cells lose the ability to turn on and off transiently the genes, e.g. cytochrome P450 enzymes that help to eliminate the toxic chemicals—and a mutation may lock in the growth-advantaged phenotype. Hence, prolonged exposure to stress results in a selection of pre-cancerous cells. Phenotypic changes representative of pre-neoplastic mutations include a decreased need for metabolites and growth factors, abnormal signal transduction, inappropriate expression of receptors for growth factors, dysregulation of cell-cycle checkpoints and resistance to apoptosis. Thus, any agent causing increased cell proliferation increases the risk of neoplastic transformation. Therefore, cancer proneness is a consequence of extensive and sustained free radical stress-related damage in diseases (Table IV).

ROS, RNS and LPO products can also modulate signalling molecules [486] and alter functions of enzymes and proteins involved in inflammation and carcinogenesis [487] such as the nuclear transcription factor NF- κ B, iNOS and cyclooxygenase-2 (COX-2). Thus, chronic inflammatory processes facilitate, through deregulation of cellular homeostasis, the initiation of normal cells and their growth and progression to malignancy, through production of various types of DNA damage, impairment of DNA repair pathways, overproduction of pro-inflammatory cytokines and reduced apoptotic rates of damaged cells [488,489].

Table IV. Examples of high cancer risk ROS overload diseases modified from [488].

Haemochromatosis	Hepatitis B	Pancreatitis
Chron's disease	Hepatitis C	Schistosoma japonicum
Ulcerative colitis	Human papilloma virus	Schistosoma hematobium
Prostatitis	Helicobacter infection	

Nutritional aspects

Different lifestyle- and environmental-related factors such as smoking, diet, alcohol, ionizing radiations, biocides, pesticides, viral infections and other health-related factors (e.g. obesity or the ageing process) may be pro-carcinogenic. In all these cases oxidative stress acts as a critical pathophysiological mechanism. The excessive consumption of calories derived from animal protein and fat combined with a poor diet in fruit, vegetables high in carotenoids and green leaf vegetables fosters the occurrence of some cancers, including, e.g. non-Hodgkin lymphoma, colon, prostate, endometrium and breast cancers [490,491]. Fruits and vegetables are rich in antioxidants such as vitamin C, vitamin E, carotenoids, natural flavonoids and other compounds able to remove oxidant species, thus providing a diet-dependent protection system for our organism [492,493]. The World Health Organization attributed insufficient consumption of fruits and vegetables to 19% of the stomach, 20% of the oesophagus, 12% of the lung and 2% of colorectal cancer cases worldwide [494].

New genomic technologies have made possible the investigation of nutritional modulation of the carcinogenesis pathway with nutrients, micronutrients and phytochemicals. Modern studies of nutrient-modulated carcinogenesis involve exploring the effect of nutrients on DNA damage and repair mechanisms. DNA methylation and other epigenetic changes, which influences gene expression and cellular phenotypes; antioxidant redox state and oxidative stress; target receptors and signal transduction pathways; cell cycle controls and check points; cell death mechanisms; and anti-angiogenic processes.

Developments in nutritional genomics, proteomics and metabolomics will facilitate to simultaneously elucidate the biological effects of dietary constituents on cell function and global gene expression. This generation of new knowledge on nutrient-gene interactions provides background for a research framework for diet and cancer prevention focused on identifying and developing new biomarkers for dietary intervention.

LPO products and cancer

While oxidative stress and LPO may play important roles in carcinogenesis, cancer cells are sensitive to the LPO products which are also mediators of oxidative stress. Among them of particular importance is 4-hydroxynonenal (HNE), which has toxic effects for the cancer cells, causing both necrosis and apoptosis, and is therefore considered as a second messenger of free radicals. On mouse colon epithelial cells, HNE has been shown to affect more Apc mutated cells, compared to normal cells, inducing therefore relatively selective apoptosis of these cells [495]. The highly reactive aldehyde 4-HNE is one of the major cytotoxic products of lipid peroxidation, but it could be found also in various tissues even under normal,

physiological conditions influencing proliferation and differentiation of the cells [70]. While physiological roles of 4-HNE have not been entirely clarified yet, this particular aldehyde seems to be an important cell growth regulating factor acting as a signalling molecule interacting with the growth regulating effects of various cytokines [71,496,497]. Unlike free radicals and other ROS, 4-HNE is able to remain stable, not metabolized, after binding to macromolecules, especially proteins. 4-HNE macromolecular conjugates might even be required for the biological activities of 4-HNE and could be detected by the use of monoclonal antibodies (Figure 13). However, one of the essential characteristics of the oxidative metabolism of tumour cells is their resistance to inducers of ROS, resulting in a relative oxidative homeostasis ('steady-state') of endogenous lipid-peroxidation products mostly below the normal values [498]. That is also why some tumour cells could be entirely '4-HNE-free', while other tumours develop in spite of abundant 4-HNE, as can be seen in Figure 12. It is interesting that dividing liver cells in partially-hepatectomized rats accumulate alpha-tocopherol during DNA replication, suggesting that a decrease of susceptibility to lipid peroxidation may be a feature of normal cell division rather than a specific feature of cancer cells [499]. In agreement with that are findings of the increased production of GSH, another key anti-oxidative defence system, in the liver of partially hepatectomized animals [500] and the association of 4-HNE with hepatitis and liver carcinogenesis [501]. Interestingly, the appearance of 4-HNE in the usual form of protein adducts that are membrane-associated in normal cells, even in case of inflammation [502], is transformed into the nuclear membrane appearance in the case of liver pathologies [501]. Similar nuclear association of HNE-protein adducts was recently observed also in ulcerogenic changes in human gastric mucosa [503,504], also indicating persistent oxidative stress and lipid peroxidation accruing in chronic inflammation as in cancer. In this respect, the pro-inflammatory cytokines granulocyte-macrophage colony stimulating factor (GM-CSF), tumour necrosis factor α (TNF α) and interleukin-1 (IL-1) are of particular interest. Namely, the gene promoter region encoding for these cytokines contain a binding site for the nuclear factor kappa B (NF- κ B), a cytosolic 'gene enhancer' protein which is activated by ROS [466], while 4-HNE was denoted to be one of the key factors regulating activity of cytokines involved in various inflammatory and fibrogenic processes [505]. Similarly, oxidative stress activates also potent gene activator protein 1 (AP-1). AP-1 activates the genes encoding for the fibrogenic growth factors (cytokines TGF β and PDGF, which are activated through AP-1) that promote mesenchymal growth leading to the formation of blood vessels and connective tissue necessary for inflammation and for cancer develop-

ment. 4-HNE, as a second messenger of ROS (free radicals), activates AP-1, followed by further TGF β synthesis and fibrogenesis [506]. Hence, inflammation and cancer could be considered as interfering processes which share two common pathophysiological mechanisms: the cytokine network and oxidative

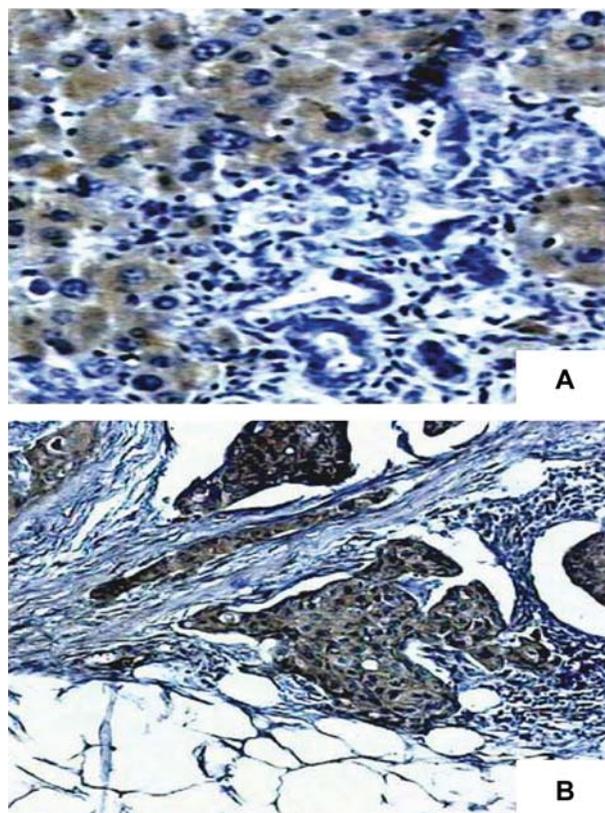


Figure 13. Immunohistochemical detection of the HNE-histidine adducts. Immunohistochemical detection of the HNE-histidine adducts in different carcinoma tissues done by specific monoclonal antibodies. The monoclonal antibodies were developed by G. Waeg's group, from culture medium of the clone 'HNE Ig4', produced by a fusion of Sp2-Ag8 myeloma cells with B-cells of a BALB-c mouse immunized with HNE-modified keyhole limpet haemocyanine (501). The antibody is specific for the HNE-histidine epitope in HNE-protein conjugates. HNE lysine and HNE cysteine give 5% and 4% cross-reactivity with HNE Ig4. Visualization of the immunohistochemical reaction was done by peroxidase labelled secondary antibody (rabbit-anti mouse) with DAB (3, 3-diaminobenzidine tetrahydrochloride) staining giving brown immunopositive reaction, with haematoxylin blue contrast staining. The slides were prepared at Rudjer Boskovic Institute (Zagreb, Croatia) and analysed by Professor K. Zarkovic at Clinical Hospital Centre of the Medical Faculty (Zagreb). (A) Immunohistochemical analysis of LEC rat liver carcinoma. The unique model of LEC rats that accumulate copper (done by Dr F. Gueraud et al., INRA, Toulouse, France) allows monitoring the experimental liver carcinogenesis associated with LPO. The liver carcinoma tissue is entirely negative for HNE (blue), while surrounding liver tissue is strongly HNE-immunopositive (brown) (magnification 400 \times). (B) Immunohistochemical analysis of human mammary carcinoma. This particular carcinoma has very pronounced stromal component, i.e. connective tissue, within which malignant cells are spread. As can be seen carcinoma cells are strongly positive for HNE (brown), while connective tissue and fat tissue of the mammary gland (lower part of the photo) are entirely negative for HNE (magnification 150 \times).

stress, while 4-HNE could at the same time promote fibrogenesis and inhibit the cancer growth.

The molecular mechanisms of the growth modifying effects of 4-HNE include the c-fos gene [507]. 4-HNE may induce an increased binding activity of the AP-1 transcription factor [506]. The c-Fos protein (product of the c-fos gene) is a mammalian transcription factor which acts by forming the heterodimer AP-1 with the transcription factor c-Jun. It is generally assumed that the existence of heterodimeric factors is important for fine tuning of transcriptional control. The corresponding genes, c-fos and c-jun, belong to the early response genes, whose induction can occur with no intervening protein synthesis, but requires only the modification of pre-existing transcription modulators. Among the early response genes c-fos is the best characterized gene. Agents that are able to induce this gene include the epidermal growth factor (EGF) and the platelet-derived growth factor (PDGF) or plain serum. The c-fos gene is necessary for cell proliferation, because c-Fos can up-regulate the cell cycle by inducing cyclin D1 which is part of the 'master switch' of the cell cycle and was found to be crucial for the regulating roles of 4-HNE and TGF β in colon carcinogenesis based on JNK up-regulation [508]. Since 4-HNE acts as bifunctional (stimulating as well as inhibiting) regulator of the c-fos expression that also modifies the effects of humoral factors which promote c-fos transcription resulting in an inhibition of cancer growth, it is likely that this aldehyde could have an essential role in 'turning inflammation against cancer'. In agreement with this are findings of association of 4-HNE and TGF β deregulation (in particular affecting the R1 receptor of TGF β) [509].

However, 4-HNE is probably not the only LPO-generated reactive aldehyde involved in colon carcinogenesis, because acrolein seems to be involved in development of benign colon tumours and their transition to carcinoma and the spread of LPO from malignant into the surrounding non-malignant tissue in relation to the cancer progression [510]. The most recent findings suggest that acrolein might be in a similar way also associated with development of prostate carcinoma, yet, in this particular case, it should be noted that acrolein might also come from spermine/spermidine oxidation, not only as a product of LPO [511].

Further studies on association of LPO and cancer development might therefore lead to novel approaches in cancer therapy based on the biomodulation of cancer and the organism's response to it (tumour-host relationship). This approach will surely involve inflammation as a key pathway of the immune response to disease, antioxidants acting as biological response modifiers of oxidative homeostasis and finally bio-regulating, not only cytotoxic, LPO products and mediators of oxidative stress, such as 4-HNE.

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References

- [1] Sies H. Oxidative stress: from basic research to clinical application. *Am J Med* 1991;91:S31-S38.
- [2] Sies H. Oxidative stress: oxidants and antioxidants. *Exp Physiol* 1997;82:291-295.
- [3] 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.
- [4] Schaur RJ. Basic aspects of the biochemical reactivity of 4-hydroxynonenal. *Mol Aspects Med* 2003;24:149-159.
- [5] Leonarduzzi G, Chiarpotto E, Biasi F, Poli G. 4-Hydroxynonenal and cholesterol oxidation products in atherosclerosis. *Mol Nutr Food Res* 2005;49:1044-1049.
- [6] Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 1956;11:298-300.
- [7] Beckman KB, Ames BN. Mitochondrial aging: open questions. *Ann NY Acad Sci* 1998;854:118-127.
- [8] Beckman KB, Ames BN. The free radical theory of aging matures. *Physiol Rev* 1998;78:547-581.
- [9] Harman D. The aging process. *Proc Natl Acad Sci USA* 1981;78:7124-7128.
- [10] Harman D. Free radicals in aging. *Mol Cell Biochem* 1988;84:155-161.
- [11] Harman D. The aging process: major risk factor for disease and death. *Proc Natl Acad Sci USA* 1991;88:5360-5363.
- [12] Harman D. Free radical theory of aging. *Mutat Res* 1992;275:257-266.
- [13] Harman D. Free radical involvement in aging. *Pathophysiology and therapeutic implications. Drugs Aging* 1993;3:60-80.
- [14] Harman D, Eddy DE, Noffsinger J. Free radical theory of aging: inhibition of amyloidosis in mice by antioxidants; possible mechanism. *J Am Geriatr Soc* 1976;24:203-210.
- [15] Grune T, Jung T, Merker K, Davies KJ. Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and 'aggresomes' during oxidative stress, aging, and disease. *Int J Biochem Cell Biol* 2004;36:2519-2530.
- [16] Sohal RS, Orr WC. Relationship between antioxidants, prooxidants, and the aging process. *Ann NY Acad Sci* 1992;663:74-84.

- [17] Fleming JE, Miquel J, Cottrell SF, Yengoyan LS, Economos AC. Is cell aging caused by respiration-dependent injury to the mitochondrial genome? *Gerontology* 1982;28:44–53.
- [18] Fleming JE, Reveillaud I, Niedzwiecki A. Role of oxidative stress in *Drosophila* aging. *Mutat Res* 1992;275:267–279.
- [19] Yan LJ, Sohal RS. Mitochondrial adenine nucleotide translocase is modified oxidatively during aging. *Proc Natl Acad Sci USA* 1998;95:12896–12901.
- [20] Hohn A, Jung T, Grimm S, Grune T. Lipofuscin-bound iron is a major intracellular source of oxidants: role in senescent cells. *Free Radic Biol Med*. 2010;48:1100–1108.
- [21] Kirkwood TB, Kowald A. Network theory of aging. *Exp Gerontol* 1997;32:395–399.
- [22] Jang YC, Remmen VH. The mitochondrial theory of aging: insight from transgenic and knockout mouse models. *Exp Gerontol* 2009;44:256–260.
- [23] McCann SM, Licinio J, Wong ML, Yu WH, Karanth S, Rettorri V. The nitric oxide hypothesis of aging. *Exp Gerontol* 1998;33:813–826.
- [24] 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.
- [25] Hannover A. Mikroskopiske undersøgelser af nervesystemet. *Kgl Danske Vidensk Kabernes Selskobe Naturv Math Ak (Copenhagen)* 1842;10:1–12.
- [26] Konneff JH. Beiträge zur kenntnis der nervenzellen der peripheren ganglien. *Mitt Naturforsch Gesellsch Bern* 1886;44:13–14.
- [27] Brizee KR, Ordy JM, Kaack B. Early appearance and regional differences in interneuronal and extraneuronal lipofuscin accumulation with age in the brain of a nonhuman primate (*Macaca mulatta*). *J Gerontol* 1974;29:366–381.
- [28] Hammer C, Braum E. Quantification of age pigments (lipofuscin). *Comp Biochem Physiol B* 1988;90:7–17.
- [29] Hartoft WS. Ceroid. *Am J Med Sci* 1965;250:324–345.
- [30] Yin D. Biochemical basis of lipofuscin, ceroid, and age pigment-like fluorophores. *Free Radic Biol Med* 1996;21:871–888.
- [31] Reichel E, Holander J, Clark HJ, Strehler BL. Lipofuscin pigment accumulation as a function of age and distribution in rodent brain. *J Gerontol* 1968;23:71–78.
- [32] Miquel J, Tappel AL, Dillard CJ, Herman MM, Bensch KG. Fluorescent products and lysosomal components in aging *Drosophila melanogaster*. *J Gerontol* 1974;29:622–637.
- [33] Sheldal JA, Tappel AL. Fluorescent products from aging *Drosophila melanogaster*. An indicator of free radical lipid peroxidation damages. *Exp Gerontol* 1974;9:33–41.
- [34] Sohal RS. Fine structural alterations with age in the fat body of the adult male housefly, *Musca domestica*. *Z. Zellforsch Mikrosk. Anatomie* 1973;1973:169–175.
- [35] Sitte N, Huber M, Grune T, Ladhoff A, Doecke WD, Von Zglinicki T, Davies KJ. Proteasome inhibition by lipofuscin/ceroid during postmitotic aging of fibroblasts. *FASEB J* 2000;14:1490–1498.
- [36] Krohne TU, Kaemmerer E, Holz FG, Kopitz J. Lipid peroxidation products reduce lysosomal protease activities in human retinal pigment epithelial cells via two different mechanisms of action. *Exp Eye Res* 2010;90:261–266.
- [37] Catalgol B, Ziaja I, Breusing N, Jung T, Hohn A, Alpertunga B, Schroeder P, Chondrogianni N, Gonos ES, Petropoulos I, Friguet B, Klotz LO, Krutmann J, Grune T. The proteasome is an integral part of solar ultraviolet radiation-induced gene expression. *J Biol Chem* 2009;284:30076–30086.
- [38] Ahmed EK, Rogowska-Wrzczesinska A, Roepstorff P, Bulteau AL, Friguet B. Protein modification and replicative senescence of WI-38 human embryonic fibroblasts. *Aging Cell* 2010;9:252–272.
- [39] Grune T, Merker K, Sandig G, Davies KJ. Selective degradation of oxidatively modified protein substrates by the proteasome. *Biochem Biophys Res Commun* 2003;305:709–718.
- [40] Friguet B, Stadtman ER, Szewda LI. Modification of glucose-6-phosphate dehydrogenase by 4-hydroxy-2-nonenal. Formation of cross-linked protein that inhibits the multicatalytic protease. *J Biol Chem* 1994;269:21639–21643.
- [41] Lippman RD. Rapid *in vivo* quantification and comparison of hydroperoxides and oxidized collagen in aging mice, rabbits and man. *Exp Gerontol* 1985;20:1–5.
- [42] Sharma SP, Wadhwa R. Effect of butylated hydroxytoluene on the life span of *Drosophila bipectinata*. *Mech Ageing Dev* 1983;23:67–71.
- [43] Rikans LE, Hornbrook KR. Lipid peroxidation, antioxidant protection and aging. *Biochim Biophys Acta* 1997;1362:116–127.
- [44] Siems W, Grune T. Intracellular metabolism of 4-hydroxynonenal. *Mol Aspects Med* 2003;24:167–175.
- [45] Voss P, Siems W. Clinical oxidation parameters of aging. *Free Radic Res* 2006;40:1339–1349.
- [46] Chia LS, Thompson JE, Moscarello MA. Changes in lipid phase behaviour in human myelin during maturation and aging. Involvement of lipid peroxidation. *FEBS Lett* 1983;157:155–158.
- [47] Sawada M, Carlson JC. Changes in superoxide radical and lipid peroxide formation in the brain, heart and liver during the lifetime of the rat. *Mech Ageing Dev* 1987;41:125–137.
- [48] Hayakawa N, Yokoyama H, Kato H, Araki T. Age-related alterations of oxidative stress markers in the mouse hippocampal CA1 sector. *Exp Mol Pathol* 2008;85:135–140.
- [49] Zhang JR, Andrus PK, Hall ED. Age-related phospholipid hydroperoxide levels in gerbil brain measured by HPLC-chemiluminescence and their relation to hydroxyl radical stress. *Brain Res* 1994;639:275–282.
- [50] Kristofikova Z, Klaschka J, Tejkalova H. Effect of aging on lipid peroxide levels induced by L-glutamic acid and estimated by means of a thiobarbituric acid test in rat brain tissue. *Exp Gerontol* 1995;30:645–657.
- [51] Padurariu M, Ciobica A, Hritcu L, Stoica B, Bild W, Stefanescu C. Changes of some oxidative stress markers in the serum of patients with mild cognitive impairment and Alzheimer's disease. *Neurosci Lett* 2010;469:6–10.
- [52] Reed TT, Pierce WM, Markesbery WR, Butterfield DA. Proteomic identification of HNE-bound proteins in early Alzheimer disease: insights into the role of lipid peroxidation in the progression of AD. *Brain Res* 2009;1274:66–76.
- [53] Mattson MP. Roles of the lipid peroxidation product 4-hydroxynonenal in obesity, the metabolic syndrome, and associated vascular and neurodegenerative disorders. *Exp Gerontol* 2009;44:625–633.
- [54] Gil L, Siems W, Mazurek B, Gross J, Schroeder P, Voss P, Grune T. Age-associated analysis of oxidative stress parameters in human plasma and erythrocytes. *Free Radic Res* 2006;40:495–505.
- [55] Ward WF, Qi W, Van Remmen H, Zackert WE, Roberts LJ, 2nd, Richardson A. Effects of age and caloric restriction on lipid peroxidation: measurement of oxidative stress by F2-isoprostane levels. *J Gerontol A Biol Sci Med Sci* 2005;60:847–851.
- [56] Mendoza-Nunez VM, Ruiz-Ramos M, Sanchez-Rodriguez MA, Retana-Ugalde R, Munoz-Sanchez JL. Aging-related oxidative stress in healthy humans. *Tohoku J Exp Med* 2007;213:261–268.
- [57] Asselin C, Bouchard B, Tardif JC, Des Rosiers C. Circulating 4-hydroxynonenal-protein thioether adducts assessed by gas chromatography-mass spectrometry are increased with disease progression and aging in spontaneously hypertensive rats. *Free Radic Biol Med* 2006;41:97–105.

- [58] Depboylu B, Dogru-Abbasoglu S, Aykac-Toker G, Uysal M. Increased susceptibility of serum and apo-B-containing lipoproteins to peroxidation in aged rats. *Clin Exp Med* 2007;7:158–163.
- [59] Andriollo-Sanchez M, Hininger-Favier I, Meunier N, Venneria E, O'Connor JM, Maiani G, Coudray C, Roussel AM. Age-related oxidative stress and antioxidant parameters in middle-aged and older European subjects: the ZENITH study. *Eur J Clin Nutr* 2005;59(Suppl 2):58–62.
- [60] Mehmetcik G, Ozdemirler G, Kanbagli O, Toker G, Uysal M. Age-related changes in plasma lipid peroxidation and antioxidant system in humans and rats. *Arch Gerontol Geriatr* 1997;25:305–310.
- [61] Zarkovic K, Zarkovic N, Schlag G, Redl H, Waeg G. Histological aspect of sepsis-induced brain changes in baboon model. Berlin: Springer; 1996.
- [62] Zarkovic K. 4-hydroxynonenal and neurodegenerative diseases. *Mol Aspects Med* 2003;24:293–303.
- [63] Urabe T, Hattori N, Yoshikawa M, Yoshino H, Uchida K, Mizuno Y. Colocalization of Bcl-2 and 4-hydroxynonenal modified proteins in microglial cells and neurons of rat brain following transient focal ischemia. *Neurosci Lett* 1998;247:159–162.
- [64] Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 1993;262:689–695.
- [65] Sayre LM, Smith MA, Perry G. Chemistry and biochemistry of oxidative stress in neurodegenerative disease. *Curr Med Chem* 2001;8:721–738.
- [66] Fridovich I. Superoxide dismutases. *Adv Enzymol Relat Areas Mol Biol* 1986;58:61–97.
- [67] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* 1991;11:81–128.
- [68] Uchida K, Stadtman ER. Modification of histidine residues in proteins by reaction with 4-hydroxynonenal. *Proc Natl Acad Sci USA* 1992;89:4544–4548.
- [69] Wataya T, Nunomura A, Smith MA, Siedlak SL, Harris PL, Shimohama S, Szewda LJ, Kaminski MA, Avila J, Price DL, Cleveland DW, Sayre LM, Perry G. High molecular weight neurofilament proteins are physiological substrates of adduction by the lipid peroxidation product hydroxynonenal. *J Biol Chem* 2002;277:4644–4648.
- [70] Zarkovic N. 4-hydroxynonenal as a bioactive marker of pathophysiological processes. *Mol Aspects Med* 2003;24:281–291.
- [71] 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.
- [72] Price DL, Sisodia SS, Gandy SE. Amyloid beta amyloidosis in Alzheimer's disease. *Curr Opin Neurol* 1995;8:268–274.
- [73] Borghi R, Patriarca S, Traverso N, Piccini A, Storace D, Garuti A, Gabriella C, Patrizio O, Massimo T. The increased activity of BACE1 correlates with oxidative stress in Alzheimer's disease. *Neurobiol Aging* 2007;28:1009–1014.
- [74] Cooper PN, Jackson M, Lennox G, Lowe J, Mann DM. Tau, ubiquitin, and alpha B-crystallin immunohistochemistry define the principal causes of degenerative frontotemporal dementia. *Arch Neurol* 1995;52:1011–1015.
- [75] Rauk A. Why is the amyloid beta peptide of Alzheimer's disease neurotoxic? *Dalton Trans* 2008;57:1273–1282.
- [76] Shringarpure R, Grune T, Sitte N, Davies KJ. 4-Hydroxynonenal-modified amyloid-beta peptide inhibits the proteasome: possible importance in Alzheimer's disease. *Cell Mol Life Sci* 2000;57:1802–1809.
- [77] Pettigrew JW, Moosy J, Withers G, McKeag D, Panchalingam K. 31P nuclear magnetic resonance study of the brain in Alzheimer's disease. *J Neuropathol Exp Neurol* 1988;47:235–248.
- [78] Morrow JD, Roberts LJ. The isoprostanes: unique bioactive products of lipid peroxidation. *Prog Lipid Res* 1997;36:1–21.
- [79] Roberts LJ, 2nd, Montine TJ, Markesbery WR, Tapper AR, Hardy P, Chemtob S, Dettbarn WD, Morrow JD. Formation of isoprostane-like compounds (neuroprostanes) *in vivo* from docosahexaenoic acid. *J Biol Chem* 1998;273:13605–13612.
- [80] Keller JN, Pang Z, Geddes JW, Begley JG, Germeyer A, Waeg G, Mattson MP. Impairment of glucose and glutamate transport and induction of mitochondrial oxidative stress and dysfunction in synaptosomes by amyloid beta-peptide: role of the lipid peroxidation product 4-hydroxynonenal. *J Neurochem* 1997;69:273–284.
- [81] Bruce-Keller AJ, Li YJ, Lovell MA, Kraemer PJ, Gary DS, Brown RR, Markesbery WR, Mattson MP. 4-Hydroxynonenal, a product of lipid peroxidation, damages cholinergic neurons and impairs visuospatial memory in rats. *J Neuropathol Exp Neurol* 1998;57:257–267.
- [82] Hwang JJ, Lee SJ, Kim TY, Cho JH, Koh JY. Zinc and 4-hydroxy-2-nonenal mediate lysosomal membrane permeabilization induced by H₂O₂ in cultured hippocampal neurons. *J Neurosci* 2008;28:3114–3122.
- [83] Kruman I, Bruce-Keller AJ, Bredesen D, Waeg G, Mattson MP. Evidence that 4-hydroxynonenal mediates oxidative stress-induced neuronal apoptosis. *J Neurosci* 1997;17:5089–5100.
- [84] Lovell MA, Ehmman WD, Mattson MP, Markesbery WR. Elevated 4-hydroxynonenal in ventricular fluid in Alzheimer's disease. *Neurobiol Aging* 1997;18:457–461.
- [85] Markesbery WR, Lovell MA. Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. *Neurobiol Aging* 1998;19:33–36.
- [86] Volkel W, Sicilia T, Pahler A, Gsell W, Tatschner T, Jellinger K, Leblhuber F, Riederer P, Lutz WK, Gotz ME. Increased brain levels of 4-hydroxy-2-nonenal glutathione conjugates in severe Alzheimer's disease. *Neurochem Int* 2006;48:679–686.
- [87] Montine TJ, Amarnath V, Martin ME, Strittmatter WJ, Graham DG. E-4-hydroxy-2-nonenal is cytotoxic and cross-links cytoskeletal proteins in P19 neuroglial cultures. *Am J Pathol* 1996;148:89–93.
- [88] Roses AD. Apolipoprotein E alleles as risk factors in Alzheimer's disease. *Annu Rev Med* 1996;47:387–400.
- [89] Montine TJ, Huang DY, Valentine WM, Amarnath V, Saunders A, Weisgraber KH, Graham DG, Strittmatter WJ. Crosslinking of apolipoprotein E by products of lipid peroxidation. *J Neuropathol Exp Neurol* 1996;55:202–210.
- [90] Lovell MA, Xie C, Markesbery WR. Acrolein is increased in Alzheimer's disease brain and is toxic to primary hippocampal cultures. *Neurobiol Aging* 2001;22:187–194.
- [91] Marnett LJ. DNA adducts of alpha,beta-unsaturated aldehydes and dicarbonyl compounds. *IARC Sci Publ* 1994;125:151–163.
- [92] 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.
- [93] 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.
- [94] Stadtman ER. Protein oxidation and aging. *Science* 1992;257:1220–1224.
- [95] 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.

- [96] Smith MA, Perry G, Richey PL, Sayre LM, Anderson VE, Beal MF, Kowall N. Oxidative damage in Alzheimer's. *Nature* 1996;382:120–121.
- [97] Duvoisin RC. The genetics of Parkinson's disease. A review. *Adv Neurol* 1993;60:306–315.
- [98] Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI, Ischiropoulos H, Trojanowski JQ, Lee VM. Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. *Science* 2000; 290:985–989.
- [99] Sofic E, Paulus W, Jellinger K, Riederer P, Youdim MB. Selective increase of iron in substantia nigra zona compacta of parkinsonian brains. *J Neurochem* 1991;56:978–982.
- [100] Khan FH, Saha M, Chakrabarti S. Dopamine induced protein damage in mitochondrial-synaptosomal fraction of rat brain. *Brain Res* 2001;895:245–249.
- [101] Agil A, Duran R, Barrero F, Morales B, Arauzo M, Alba F, Miranda MT, Prieto I, Ramirez M, Vives F. Plasma lipid peroxidation in sporadic Parkinson's disease. Role of the L-dopa. *J Neurol Sci* 2006;240:31–36.
- [102] Alam ZI, Jenner A, Daniel SE, Lees AJ, Cairns N, Marsden CD, Jenner P, Halliwell B. Oxidative DNA damage in the parkinsonian brain: an apparent selective increase in 8-hydroxyguanine levels in substantia nigra. *J Neurochem* 1997; 69:1196–1203.
- [103] Jenner P. Oxidative stress in Parkinson's disease. *Ann Neurol* 2003;53(Suppl 3):S26–36;discussion S36–28.
- [104] Leiphon LJ, Picklo MJ, Sr. Inhibition of aldehyde detoxification in CNS mitochondria by fungicides. *Neurotoxicology* 2007;28:143–149.
- [105] Aoyama K, Matsubara K, Kobayashi S. Aging and oxidative stress in progressive supranuclear palsy. *Eur J Neurol* 2006;13:89–92.
- [106] Fray AE, Ince PG, Banner SJ, Milton ID, Usher PA, Cookson MR, Shaw PJ. The expression of the glial glutamate transporter protein EAAT2 in motor neuron disease: an immunohistochemical study. *Eur J Neurosci* 1998;10:2481–2489.
- [107] Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX, Rahmani Z, Krizus A, McKenna-Yasek D, Cayabyab A, Gaston SM, Berger R, Tanzi RE, Halperin JJ, Herzfeldt B, Van den Bergh R, Hung WY, Bird T, Deng G, Mulder DW, Smyth C, Laing NG, Soriano E, Pericak-Vance MA, Haines J, Rouleau GA, Gusella JS, Horvitz HR, Brown RH. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 1993;362:59–62.
- [108] Borchelt DR, Lee MK, Slunt HS, Guarnieri M, Xu ZS, Wong PC, Brown RH, Price DL, Sisodia SS, Cleveland DW. Superoxide-dismutase-1 with mutations linked to familial amyotrophic-lateral-sclerosis possesses significant activity. *Proc Natl Acad Sci USA* 1994;91:8292–8296.
- [109] Wiedau-Pazos M, Goto JJ, Rabizadeh S, Gralla EB, Roe JA, Lee MK, Valentine JS, Bredesen DE. Altered reactivity of superoxide dismutase in familial amyotrophic lateral sclerosis. *Science* 1996;271:515–518.
- [110] Beckman JS, Carson M, Smith CD, Koppenol WH. Als, sod and peroxynitrite. *Nature* 1993;364:584–584.
- [111] Shibata N, Hirano A, Kobayashi M, Siddique T, Deng HX, Hung WY, Kato T, Asayama K. Intense superoxide dismutase-1 immunoreactivity in intracytoplasmic hyaline inclusions of familial amyotrophic lateral sclerosis with posterior column involvement. *J Neuropath Exp Neur* 1996;55: 481–490.
- [112] Pedersen WA, Fu WM, Keller JN, Markesbery WR, Appel S, Smith RG, Kasarskis E, Mattson MP. Protein modification by the lipid peroxidation product 4-hydroxynonenal in the spinal cords of amyotrophic lateral sclerosis patients. *Ann Neurol* 1998;44:819–824.
- [113] Shibata N, Nagai R, Miyata S, Jono T, Horiuchi S, Hirano A, Kato S, Sasaki S, Asayama K, Kobayashi M. Nonoxidative protein glycation is implicated in familial amyotrophic lateral sclerosis with superoxide dismutase-1 mutation. *Acta Neuropathol* 2000;100:275–284.
- [114] Shibata N, Yamada S, Uchida K, Hirano A, Sakoda S, Fujimura H, Sasaki S, Iwata M, Toi S, Kawaguchi M, Yamamoto T, Kobayashi M. Accumulation of protein-bound 4-hydroxy-2-hexenal in spinal cords from patients with sporadic amyotrophic lateral sclerosis. *Brain Research* 2004; 1019:170–177.
- [115] Stocker R, Keaney JF, Jr. New insights on oxidative stress in the artery wall. *J Thromb Haemost* 2005;3:1825–1834.
- [116] Scott J. Pathophysiology and biochemistry of cardiovascular disease. *Curr Opin Genet Dev* 2004;14:271–279.
- [117] Hanna IR, Taniyama Y, Szocs K, Rocic P, Griendling KK. NAD(P)H oxidase-derived reactive oxygen species as mediators of angiotensin II signaling. *Antioxid Redox Signal* 2002;4:899–914.
- [118] Irani, K. Oxidant signaling in vascular cell growth, death, and survival : a review of the roles of reactive oxygen species in smooth muscle and endothelial cell mitogenic and apoptotic signaling. *Circ Res* 2000;87:179–183.
- [119] Clempus RE, Griendling KK. Reactive oxygen species signaling in vascular smooth muscle cells. *Cardiovasc Res* 2006;71:216–225.
- [120] Yung LM, Leung FP, Yao X, Chen ZY, Huang Y. Reactive oxygen species in vascular wall. *Cardiovasc Hematol Disord Drug Targets* 2006;6:1–19.
- [121] Leitinger N. Oxidized phospholipids as modulators of inflammation in atherosclerosis. *Curr Opin Lipidol* 2003; 14:421–430.
- [122] McEwen JE, Zimniak P, Mehta JL, Shmookler Reis RJ. Molecular pathology of aging and its implications for senescent coronary atherosclerosis. *Curr Opin Cardiol* 2005;20: 399–406.
- [123] Berliner JA, Gharavi NM. Endothelial cell regulation by phospholipid oxidation products. *Free Radic Biol Med* 2008;45:119–123.
- [124] Berliner JA, Leitinger N, Tsimikas S. The role of oxidized phospholipids in atherosclerosis. *J Lipid Res* 2009;50(Suppl): 207–212.
- [125] Ashraf MZ, Kar NS, Podrez EA. Oxidized phospholipids: biomarker for cardiovascular diseases. *Int J Biochem Cell Biol* 2009;41:1241–1244.
- [126] Szapacs ME, Kim HY, Porter NA, Liebler DC. Identification of proteins adducted by lipid peroxidation products in plasma and modifications of apolipoprotein A1 with a novel biotinylated phospholipid probe. *J Proteome Res* 2008; 7:4237–4246.
- [127] Watson AD, Berliner JA, Hama SY, La Du BN, Faull KF, Fogelman AM, Navab M. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J Clin Invest* 1995;96:2882–2891.
- [128] Stremmler KE, Stafforini DM, Prescott SM, Zimmerman GA, McIntyre TM. An oxidized derivative of phosphatidylcholine is a substrate for the platelet-activating factor acetylhydrolase from human plasma. *J Biol Chem* 1989;264: 5331–5334.
- [129] Podrez EA, Poliakov E, Shen Z, Zhang R, Deng Y, Sun M, Finton PJ, Shan L, Gugiu B, Fox PL, Hoff HF, Salomon RG, Hazen SL. Identification of a novel family of oxidized phospholipids that serve as ligands for the macrophage scavenger receptor CD36. *J Biol Chem* 2002;277:38503–38516.
- [130] Boullier A, Gillotte KL, Horkko S, Green SR, Friedman P, Dennis EA, Witztum JL, Steinberg D, Quehenberger O. The binding of oxidized low density lipoprotein to mouse CD36 is mediated in part by oxidized phospholipids that are associated with both the lipid and protein moieties of the lipoprotein. *J Biol Chem* 2000;275:9163–9169.

- [131] Podrez EA, Byzova TV, Febbraio M, Salomon RG, Ma Y, Valiyaveetil M, Poliakov E, Sun M, Finton PJ, Curtis BR, Chen J, Zhang R, Silverstein RL, Hazen SL. Platelet CD36 links hyperlipidemia, oxidant stress and a prothrombotic phenotype. *Nat Med* 2007;13:1086–1095.
- [132] Ashraf MZ, Kar NS, Chen X, Choi J, Salomon RG, Febbraio M, Podrez EA. Specific oxidized phospholipids inhibit scavenger receptor bi-mediated selective uptake of cholesteryl esters. *J Biol Chem* 2008;283:10408–10414.
- [133] Prescott SM, Zimmerman GA, Stafforini DM, McIntyre TM. Platelet-activating factor and related lipid mediators. *Annu Rev Biochem* 2000;69:419–445.
- [134] Walton KA, Hsieh X, Gharavi N, Wang S, Wang G, Yeh M, Cole AL, Berliner JA. Receptors involved in the oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine-mediated synthesis of interleukin-8. A role for Toll-like receptor 4 and a glycosylphosphatidylinositol-anchored protein. *J Biol Chem* 2003;278:29661–29666.
- [135] Furnkranz A, Schober A, Bochkov VN, Bashtrykov P, Kronke G, Kadl A, Binder BR, Weber C, Leitinger N. Oxidized phospholipids trigger atherogenic inflammation in murine arteries. *Arterioscler Thromb Vasc Biol* 2005;25:633–638.
- [136] Kadl A, Galkina E, Leitinger N. Induction of CCR2-dependent macrophage accumulation by oxidized phospholipids in the air-pouch model of inflammation. *Arthritis Rheum* 2009;60:1362–1371.
- [137] Bochkov VN, Mechtcheriakova D, Lucerna M, Huber J, Malli R, Graier WF, Hofer E, Binder BR, Leitinger N. Oxidized phospholipids stimulate tissue factor expression in human endothelial cells via activation of ERK/EGR-1 and Ca(++)/NFAT. *Blood* 2002;99:199–206.
- [138] Gargalovic PS, Imura M, Zhang B, Gharavi NM, Clark MJ, Pagnon J, Yang WP, He A, Truong A, Patel S, Nelson SF, Horvath S, Berliner JA, Kirchgessner TG, Lusis AJ. Identification of inflammatory gene modules based on variations of human endothelial cell responses to oxidized lipids. *Proc Natl Acad Sci USA* 2006;103:12741–12746.
- [139] de Assis EF, Silva AR, Caiado LF, Marathe GK, Zimmerman GA, Prescott SM, McIntyre TM, Bozza PT, de Castro-Faria-Neto HC. Synergism between platelet-activating factor-like phospholipids and peroxisome proliferator-activated receptor gamma agonists generated during low density lipoprotein oxidation that induces lipid body formation in leukocytes. *J Immunol* 2003;171:2090–2098.
- [140] Cherepanova OA, Pidkova NA, Sarmiento OF, Yoshida T, Gan Q, Adiguzel E, Bendeck MP, Berliner J, Leitinger N, Owens GK. Oxidized phospholipids induce type VIII collagen expression and vascular smooth muscle cell migration. *Circ Res* 2009;104:609–618.
- [141] Rouhanizadeh M, Hwang J, Clempus RE, Marcu L, Lassegue B, Sevanian A, Hsiai TK. Oxidized-1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine induces vascular endothelial superoxide production: implication of NADPH oxidase. *Free Radic Biol Med* 2005;39:1512–1522.
- [142] Lee S, Li R, Kim B, Palvolgyi R, Ho T, Yang QZ, Xu J, Szeto WL, Honda H, Berliner JA. Ox-PAPC activation of PMET system increases expression of heme oxygenase-1 in human aortic endothelial cell. *J Lipid Res* 2009;50:265–274.
- [143] Oskolkova OV, Afonyushkin T, Leitner A, von Schlieffen E, Gargalovic PS, Lusis AJ, Binder BR, Bochkov VN. ATF4-dependent transcription is a key mechanism in VEGF up-regulation by oxidized phospholipids: critical role of oxidized sn-2 residues in activation of unfolded protein response. *Blood* 2008;112:330–339.
- [144] Chen R, Yang L, McIntyre TM. Cytotoxic phospholipid oxidation products. Cell death from mitochondrial damage and the intrinsic caspase cascade. *J Biol Chem* 2007;282:24842–24850.
- [145] Fruhwirth GO, Hermetter A. Mediation of apoptosis by oxidized phospholipids. *Subcell Biochem* 2008;49:351–367.
- [146] Greenberg ME, Sun M, Zhang R, Febbraio M, Silverstein R, Hazen SL. Oxidized phosphatidylserine-CD36 interactions play an essential role in macrophage-dependent phagocytosis of apoptotic cells. *J Exp Med* 2006;203:2613–2625.
- [147] Tsimikas S, Tsironis LD, Tselepis AD. New insights into the role of lipoprotein(a)-associated lipoprotein-associated phospholipase A2 in atherosclerosis and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2007;27:2094–2099.
- [148] Tsimikas S, Witztum JL. The role of oxidized phospholipids in mediating lipoprotein(a) atherogenicity. *Curr Opin Lipidol* 2008;19:369–377.
- [149] Frostegard J. Low level natural antibodies against phosphocholine: a novel risk marker and potential mechanism in atherosclerosis and cardiovascular disease. *Clin Immunol* 2010;134:47–54.
- [150] Esterbauer H. Cytotoxicity and genotoxicity of lipid-oxidation products. *Am J Clin Nutr* 1993;57:779S–785S; discussion 785S–786S.
- [151] Van Kuijk FJ, Holte LL, Dratz EA. 4-Hydroxyhexenal: a lipid peroxidation product derived from oxidized docosahexaenoic acid. *Biochim Biophys Acta* 1990;1043:116–118.
- [152] Thornalley PJ. Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts. *Arch Biochem Biophys* 2003;419:31–40.
- [153] Kaunitz H, Johnson RE, Pegus L. A long term nutritional study with fresh and mildly oxidized vegetables and animal fats. *J Am Oil Chem Soc* 1965;42:770–774.
- [154] Yoshioka M, Kaneda T. Study on the toxicity of the autoxidized oil. III. The toxicity of hydroxyperoxyalkenals. *Yukagaku* 1974;23:321–326.
- [155] Siu GM, Draper HH, Valli VE. Oral toxicity of malonaldehyde: a 90-day study on mice. *J Toxicol Environ Health* 1983;11:105–119.
- [156] Xanthis A, Hatzitolios A, Koliakos G, Tatola V. Advanced glycosylation end products and nutrition—a possible relation with diabetic atherosclerosis and how to prevent it. *J Food Sci* 2007;72:125–129.
- [157] Cai W, He JC, Zhu L, Chen X, Wallenstein S, Striker GE, Vlassara H. Reduced oxidant stress and extended lifespan in mice exposed to a low glycotoxin diet: association with increased AGER1 expression. *Am J Pathol* 2007;170:1893–1902.
- [158] Chisolm GM, Steinberg D. The oxidative modification hypothesis of atherogenesis: an overview. *Free Radic Biol Med* 2000;28:1815–1826.
- [159] Steinbrecher UP. Receptors for oxidized low density lipoprotein. *Biochim Biophys Acta* 1999;1436:279–298.
- [160] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;362:801–809.
- [161] Brown BE, Dean RT, Davies MJ. Glycation of low-density lipoproteins by methylglyoxal and glycolaldehyde gives rise to the *in vitro* formation of lipid-laden cells. *Diabetologia* 2005;48:361–369.
- [162] Petersen DR, Doorn JA. Reactions of 4-hydroxynonenal with proteins and cellular targets. *Free Radic Biol Med* 2004;37:937–945.
- [163] Vindis C, Escargueil-Blanc I, Elbaz M, Marcheix B, Grazide MH, Uchida K, Salvayre R, Negre-Salvayre A. Desensitization of platelet-derived growth factor receptor-beta by oxidized lipids in vascular cells and atherosclerotic lesions: prevention by aldehyde scavengers. *Circ Res* 2006;98:785–792.
- [164] Suc I, Meilhac O, Lajoie-Mazenc I, Vandaele J, Jurgens G, Salvayre R, Negre-Salvayre A. Activation of EGF receptor by oxidized LDL. *FASEB J* 1998;12:665–671.
- [165] Escargueil-Blanc I, Salvayre R, Vacaresse N, Jurgens G, Darblade B, Arnal JF, Parthasarathy S, Negre-Salvayre A. Mildly oxidized

- LDL induces activation of platelet-derived growth factor beta-receptor pathway. *Circulation* 2001;104:1814–1821.
- [166] Liu W, Akhand AA, Kato M, Yokoyama I, Miyata T, Kurokawa K, Uchida K, Nakashima I. 4-hydroxynonenal triggers an epidermal growth factor receptor-linked signal pathway for growth inhibition. *J Cell Sci* 1999;112:2409–2417.
- [167] Portero-Otin M, Pamplona R, Bellmunt MJ, Ruiz MC, Prat, Salvayre R, Negre-Salvayre A. Advanced glycation end product precursors impair epidermal growth factor receptor signaling. *Diabetes* 2002;51:1535–1542.
- [168] Cantero AV, Portero-Otin M, Ayala V, Auge N, Sanson M, Elbaz M, Thiers JC, Pamplona R, Salvayre R, Negre-Salvayre A. Methylglyoxal induces advanced glycation end product (AGEs) formation and dysfunction of PDGF receptor-beta: implications for diabetic atherosclerosis. *FASEB J* 2007; 21:3096–3106.
- [169] Je JH, Lee JY, Jung KJ, Sung B, Go EK, Yu BP, Chung HY. NF-kappaB activation mechanism of 4-hydroxyhexenal via NIK/IKK and p38 MAPK pathway. *FEBS Lett* 2004;566: 183–189.
- [170] Nitti M, Domenicotti C, d'Abramo C, Assereto S, Cottalasso D, Melloni E, Poli G, Biasi F, Marinari UM, Pronzato MA. Activation of PKC-beta isoforms mediates HNE-induced MCP-1 release by macrophages. *Biochem Biophys Res Commun* 2002;294:547–552.
- [171] Page S, Fischer C, Baumgartner B, Haas M, Kreusel U, Loidl G, Hayn M, Ziegler-Heitbrock HW, Neumeier D, Brand K. 4-Hydroxynonenal prevents NF-kappaB activation and tumor necrosis factor expression by inhibiting I-kappaB phosphorylation and subsequent proteolysis. *J Biol Chem* 1999;274:11611–11618.
- [172] Donath B, Fischer C, Page S, Prebeck S, Jilg N, Weber M, da Costa C, Neumeier D, Miethke T, Brand K. Chlamydia pneumoniae activates IKK/I kappa B-mediated signaling, which is inhibited by 4-HNE and following primary exposure. *Atherosclerosis* 2002;165:79–88.
- [173] Valacchi G, Pagnin E, Phung A, Nardini M, Schock BC, Cross CE, van der Vliet A. Inhibition of NFkappaB activation and IL-8 expression in human bronchial epithelial cells by acrolein. *Antioxid Redox Signal* 2005;7:25–31.
- [174] Chen ZH, Saito Y, Yoshida Y, Sekine A, Noguchi N, Niki E. 4-Hydroxynonenal induces adaptive response and enhances PC12 cell tolerance primarily through induction of thioredoxin reductase 1 via activation of Nrf2. *J Biol Chem* 2005;280:41921–41927.
- [175] Sanson M, Auge N, Vindis C, Muller C, Bando Y, Thiers JC, Marachet MA, Zarkovic K, Sawa Y, Salvayre R, Negre-Salvayre A. Oxidized low-density lipoproteins trigger endoplasmic reticulum stress in vascular cells: prevention by oxygen-regulated protein 150 expression. *Circ Res* 2009;104: 328–336.
- [176] Leonarduzzi G, Gamba P, Sottero B, Kadl A, Robbesyn F, Calogero RA, Biasi F, Chiarpotto E, Leitinger N, Sevanian A, Poli G. Oxysterol-induced up-regulation of MCP-1 expression and synthesis in macrophage cells. *Free Radic Biol Med* 2005;39:1152–1161.
- [177] Yang Y, Sharma R, Sharma A, Awasthi S, Awasthi YC. Lipid peroxidation and cell cycle signaling: 4-hydroxynonenal, a key molecule in stress mediated signaling. *Acta Biochim Pol* 2003;50:319–336.
- [178] Fukunaga M, Miyata S, Higo S, Hamada Y, Ueyama S, Kasuga M. Methylglyoxal induces apoptosis through oxidative stress-mediated activation of p38 mitogen-activated protein kinase in rat Schwann cells. *Ann NY Acad Sci* 2005;1043:151–157.
- [179] Chan WH, Wu HJ, Shiao NH. Apoptotic signaling in methylglyoxal-treated human osteoblasts involves oxidative stress, c-Jun N-terminal kinase, caspase-3, and p21-activated kinase 2. *J Cell Biochem* 2007;100:1056–1069.
- [180] Kristal BS, Park BK, Yu BP. 4-Hydroxyhexenal is a potent inducer of the mitochondrial permeability transition. *J Biol Chem* 1996;271:6033–6038.
- [181] Chen JJ, Bertrand H, Yu BP. Inhibition of adenine nucleotide translocator by lipid peroxidation products. *Free Radic Biol Med* 1995;19:583–590.
- [182] Palinski W, Rosenfeld ME, Yla-Herttuala S, Gurtner GC, Socher SS, Butler SW, Parthasarathy S, Carew TE, Steinberg D, Witztum JL. Low density lipoprotein undergoes oxidative modification in vivo. *Proc Natl Acad Sci USA* 1989;86:1372–1376.
- [183] Torzewski M, Klouche M, Hock J, Messner M, Dorweiler B, Torzewski J, Gabbert HE, Bhakdi S. Immunohistochemical demonstration of enzymatically modified human LDL and its colocalization with the terminal complement complex in the early atherosclerotic lesion. *Arterioscler Thromb Vasc Biol* 1998;18:369–378.
- [184] Fredrikson GN, Berglund G, Alm R, Nilsson JA, Shah PK, Nilsson J. Identification of autoantibodies in human plasma recognizing an apoB-100 LDL receptor binding site peptide. *J Lipid Res* 2006;47:2049–2054.
- [185] Tsimikas S. Oxidized low-density lipoprotein biomarkers in atherosclerosis. *Curr Atheroscler Rep* 2006;8:55–61.
- [186] Fraley AE, Tsimikas S. Clinical applications of circulating oxidized low-density lipoprotein biomarkers in cardiovascular disease. *Curr Opin Lipidol* 2006;17:502–509.
- [187] Rong JX, Shen L, Chang YH, Richters A, Hodis HN, Sevanian A. Cholesterol oxidation products induce vascular foam cell lesion formation in hypercholesterolemic New Zealand white rabbits. *Arterioscler Thromb Vasc Biol* 1999;19:2179–2188.
- [188] Hodis HN, Kramsch DM, Avogaro P, Bittolo-Bon G, Cazzolato G, Hwang J, Peterson H, Sevanian A. Biochemical and cytotoxic characteristics of an *in vivo* circulating oxidized low density lipoprotein (LDL-). *J Lipid Res* 1994; 35:669–677.
- [189] Brown AJ, Jessup W. Oxysterols and atherosclerosis. *Atherosclerosis* 1999;142:1–28.
- [190] Poli G, Sottero B, Gargiulo S, Leonarduzzi G. Cholesterol oxidation products in the vascular remodeling due to atherosclerosis. *Mol Aspects Med* 2009;30:180–189.
- [191] Steinberg D. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nat Med* 2002;8:1211–1217.
- [192] Lemaire S, Lizard G, Monier S, Miguet C, Gueldry S, Volot F, Gambert P, Neel D. Different patterns of IL-1beta secretion, adhesion molecule expression and apoptosis induction in human endothelial cells treated with 7alpha-, 7beta-hydroxycholesterol, or 7-ketocholesterol. *FEBS Lett* 1998; 440:434–439.
- [193] Naito Y, Shimozawa M, Manabe H, Nakabe N, Katada K, Kokura S, Yoshida N, Ichikawa H, Kon T, Yoshikawa T. Azelnidipine, a new calcium channel blocker, inhibits endothelial inflammatory response by reducing intracellular levels of reactive oxygen species. *Eur J Pharmacol* 2006; 546:11–18.
- [194] Prunet C, Montange T, Vejux A, Laubriet A, Rohmer JF, Riedinger JM, Athias A, Lemaire-Ewing S, Neel D, Petit JM, Steinmetz E, Brenot R, Gambert P, Lizard G. Multiplexed flow cytometric analyses of pro- and anti-inflammatory cytokines in the culture media of oxysterol-treated human monocyte cells and in the sera of atherosclerotic patients. *Cytometry A* 2006;69:359–373.
- [195] Leonarduzzi G, Sevanian A, Sottero B, Arkan MC, Biasi F, Chiarpotto E, Basaga H, Poli G. Up-regulation of the fibrogenic cytokine TGF-beta1 by oxysterols: a mechanistic link between cholesterol and atherosclerosis. *FASEB J* 2001;15:1619–1621.
- [196] Adachi J, Ishii K, Tomita M, Fujita T, Nurhantari Y, Nagasaki Y, Ueno Y. Consecutive administration of paraquat to rats

- induces enhanced cholesterol peroxidation and lung injury. *Arch Toxicol* 2003;77:353–357.
- [197] Ferre N, Martinez-Clemente M, Lopez-Parra M, Gonzalez-Periz A, Horrillo R, Planaguma A, Camps J, Joven J, Tres A, Guardiola F, Bataller R, Arroyo V, Claria J. Increased susceptibility to exacerbated liver injury in hypercholesterolemic ApoE-deficient mice: potential involvement of oxysterols. *Am J Physiol Gastrointest Liver Physiol* 2009;296:553–562.
- [198] Vejux A, Lizard G. Cytotoxic effects of oxysterols associated with human diseases: induction of cell death (apoptosis and/or oncosis), oxidative and inflammatory activities, and phospholipidosis. *Mol Aspects Med* 2009;30:153–170.
- [199] Berthier A, Lemaire-Ewing S, Prunet C, Monier S, Athias A, Bessede G, Pais de Barros JP, Laubriet A, Gambert P, Lizard G, Neel D. Involvement of a calcium-dependent dephosphorylation of BAD associated with the localization of Trpc-1 within lipid rafts in 7-ketocholesterol-induced THP-1 cell apoptosis. *Cell Death Differ* 2004;11:897–905.
- [200] Berthier A, Lemaire-Ewing S, Prunet C, Montange T, Vejux A, Pais de Barros JP, Monier S, Gambert P, Lizard G, Neel D. 7-Ketocholesterol-induced apoptosis. Involvement of several pro-apoptotic but also anti-apoptotic calcium-dependent transduction pathways. *FEBS J* 2005;272:3093–3104.
- [201] Leonarduzzi G, Gamba P, Gargiulo S, Sottero B, Kadl A, Biasi F, Chiarpotto E, Leitinger N, Vendemiale G, Serviddio G, Poli G. Oxidation as a crucial reaction for cholesterol to induce tissue degeneration: CD36 overexpression in human promonocytic cells treated with a biologically relevant oxysterol mixture. *Aging Cell* 2008;7:375–382.
- [202] Yun MR, Im DS, Lee SJ, Park HM, Bae SS, Lee WS, Kim CD. 4-Hydroxynonenal enhances CD36 expression on murine macrophages via p38 MAPK-mediated activation of 5-lipoxygenase. *Free Radic Biol Med* 2009;46:692–698.
- [203] Noris M, Perico N, Remuzzi G. Mechanisms of disease: preeclampsia. *Nat Clin Pract Nephrol* 2005;1:98–114; quiz 120.
- [204] Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science* 2005;308:1592–1594.
- [205] Samadi AR, Mayberry RM, Zaidi AA, Pleasant JC, McGhee N, Jr, Rice RJ. Maternal hypertension and associated pregnancy complications among African-American and other women in the United States. *Obstet Gynecol* 1996;87:557–563.
- [206] Saftlas AF, Olson DR, Franks AL, Atrash HK, Pokras R. Epidemiology of preeclampsia and eclampsia in the United States, 1979–1986. *Am J Obstet Gynecol* 1990;163:460–465.
- [207] MacKay AP, Berg CJ, Atrash HK. Pregnancy-related mortality from preeclampsia and eclampsia. *Obstet Gynecol* 2001;97:533–538.
- [208] Paruk F, Moodley J. Maternal and neonatal outcome in early- and late-onset pre-eclampsia. *Semin Neonatol* 2000;5:197–207.
- [209] Redman CW, Sargent IL. Placental stress and pre-eclampsia: a revised view. *Placenta* 2009;30(Suppl A):38–42.
- [210] Webster RP, Roberts VH, Myatt L. Protein nitration in placenta - functional significance. *Placenta* 2008;29:985–994.
- [211] Myatt L. Review: reactive oxygen and nitrogen species and functional adaptation of the placenta. *Placenta* 2010;31(Suppl):66–69.
- [212] Chekir C, Nakatsuka M, Noguchi S, Konishi H, Kamada Y, Sasaki A, Hao L, Hiramatsu Y. Accumulation of advanced glycation end products in women with preeclampsia: possible involvement of placental oxidative and nitrative stress. *Placenta* 2006;27:225–233.
- [213] Uchida K. Lipid peroxidation and redox-sensitive signaling pathways. *Curr Atheroscler Rep* 2007;9:216–221.
- [214] Leitinger N. Oxidized phospholipids as triggers of inflammation in atherosclerosis. *Mol Nutr Food Res* 2005;49:1063–1071.
- [215] Bochkov VN, Philippova M, Oskolkova O, Kadl A, Furnkranz A, Karabeg E, Afonyushkin T, Gruber F, Breuss J, Minchenko A, Mechtcheriakova D, Hohensinner P, Rychli K, Wojta J, Resink T, Erne P, Binder BR, Leitinger N. Oxidized phospholipids stimulate angiogenesis via autocrine mechanisms, implicating a novel role for lipid oxidation in the evolution of atherosclerotic lesions. *Circ Res* 2006;99:900–908.
- [216] Mahdy Z, Otun HA, Dunlop W, Gillespie JI. The responsiveness of isolated human hand vein endothelial cells in normal pregnancy and in pre-eclampsia. *J Physiol* 1998;508:609–617.
- [217] Lyall F, Greer IA. Pre-eclampsia: a multifaceted vascular disorder of pregnancy. *J Hypertens* 1994;12:1339–1345.
- [218] Poston L. Maternal vascular function in pregnancy. *J Hum Hypertens* 1996;10:391–394.
- [219] Savvidou MD, Hingorani AD, Tsikas D, Frolich JC, Vallance P, Nicolaides KH. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. *Lancet* 2003;361:1511–1517.
- [220] Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003;111:649–658.
- [221] Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, Bdelah Y, Lim KH, Yuan HT, Libermann TA, Stillman IE, Roberts D, D'Amore PA, Epstein FH, Sellke FW, Romero R, Sukhatme VP, Letarte M, Karumanchi SA. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med* 2006;12:642–649.
- [222] Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R, Karumanchi SA. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* 2006;355:992–1005.
- [223] Cudmore M, Ahmad S, Al-Ani B, Fujisawa T, Coxall H, Chudasama K, Devey LR, Wigmore SJ, Abbas A, Hewett PW, Ahmed A. Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1. *Circulation* 2007;115:1789–1797.
- [224] Young BC, Levine RJ, Karumanchi SA. Pathogenesis of preeclampsia. *Annu Rev Pathol* 2010;5:173–192.
- [225] Mills TA, Wareing M, Shennan AH, Poston L, Baker PN, Greenwood SL. Acute and chronic modulation of placental chorionic plate artery reactivity by reactive oxygen species. *Free Radic Biol Med* 2009;47:159–166.
- [226] Trudinger BJ, Giles WB, Cook CM. Flow velocity waveforms in the maternal uteroplacental and fetal umbilical placental circulations. *Am J Obstet Gynecol* 1985;152:155–163.
- [227] Rayman MP, Barlis J, Evans RW, Redman CW, King LJ. Abnormal iron parameters in the pregnancy syndrome preeclampsia. *Am J Obstet Gynecol* 2002;187:412–418.
- [228] Gupta S, Aziz N, Sekhon L, Agarwal R, Mansour G, Li J, Agarwal A. Lipid peroxidation and antioxidant status in preeclampsia: a systematic review. *Obstet Gynecol Surv* 2009;64:750–759.
- [229] Hnat MD, Meadows JW, Brockman DE, Pitzer B, Lyall F, Myatt L. Heat shock protein-70 and 4-hydroxy-2-nonenal adducts in human placental villous tissue of normotensive, preeclamptic and intrauterine growth restricted pregnancies. *Am J Obstet Gynecol* 2005;193:836–840.
- [230] Rudra CB, Qiu C, David RM, Bralley JA, Walsh SW, Williams MA. A prospective study of early-pregnancy plasma malondialdehyde concentration and risk of preeclampsia. *Clin Biochem* 2006;39:722–726.
- [231] Mistry HD, Wilson V, Ramsay MM, Symonds ME, Broughton Pipkin F. Reduced selenium concentrations and

- glutathione peroxidase activity in preeclamptic pregnancies. *Hypertension* 2008;52:881–888.
- [232] Mehendale S, Kilari A, Dangat K, Taralekar V, Mahadik S, Joshi S. Fatty acids, antioxidants, and oxidative stress in pre-eclampsia. *Int J Gynaecol Obstet* 2008;100:234–238.
- [233] Kaur G, Mishra S, Sehgal A, Prasad R. Alterations in lipid peroxidation and antioxidant status in pregnancy with preeclampsia. *Mol Cell Biochem* 2008;313:37–44.
- [234] Biri A, Bozkurt N, Gunaydin G, Korucuoglu U, Durak I, Kavutcu M. Antioxidant enzyme activities and lipid peroxidation in preeclampsia. *Int J Gynaecol Obstet* 2007;96:196–197.
- [235] Chamy VM, Lepe J, Catalan A, Retamal D, Escobar JA, Madrid EM. Oxidative stress is closely related to clinical severity of pre-eclampsia. *Biol Res* 2006;39:229–236.
- [236] Balogh LM, Roberts AG, Shireman LM, Greene RJ, Atkins WM. The stereochemical course of 4-hydroxy-2-nonenal metabolism by glutathione S-transferases. *J Biol Chem* 2008;283:16702–16710.
- [237] Awasthi YC, Yang Y, Tiwari NK, Patrick B, Sharma A, Li J, Awasthi S. Regulation of 4-hydroxynonenal-mediated signaling by glutathione S-transferases. *Free Radic Biol Med* 2004;37:607–619.
- [238] Rayman MP, Bode P, Redman CW. Low selenium status is associated with the occurrence of the pregnancy disease preeclampsia in women from the United Kingdom. *Am J Obstet Gynecol* 2003;189:1343–1349.
- [239] Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. *Free Radic Biol Med* 2007;43:4–15.
- [240] Atkinson J, Harroun T, Wassall SR, Stillwell W, Katsaras J. The location and behavior of alpha-tocopherol in membranes. *Mol Nutr Food Res* 2010;54:641–651.
- [241] Serdar Z, Gur E, Colakoethullary M, Develioethlu O, Sarandol E. Lipid and protein oxidation and antioxidant function in women with mild and severe preeclampsia. *Arch Gynecol Obstet* 2003;268:19–25.
- [242] Gratacos E, Casals E, Deulofeu R, Cararach V, Alonso PL, Fortuny A. Lipid peroxide and vitamin E patterns in pregnant women with different types of hypertension in pregnancy. *Am J Obstet Gynecol* 1998;178:1072–1076.
- [243] Llurba E, Gratacos E, Martin-Gallan P, Cabero L, Dominguez CA comprehensive study of oxidative stress and antioxidant status in preeclampsia and normal pregnancy. *Free Radic Biol Med* 2004;37:557–570.
- [244] El-Salahy EM, Ahmed MI, El-Gharieb A, Tawfik H. New scope in angiogenesis: role of vascular endothelial growth factor (VEGF), NO, lipid peroxidation, and vitamin E in the pathophysiology of pre-eclampsia among Egyptian females. *Clin Biochem* 2001;34:323–329.
- [245] Yanik FF, Amanvermez R, Yanik A, Celik C, Kokcu A. Preeclampsia associated with increased lipid peroxidation and decreased serum vitamin E levels. *Int J Gynaecol Obstet* 1999;64:27–33.
- [246] Ishihara O, Hayashi M, Osawa H, Kobayashi K, Takeda S, Vessby B, Basu S. Isoprostanes, prostaglandins and tocopherols in pre-eclampsia, normal pregnancy and non-pregnancy. *Free Radic Res* 2004;38:913–918.
- [247] Kim YH, Kim CH, Cho MK, Kim KM, Lee SY, Ahn BW, Yang SY, Kim SM, Song TB. Total peroxyl radical-trapping ability and anti-oxidant vitamins of the umbilical venous plasma and the placenta in pre-eclampsia. *J Obstet Gynaecol Res* 2006;32:32–41.
- [248] Roberts JM, Edep ME, Goldfien A, Taylor RN. Sera from preeclamptic women specifically activate human umbilical vein endothelial cells *in vitro*: morphological and biochemical evidence. *Am J Reprod Immunol* 1992;27:101–108.
- [249] Robinson NJ, Minchell LJ, Myers JE, Hubel CA, Crocker IP. A potential role for free fatty acids in the pathogenesis of preeclampsia. *J Hypertens* 2009;27:1293–1302.
- [250] Takacs P, Kauma SW, Sholley MM, Walsh SW, Dinsmoor MJ, Green K. Increased circulating lipid peroxides in severe preeclampsia activate NF-kappaB and upregulate ICAM-1 in vascular endothelial cells. *FASEB J* 2001;15:279–281.
- [251] Scalera F, Fischer T, Schlembach D, Beinder E. Serum from healthy pregnant women reduces oxidative stress in human umbilical vein endothelial cells. *Clin Sci (Lond)* 2002;103:53–57.
- [252] Herbst U, Toborek M, Kaiser S, Mattson MP, Hennig B. 4-Hydroxynonenal induces dysfunction and apoptosis of cultured endothelial cells. *J Cell Physiol* 1999;181:295–303.
- [253] Tastekin A, Ors R, Demircan B, Saricam Z, Inceg M, Akcay F. Oxidative stress in infants born to preeclamptic mothers. *Pediatr Int* 2005;47:658–662.
- [254] Braekke K, Harsem NK, Staff AC. Oxidative stress and antioxidant status in fetal circulation in preeclampsia. *Pediatr Res* 2006;60:560–564.
- [255] Howlader MZ, Parveen S, Tamanna S, Khan TA, Begum F. Oxidative stress and antioxidant status in neonates born to pre-eclamptic mother. *J Trop Pediatr* 2009;55:363–367.
- [256] Gitto E, Pellegrino S, Gitto P, Barberi I, Reiter RJ. Oxidative stress of the newborn in the pre- and postnatal period and the clinical utility of melatonin. *J Pineal Res* 2009;46:128–139.
- [257] Steinert JR, Wyatt AW, Jacob R, Mann GE. Redox modulation of Ca²⁺ signaling in human endothelial and smooth muscle cells in pre-eclampsia. *Antioxid Redox Signal* 2009;11:1149–1163.
- [258] Romanowicz L, Bankowski E. Lipid compounds of the umbilical cord artery and their alterations in preeclampsia. *Atherosclerosis* 2009;204:44–51.
- [259] Barker DJ. Fetal origins of coronary heart disease. *BMJ* 1995;311:171–174.
- [260] Barker DJ. Fetal programming of coronary heart disease. *Trends Endocrinol Metab* 2002;13:364–368.
- [261] Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet* 1993;341:938–941.
- [262] Palinski W, Napoli C. The fetal origins of atherosclerosis: maternal hypercholesterolemia, and cholesterol-lowering or antioxidant treatment during pregnancy influence in utero programming and postnatal susceptibility to atherogenesis. *FASEB J* 2002;16:1348–1360.
- [263] Myatt L. Placental adaptive responses and fetal programming. *J Physiol* 2006;572:25–30.
- [264] Steinert JR, Poston L, Mann GE, Jacob R. Abnormalities in intracellular Ca²⁺ regulation in fetal vascular smooth muscle in pre-eclampsia: enhanced sensitivity to arachidonic acid. *FASEB J* 2003;17:307–309.
- [265] Steinert JR, Wyatt AW, Poston L, Jacob R, Mann GE. Preeclampsia is associated with altered Ca²⁺ regulation and NO production in human fetal venous endothelial cells. *FASEB J* 2002;16:721–723.
- [266] Akar F, Ark M, Uydes BS, Soysal ME, Saracoglu F, Abacioglu N, Van de Voorde J, Kanzik I. Nitric oxide production by human umbilical vessels in severe pre-eclampsia. *J Hypertens* 1994;12:1235–1241.
- [267] Seidman DS, Laor A, Gale R, Stevenson DK, Mashiach S, Danon YL. Pre-eclampsia and offspring's blood pressure, cognitive ability and physical development at 17-years-of-age. *Br J Obstet Gynaecol* 1991;98:1009–1014.
- [268] Tenhola S, Rahiala E, Halonen P, Vanninen E, Voutilainen R. Maternal preeclampsia predicts elevated blood pressure in 12-year-old children: evaluation by ambulatory blood pressure monitoring. *Pediatr Res* 2006;59:320–324.
- [269] Tenhola S, Rahiala E, Martikainen A, Halonen P, Voutilainen R. Blood pressure, serum lipids, fasting insulin, and adrenal hormones in 12-year-old children born with maternal preeclampsia. *J Clin Endocrinol Metab* 2003;88:1217–1222.

- [270] Vatten LJ, Romundstad PR, Holmen TL, Hsieh CC, Trichopoulos D, Stuver SO. Intrauterine exposure to preeclampsia and adolescent blood pressure, body size, and age at menarche in female offspring. *Obstet Gynecol* 2003;101:529–533.
- [271] Oglænd B, Forman MR, Romundstad PR, Nilsen ST, Vatten LJ. Blood pressure in early adolescence in the offspring of preeclamptic and normotensive pregnancies. *J Hypertens* 2009;27:2051–2054.
- [272] Kajantie E, Eriksson JG, Osmond C, Thornburg K, Barker DJ. Pre-eclampsia is associated with increased risk of stroke in the adult offspring: the Helsinki birth cohort study. *Stroke* 2009;40:1176–1180.
- [273] Ferreira I, Peeters LL, Stehouwer CD. Preeclampsia and increased blood pressure in the offspring: meta-analysis and critical review of the evidence. *J Hypertens* 2009;27:1955–1959.
- [274] Bellamy L, Casas JP, Hingorani AD, Williams DJ. Preeclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ* 2007;335:974.
- [275] Afzal-Ahmed I, Mann GE, Shennan AH, Poston L, Naftalin RJ. Preeclampsia inactivates glucose-6-phosphate dehydrogenase and impairs the redox status of erythrocytes and fetal endothelial cells. *Free Radic Biol Med* 2007;42:1781–1790.
- [276] Sobrevia L, Cesare P, Yudilevich DL, Mann GE. Diabetes-induced activation of system γ^+ and nitric oxide synthase in human endothelial cells: association with membrane hyperpolarization. *J Physiol* 1995;489:183–192.
- [277] Sobrevia L, Yudilevich DL, Mann GE. Elevated D-glucose induces insulin insensitivity in human umbilical endothelial cells isolated from gestational diabetic pregnancies. *J Physiol* 1998;506:219–230.
- [278] Casanello P, Sobrevia L. Intrauterine growth retardation is associated with reduced activity and expression of the cationic amino acid transport systems γ^+ /hCAT-1 and γ^+ /hCAT-2B and lower activity of nitric oxide synthase in human umbilical vein endothelial cells. *Circ Res* 2002;91:127–134.
- [279] Esplin MS, Fausett MB, Fraser A, Kerber R, Mineau G, Carrillo J, Varner MW. Paternal and maternal components of the predisposition to preeclampsia. *N Engl J Med* 2001;344:867–872.
- [280] Lodovici M, Bigagli E, Bardini G, Rotella CM. Lipoperoxidation and antioxidant capacity in patients with poorly controlled type 2 diabetes. *Toxicol Ind Health* 2009;25:337–341.
- [281] Goodarzi MT, Varmaziar L, Navidi AA, Parivar K. Study of oxidative stress in type 2 diabetic patients and its relationship with glycated hemoglobin. *Saudi Med J* 2008;29:503–506.
- [282] Ramakrishna V, Jaikhanani R. Oxidative stress in non-insulin-dependent diabetes mellitus (NIDDM) patients. *Acta Diabetol* 2008;45:41–46.
- [283] Ahmed FN, Naqvi FN, Shafiq F. Lipid peroxidation and serum antioxidant enzymes in patients with type 2 diabetes mellitus. *Ann NY Acad Sci* 2006;1084:481–489.
- [284] Aydin A, Orhan H, Sayal A, Ozata M, Sahin G, Isimer A. Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control. *Clin Biochem* 2001;34:65–70.
- [285] Negre-Salvayre A, Salvayre R, Auge N, Pamplona R, Portero-Otin M. Hyperglycemia and glycation in diabetic complications. *Antioxid Redox Signal* 2009;11:3071–3109.
- [286] Han Y, Randell E, Vasdev S, Gill V, Gadag V, Newhook LA, Grant M, Hagerty D. Plasma methylglyoxal and glyoxal are elevated and related to early membrane alteration in young, complication-free patients with Type 1 diabetes. *Mol Cell Biochem* 2007;305:123–131.
- [287] Oranje WA, Rondas-Colbers GJ, Swennen GN, Wolffenbuttel BH. Lipid peroxidation in type 2 diabetes: relationship with macrovascular disease? *Neth J Med* 1998;53:61–68.
- [288] Selvaraj N, Bobby Z, Koner BC, Das AK. Reassessing the increased glycation of hemoglobin in nondiabetic chronic renal failure patients: a hypothesis on the role of lipid peroxides. *Clin Chim Acta* 2005;360:108–113.
- [289] Mezzetti A, Cipollone F, Cuccurullo F. Oxidative stress and cardiovascular complications in diabetes: isoprostanes as new markers on an old paradigm. *Cardiovasc Res* 2000;47:475–488.
- [290] Inouye M, Mio T, Sumino K. Glycated hemoglobin and lipid peroxidation in erythrocytes of diabetic patients. *Metabolism* 1999;48:205–209.
- [291] Inouye M, Hashimoto H, Mio T, Sumino K. Levels of lipid peroxidation product and glycated hemoglobin A1c in the erythrocytes of diabetic patients. *Clin Chim Acta* 1998;276:163–172.
- [292] Weng SW, Lin TK, Liou CW, Chen SD, Wei YH, Lee HC, Chen IY, Hsieh CJ, Wang PW. Peripheral blood mitochondrial DNA content and dysregulation of glucose metabolism. *Diabetes Res Clin Pract* 2009;83:94–99.
- [293] Stentz FB, Umpierrez GE, Cuervo R, Kitabchi AE. Proinflammatory cytokines, markers of cardiovascular risks, oxidative stress, and lipid peroxidation in patients with hyperglycemic crises. *Diabetes* 2004;53:2079–2086.
- [294] Lee DM, Hoffman WH, Carl GF, Khichi M, Cornwell PE. Lipid peroxidation and antioxidant vitamins prior to, during, and after correction of diabetic ketoacidosis. *J Diabetes Complications* 2002;16:294–300.
- [295] Peuchant E, Delmas-Beauvieux MC, Couchouron A, Dubourg L, Thomas MJ, Perromat A, Clerc M, Gin H. Short-term insulin therapy and normoglycemia. Effects on erythrocyte lipid peroxidation in NIDDM patients. *Diabetes Care* 1997;20:202–207.
- [296] Sorrentino SA, Besler C, Rohrer L, Meyer M, Heinrich K, Bahlmann FH, Mueller M, Horvath T, Doerries C, Heineemann M, Flemmer S, Markowski A, Manes C, Bahr MJ, Haller H, von Eckardstein A, Drexler H, Landmesser U. Endothelial-vasoprotective effects of high-density lipoprotein are impaired in patients with type 2 diabetes mellitus but are improved after extended-release niacin therapy. *Circulation* 2010;121:110–122.
- [297] Davison GW, Ashton T, George L, Young IS, McEneny J, Davies B, Jackson SK, Peters JR, Bailey DM. Molecular detection of exercise-induced free radicals following ascorbate prophylaxis in type 1 diabetes mellitus: a randomised controlled trial. *Diabetologia* 2008;51:2049–2059.
- [298] Anderson RA, Evans ML, Ellis GR, Graham J, Morris K, Jackson SK, Lewis MJ, Rees A, Frenneaux MP. The relationships between post-prandial lipaemia, endothelial function and oxidative stress in healthy individuals and patients with type 2 diabetes. *Atherosclerosis* 2001;154:475–483.
- [299] Jain SK. Hyperglycemia can cause membrane lipid peroxidation and osmotic fragility in human red blood cells. *J Biol Chem* 1989;264:21340–21345.
- [300] Jain SK, McVie R, Duett J, Herbst JJ. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. *Diabetes* 1989;38:1539–1543.
- [301] Gillery P, Monboisse JC, Maquart FX, Borel JP. Glycation of proteins as a source of superoxide. *Diabete Metab* 1988;14:25–30.
- [302] Hunt JV, Smith CC, Wolff SP. Autooxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. *Diabetes* 1990;39:1420–1424.
- [303] Serin O, Konukoglu D, Firtina S, Mavis O. Serum oxidized low density lipoprotein, paraoxonase 1 and lipid peroxidation levels during oral glucose tolerance test. *Horm Metab Res* 2007;39:207–211.

- [304] Manuel YKB, de Vos C, van Campenhout A, Vinckx M, Abrams P, van Campenhout C. Divergent *in vitro* and *in vivo* lipid peroxidation in the postprandial phase of patients with type I diabetes mellitus. *Eur J Clin Nutr* 2008;62:401–410.
- [305] Hu Y, Block G, Norkus EP, Morrow JD, Dietrich M, Hudes M. Relations of glycemic index and glycemic load with plasma oxidative stress markers. *Am J Clin Nutr* 2006;84:70–76; quiz 266–267.
- [306] Ohmura C, Watada H, Azuma K, Shimizu T, Kanazawa A, Ikeda F, Yoshihara T, Fujitani Y, Hirose T, Tanaka Y, Kawamori R. Aldose reductase inhibitor, epalrestat, reduces lipid hydroperoxides in type 2 diabetes. *Endocr J* 2009;56:149–156.
- [307] Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000;404:787–790.
- [308] Du X, Matsumura T, Edelstein D, Rossetti L, Zsengeller Z, Szabo C, Brownlee M. Inhibition of GAPDH activity by poly (ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest* 2003;112:1049–1057.
- [309] Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005;54:1615–1625.
- [310] Schrauwen P, Schrauwen-Hinderling V, Hoeks J, Hesselink MK. Mitochondrial dysfunction and lipotoxicity. *Biochim Biophys Acta* 2010;1801:266–271.
- [311] Schrauwen-Hinderling VB, Kooi ME, Hesselink MK, Moonen-Kornips E, Schaart G, Mustard KJ, Hardie DG, Saris WH, Nicolay K, Schrauwen P. Intramyocellular lipid content and molecular adaptations in response to a 1-week high-fat diet. *Obes Res* 2005;13:2088–2094.
- [312] Raubenheimer PJ. Impaired mitochondrial activity and insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med* 2004;350:2419–2421; author reply 2419–2421.
- [313] Short KR, Nair KS, Stump CS. Impaired mitochondrial activity and insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med* 2004;350:2419–2421; author reply 2419–2421.
- [314] Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 2003;300:1140–1142.
- [315] Richardson DK, Kashyap S, Bajaj M, Cusi K, Mandarino SJ, Finlayson J, DeFronzo RA, Jenkinson CP, Mandarino LJ. Lipid infusion decreases the expression of nuclear encoded mitochondrial genes and increases the expression of extracellular matrix genes in human skeletal muscle. *J Biol Chem* 2005;280:10290–10297.
- [316] Schrauwen P, Mensink M, Schaart G, Moonen-Kornips E, Sels JP, Blaak EE, Russell AP, Hesselink MK. Reduced skeletal muscle uncoupling protein-3 content in prediabetic subjects and type 2 diabetic patients: restoration by rosiglitazone treatment. *J Clin Endocrinol Metab* 2006;91:1520–1525.
- [317] Boveris A, Cadenas E. Mitochondrial production of hydrogen peroxide regulation by nitric oxide and the role of ubiquinone. *IUBMB Life* 2000;50:245–250.
- [318] Herrero A, Barja G. Localization of the site of oxygen radical generation inside the complex I of heart and nonsynaptic brain mammalian mitochondria. *J Bioenerg Biomembr* 2000;32:609–615.
- [319] Barja G, Herrero A. Localization at complex I and mechanism of the higher free radical production of brain nonsynaptic mitochondria in the short-lived rat than in the longevous pigeon. *J Bioenerg Biomembr* 1998;30:235–243.
- [320] Genova ML, Ventura B, Giuliano G, Bovina C, Formiggini G, Parenti Castelli G, Lenaz G. The site of production of superoxide radical in mitochondrial Complex I is not a bound ubiquinone but presumably iron-sulfur cluster N2. *FEBS Lett* 2001;505:364–368.
- [321] Ho JK, Duclos RI, Jr, Hamilton JA. Interactions of acyl carnitines with model membranes: a (13)C-NMR study. *J Lipid Res* 2002;43:1429–1439.
- [322] Brownlee M. Negative consequences of glycation. *Metabolism* 2000;49:9–13.
- [323] Nathan DM. Some answers, more controversy, from UKPDS. United Kingdom Prospective Diabetes Study. *Lancet* 1998;352:832–833.
- [324] Russell AP, Gastaldi G, Bobbioni-Harsch E, Arboit P, Gobelet C, Deriaz O, Golay A, Witztum JL, Giacobino JP. Lipid peroxidation in skeletal muscle of obese as compared to endurance-trained humans: a case of good vs. bad lipids? *FEBS Lett* 2003;551:104–106.
- [325] Hulbert AJ, Pamplona R, Buffenstein R, Buttemer WA. Life and death: metabolic rate, membrane composition, and life span of animals. *Physiol Rev* 2007;87:1175–1213.
- [326] Bonnard C, Durand A, Peyrol S, Chanseume E, Chauvin MA, Morio B, Vidal H, Rieusset J. Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. *J Clin Invest* 2008;118:789–800.
- [327] Korshunov SS, Skulachev VP, Starkov AA. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Lett* 1997;416:15–18.
- [328] Amara CE, Shankland EG, Jubrias SA, Marcinek DJ, Kushmerick MJ, Conley KE. Mild mitochondrial uncoupling impacts cellular aging in human muscles *in vivo*. *Proc Natl Acad Sci USA* 2007;104:1057–1062.
- [329] Borst P, Loos JA, Christ EJ, Slater EC. Uncoupling activity of long-chain fatty acids. *Biochim Biophys Acta* 1962;62:509–518.
- [330] Echtay KS, Esteves TC, Pakay JL, Jekabsons MB, Lambert AJ, Portero-Otin M, Pamplona R, Vidal-Puig AJ, Wang S, Roebuck SJ, Brand MD. A signalling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling. *EMBO J* 2003;22:4103–4110.
- [331] Portero-Otin M, Pamplona R, Boada J, Jove M, Gonzalo H, Buleon M, Linz W, Schafer S, Tack I, Girolami JP. Inhibition of renin angiotensin system decreases renal protein oxidative damage in diabetic rats. *Biochem Biophys Res Commun* 2008;368:528–535.
- [332] Goglia F, Skulachev VP. A function for novel uncoupling proteins: antioxidant defense of mitochondrial matrix by translocating fatty acid peroxides from the inner to the outer membrane leaflet. *FASEB J* 2003;17:1585–1591.
- [333] Brand MD, Pamplona R, Portero-Otin M, Requena JR, Roebuck SJ, Buckingham JA, Clapham JC, Cadenas S. Oxidative damage and phospholipid fatty acyl composition in skeletal muscle mitochondria from mice underexpressing or overexpressing uncoupling protein 3. *Biochem J* 2002;368:597–603.
- [334] Hoeks J, Hesselink MK, Sluiter W, Schaart G, Willems J, Morrisson A, Clapham JC, Saris WH, Schrauwen P. The effect of high-fat feeding on intramuscular lipid and lipid peroxidation levels in UCP3-ablated mice. *FEBS Lett* 2006;580:1371–1375.
- [335] Schrauwen P, Hesselink MK, Blaak EE, Borghouts LB, Schaart G, Saris WH, Keizer HA. Uncoupling protein 3 content is decreased in skeletal muscle of patients with type 2 diabetes. *Diabetes* 2001;50:2870–2873.
- [336] Bryant RW, Bailey JM. Altered lipoygenase metabolism and decreased glutathione peroxidase activity in platelets from selenium-deficient rats. *Biochem Biophys Res Commun* 1980;92:268–276.
- [337] 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.
- [338] 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.
- [339] Bacot S, Bernoud-Hubac N, Baddas N, Chantegrel B, Deshayes C, Doutheau A, Lagarde M, Guichardant M. Covalent binding of hydroxy-alkenals 4-HDDE, 4-HHE, and 4-HNE to ethanolamine phospholipid subclasses. *J Lipid Res* 2003;44:917–926.
- [340] Riahi Y, Sin-Malia Y, Cohen G, Alpert E, Gruzman A, Eckel J, Staels B, Guichardant M, Sasson S. The natural protective mechanism against hyperglycemia in vascular endothelial cells: roles of the lipid peroxidation product 4-hydroxydodecadial (4-HDDE) and PPAR δ . *Diabetes* 2010;59:808–818.
- [341] Schneider C. An update on products and mechanisms of lipid peroxidation. *Mol Nutr Food Res* 2009;53:315–321.
- [342] 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.
- [343] Tejero I, Gonzalez-Lafont A, Lluch JM, Eriksson LA. Theoretical modeling of hydroxyl-radical-induced lipid peroxidation reactions. *J Phys Chem B* 2007;111:5684–5693.
- [344] 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.
- [345] 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.
- [346] Pryor WA, Porter NA. Suggested mechanisms for the production of 4-hydroxy-2-nonenal from the autoxidation of polyunsaturated fatty acids. *Free Radic Biol Med* 1990;8: 541–543.
- [347] Guichardant M, Bacot S, Moliere P, Lagarde M. Hydroxy-alkenals from the peroxidation of n-3 and n-6 fatty acids and urinary metabolites. Prostaglandins Leukot Essent Fatty Acids 2006;75:179–182.
- [348] Coleman JD, Prabhu KS, Thompson JT, Reddy PS, Peters JM, Peterson BR, Reddy CC, Vanden Heuvel JP. The oxidative stress mediator 4-hydroxynonenal is an intracellular agonist of the nuclear receptor peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta). *Free Radic Biol Med* 2007;42:1155–1164.
- [349] Alpert E, Gruzman A, Riahi Y, Blejter R, Aharoni P, Weisinger G, Eckel J, Kaiser N, Sasson S. Delayed autoregulation of glucose transport in vascular endothelial cells. *Diabetologia* 2005;48:752–755.
- [350] Alpert E, Gruzman A, Totary H, Kaiser N, Reich R, Sasson S. A natural protective mechanism against hyperglycaemia in vascular endothelial and smooth-muscle cells: role of glucose and 12-hydroxyeicosatetraenoic acid. *Biochem J* 2002;362:413–422.
- [351] Patricia MK, Kim JA, Harper CM, Shih PT, Berliner JA, Natarajan R, Nadler JL, Hedrick CC. Lipoxygenase products increase monocyte adhesion to human aortic endothelial cells. *Arterioscler Thromb Vasc Biol* 1999;19: 2615–2622.
- [352] Bleich D, Chen S, Zipser B, Sun D, Funk CD, Nadler JL. Resistance to type 1 diabetes induction in 12-lipoxygenase knockout mice. *J Clin Invest* 1999;103:1431–1436.
- [353] Uchida K. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. *Prog Lipid Res* 2003;42:318–343.
- [354] Requena JR, Fu MX, Ahmed MU, Jenkins AJ, Lyons TJ, Thorpe SR. Lipoxidation products as biomarkers of oxidative damage to proteins during lipid peroxidation reactions. *Nephrol Dial Transplant* 1996;11(Suppl 5):48–53.
- [355] Eckl PM, Ortner A, Esterbauer H. Genotoxic properties of 4-hydroxyalkenals and analogous aldehydes. *Mutat Res* 1993;290:183–192.
- [356] Tokuyama Y, Sturis J, DePaoli AM, Takeda J, Stoffel M, Tang J, Sun X, Polonsky KS, Bell GI. Evolution of beta-cell dysfunction in the male Zucker diabetic fatty rat. *Diabetes* 1995;44:1447–1457.
- [357] Laybutt DR, Sharma A, Sgroi DC, Gaudet J, Bonner-Weir S, Weir GC. Genetic regulation of metabolic pathways in beta-cells disrupted by hyperglycemia. *J Biol Chem* 2002;277:10912–10921.
- [358] Natarajan R, Gu JL, Rossi J, Gonzales N, Lanting L, Xu L, Nadler J. Elevated glucose and angiotensin II increase 12-lipoxygenase activity and expression in porcine aortic smooth muscle cells. *Proc Natl Acad Sci USA* 1993;90:4947–4951.
- [359] Toyokuni S, Yamada S, Kashima M, Ihara Y, Yamada Y, Tanaka T, Hiai H, Seino Y, Uchida K. Serum 4-hydroxy-2-nonenal-modified albumin is elevated in patients with type 2 diabetes mellitus. *Antioxid Redox Signal* 2000;2:681–685.
- [360] Orioli M, Aldini G, Benfatto MC, Facino RM, Carini M. HNE Michael adducts to histidine and histidine-containing peptides as biomarkers of lipid-derived carbonyl stress in urines: LC-MS/MS profiling in Zucker obese rats. *Anal Chem* 2007;79:9174–9184.
- [361] Bleich D, Chen S, Gu JL, Nadler JL. The role of 12-lipoxygenase in pancreatic - cells (Review). *Int J Mol Med* 1998;1: 265–272.
- [362] Prasad KM, Thimmalapura PR, Woode EA, Nadler JL. Evidence that increased 12-lipoxygenase expression impairs pancreatic beta cell function and viability. *Biochem Biophys Res Commun* 2003;308:427–432.
- [363] Miwa I, Ichimura N, Sugiura M, Hamada Y, Taniguchi S. Inhibition of glucose-induced insulin secretion by 4-hydroxy-2-nonenal and other lipid peroxidation products. *Endocrinology* 2000;141:2767–2772.
- [364] Nunemaker CS, Chen M, Pei H, Kimble SD, Keller SR, Carter JD, Yang Z, Smith KM, Wu R, Bevard MH, Garmey JC, Nadler JL. 12-Lipoxygenase-knockout mice are resistant to inflammatory effects of obesity induced by Western diet. *Am J Physiol Endocrinol Metab* 2008;295:1065–1075.
- [365] Chen M, Yang ZD, Smith KM, Carter JD, Nadler JL. Activation of 12-lipoxygenase in proinflammatory cytokine-mediated beta cell toxicity. *Diabetologia* 2005;48:486–495.
- [366] Barrera G, Pizzimenti S, Laurora S, Briatore F, Toaldo C, Dianzani MU. 4-hydroxynonenal and cell cycle. *Biofactors* 2005;24:151–157.
- [367] McDuffie M, Maybee NA, Keller SR, Stevens BK, Garmey JC, Morris MA, Kropf E, Rival C, Ma K, Carter JD, Tersey SA, Nunemaker CS, Nadler JL. Nonobese diabetic (NOD) mice congenic for a targeted deletion of 12/15-lipoxygenase are protected from autoimmune diabetes. *Diabetes* 2008;57: 199–208.
- [368] Suarez-Pinzon WL, Strynadka K, Rabinovitch A. Destruction of rat pancreatic islet beta-cells by cytokines involves the production of cytotoxic aldehydes. *Endocrinology* 1996; 137:5290–5296.
- [369] Numazawa S, Sakaguchi H, Aoki R, Taira T, Yoshida T. Regulation of the susceptibility to oxidative stress by cysteine availability in pancreatic beta-cells. *Am J Physiol Cell Physiol* 2008;295:468–474.
- [370] Cahuana GM, Tejedo JR, Jimenez J, Ramirez R, Sobrino F, Bedoya FJ. Involvement of advanced lipoxidation end products (ALEs) and protein oxidation in the apoptotic actions of nitric oxide in insulin secreting RINm5F cells. *Biochem Pharmacol* 2003;66:1963–1971.
- [371] Srivastava S, Chandra A, Wang LF, Seifert WE, Jr, DaGue BB, Ansari NH, Srivastava SK, Bhatnagar A. Metabolism of the lipid peroxidation product, 4-hydroxy-trans-2-nonenal, in isolated perfused rat heart. *J Biol Chem* 1998;273: 10893–10900.
- [372] Murphy TC, Poppe C, Porter JE, Montine TJ, Picklo MJ, Sr. 4-Hydroxy-trans-2-nonenal is a gamma-hydroxy-

- butyrate receptor ligand in the cerebral cortex and hippocampus. *J Neurochem* 2004;89:1462–1470.
- [373] Kaiser N, Leibowitz G. Failure of beta-cell adaptation in type 2 diabetes: lessons from animal models. *Front Biosci* 2009;14:1099–1115.
- [374] Modak MA, Parab PB, Ghaskadbi SS. Pancreatic islets are very poor in rectifying oxidative DNA damage. *Pancreas* 2009;38:23–29.
- [375] Lenzen S, Drinkgern J, Tiedge M. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. *Free Radic Biol Med* 1996;20:463–466.
- [376] Wu L, Nicholson W, Knobel SM, Steffner RJ, May JM, Piston DW, Powers AC. Oxidative stress is a mediator of glucose toxicity in insulin-secreting pancreatic islet cell lines. *J Biol Chem* 2004;279:12126–12134.
- [377] Traverso N, Menini S, Odetti P, Pronzato MA, Cottalasso D, Marinari UM. Diabetes impairs the enzymatic disposal of 4-hydroxynonenal in rat liver. *Free Radic Biol Med* 2002;32:350–359.
- [378] Milicevic Z, Raz I, Beattie SD, Campaigne BN, Sarwat S, Gromniak E, Kowalska I, Galic E, Tan M, Hanefeld M. Natural history of cardiovascular disease in patients with diabetes: role of hyperglycemia. *Diabetes Care* 2008;31(Suppl 2):155–160.
- [379] Yu Y, Lyons TJ. A lethal tetrad in diabetes: hyperglycemia, dyslipidemia, oxidative stress, and endothelial dysfunction. *Am J Med Sci* 2005;330:227–232.
- [380] Yamagishi SI, Nakamura K, Matsui T, Ueda SI, Imaizumi T. Role of postprandial hyperglycaemia in cardiovascular disease in diabetes. *Int J Clin Pract* 2007;61:83–87.
- [381] Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 2000;321:405–412.
- [382] Minekura H, Kumagai T, Kawamoto Y, Nara F, Uchida K. 4-Hydroxy-2-nonenal is a powerful endogenous inhibitor of endothelial response. *Biochem Biophys Res Commun* 2001;282:557–561.
- [383] Whitsett J, Picklo MJ, Sr, Vasquez-Vivar J. 4-Hydroxy-2-nonenal increases superoxide anion radical in endothelial cells via stimulated GTP cyclohydrolase proteasomal degradation. *Arterioscler Thromb Vasc Biol* 2007;27:2340–2347.
- [384] Yun MR, Im DS, Lee SJ, Woo JW, Hong KW, Bae SS, Kim CD. 4-hydroxynonenal contributes to macrophage foam cell formation through increased expression of class A scavenger receptor at the level of translation. *Free Radic Biol Med* 2008;45:177–183.
- [385] Annangudi SP, Deng Y, Gu X, Zhang W, Crabb JW, Salomon RG. Low-density lipoprotein has an enormous capacity to bind (E)-4-hydroxynon-2-enal (HNE): detection and characterization of lysyl and histidyl adducts containing multiple molecules of HNE. *Chem Res Toxicol* 2008;21:1384–1395.
- [386] Kakishita H, Hattori Y. Vascular smooth muscle cell activation and growth by 4-hydroxynonenal. *Life Sci* 2001;69:689–697.
- [387] Watanabe T, Pakala R, Katagiri T, Benedict CR. Oxidized low-density lipoproteins potentiate the mitogenic effect of 5-hydroxytryptamine on vascular smooth muscle cells. *Jpn Heart J* 2002;43:35–42.
- [388] Ramana KV, Bhatnagar A, Srivastava S, Yadav UC, Awasthi S, Awasthi YC, Srivastava SK. Mitogenic responses of vascular smooth muscle cells to lipid peroxidation-derived aldehyde 4-hydroxy-trans-2-nonenal (HNE): role of aldose reductase-catalyzed reduction of the HNE-glutathione conjugates in regulating cell growth. *J Biol Chem* 2006;281:17652–17660.
- [389] Lee JY, Jung GY, Heo HJ, Yun MR, Park JY, Bae SS, Hong KW, Lee WS, Kim CD. 4-Hydroxynonenal induces vascular smooth muscle cell apoptosis through mitochondrial generation of reactive oxygen species. *Toxicol Lett* 2006;166:212–221.
- [390] 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.
- [391] Li WG, Stoll LL, Rice JB, Xu SP, Miller FJ, Jr, Chatterjee P, Hu L, Oberley LW, Spector AA, Weintraub NL. Activation of NAD(P)H oxidase by lipid hydroperoxides: mechanism of oxidant-mediated smooth muscle cytotoxicity. *Free Radic Biol Med* 2003;34:937–946.
- [392] Akiba S, Kumazawa S, Yamaguchi H, Hontani N, Matsumoto T, Ikeda T, Oka M, Sato T. Acceleration of matrix metalloproteinase-1 production and activation of platelet-derived growth factor receptor beta in human coronary smooth muscle cells by oxidized LDL and 4-hydroxynonenal. *Biochim Biophys Acta* 2006;1763:797–804.
- [393] Vindis C, Escargueil-Blanc I, Uchida K, Elbaz M, Salvayre R, Negre-Salvayre A. Lipid oxidation products and oxidized low-density lipoproteins impair platelet-derived growth factor receptor activity in smooth muscle cells: implication in atherosclerosis. *Redox Rep* 2007;12:96–100.
- [394] Lee SJ, Seo KW, Yun MR, Bae SS, Lee WS, Hong KW, Kim CD. 4-Hydroxynonenal enhances MMP-2 production in vascular smooth muscle cells via mitochondrial ROS-mediated activation of the Akt/NF-kappaB signaling pathways. *Free Radic Biol Med* 2008;45:1487–1492.
- [395] Seo KW, Lee SJ, Kim CE, Yun MR, Park HM, Yun JW, Bae SS, Kim CD. Participation of 5-lipoxygenase-derived LTB(4) in 4-hydroxynonenal-enhanced MMP-2 production in vascular smooth muscle cells. *Atherosclerosis* 2010;208:56–61.
- [396] Yamanouchi J, Takatori A, Itagaki S, Kawamura S, Yoshikawa Y. APA hamster model for diabetic atherosclerosis. 2. Analysis of lipids and lipoproteins. *Exp Anim* 2000;49:267–274.
- [397] Meng J, Sakata N, Takebayashi S, Asano T, Futata T, Nagai R, Ikeda K, Horiuchi S, Myint T, Taniguchi N. Glycoxidation in aortic collagen from STZ-induced diabetic rats and its relevance to vascular damage. *Atherosclerosis* 1998;136:355–365.
- [398] Polak M, Zagorski Z. Lipid peroxidation in diabetic retinopathy. *Ann Univ Mariae Curie Sklodowska Med* 2004;59:434–437.
- [399] Furfuro AL, Menini S, Patriarca S, Pesce C, Odetti P, Cottalasso D, Marinari UM, Pronzato MA, Traverso N. HNE-dependent molecular damage in diabetic nephropathy and its possible prevention by N-acetyl-cysteine and oserutin. *Biofactors* 2005;24:291–298.
- [400] Horie K, Miyata T, Maeda K, Miyata S, Sugiyama S, Sakai H, van Ypersele de Strihou C, Monnier VM, Witztum JL, Kurokawa K. Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. Implication for glycoxidative stress in the pathogenesis of diabetic nephropathy. *J Clin Invest* 1997;100:2995–3004.
- [401] Akude E, Zhrebetskaya E, Roy Chowdhury SK, Girling K, Fernyhough P. 4-Hydroxy-2-nonenal induces mitochondrial dysfunction and aberrant axonal outgrowth in adult sensory neurons that mimics features of diabetic neuropathy. *Neurotox Res* 2010;17:28–38.
- [402] Zhrebetskaya E, Akude E, Smith DR, Fernyhough P. Development of selective axonopathy in adult sensory neurons isolated from diabetic rats: role of glucose-induced oxidative stress. *Diabetes* 2009;58:1356–1364; 2009.
- [403] Beretta G, Artali R, Regazzoni L, Panigati M, Facino RM. Glycyl-histidyl-lysine (GHK) is a quencher of alpha,beta-4-hydroxy-trans-2-nonenal: a comparison with carnosine. Insights into the mechanism of reaction by electrospray ionization mass spectrometry, 1H NMR, and computational techniques. *Chem Res Toxicol* 2007;20:1309–1314.

- [404] Dwivedi S, Sharma A, Patrick B, Sharma R, Awasthi YC. Role of 4-hydroxynonenal and its metabolites in signaling. *Redox Rep* 2007;12:4–10.
- [405] Chen ZH, Niki E. 4-hydroxynonenal (4-HNE) has been widely accepted as an inducer of oxidative stress. Is this the whole truth about it or can 4-HNE also exert protective effects? *IUBMB Life* 2006;58:372–373.
- [406] Forman BM, Chen J, Evans RM. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc Natl Acad Sci USA* 1997;94:4312–4317.
- [407] 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.
- [408] Grimsrud PA, Picklo MJ, Sr, Griffin TJ, Bernlohr DA. Carbonylation of adipose proteins in obesity and insulin resistance: identification of adipocyte fatty acid-binding protein as a cellular target of 4-hydroxynonenal. *Mol Cell Proteomics* 2007;6:624–637.
- [409] Reagan LP, Magarinos AM, Yee DK, Swzeda LI, Van Bueren A, McCall AL, McEwen BS. Oxidative stress and HNE conjugation of GLUT3 are increased in the hippocampus of diabetic rats subjected to stress. *Brain Res* 2000;862:292–300.
- [410] Sasson S, Eckel J. Disparate effects of 12-lipoxygenase and 12-hydroxyeicosatetraenoic acid in vascular endothelial and smooth muscle cells and in cardiomyocytes. *Arch Physiol Biochem* 2006;112:119–129.
- [411] Totary-Jain H, Naveh-Manly T, Riahi Y, Kaiser N, Eckel J, Sasson S. Calreticulin destabilizes glucose transporter-1 mRNA in vascular endothelial and smooth muscle cells under high-glucose conditions. *Circ Res* 2005;97:1001–1008.
- [412] Araya Y, Kasuga J, Toyota K, Hirakawa Y, Oyama T, Makishima M, Morikawa K, Hashimoto Y, Miyachi H. Structure-based design and synthesis of fluorescent PPARalpha/delta co-agonist and its application as a probe for fluorescent polarization assay of PPARdelta ligands. *Chem Pharm Bull (Tokyo)* 2008;56:1357–1359.
- [413] Willson TM, Brown PJ, Sternbach DD, Henke BR. The PPARs: from orphan receptors to drug discovery. *J Med Chem* 2000;43:527–550.
- [414] Third report of the National Cholesterol Education Program (NCEP). Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation* 2002;106:3143–3421.
- [415] Himmelfarb J. Oxidative stress in hemodialysis. *Contrib Nephrol* 2008;161:132–137.
- [416] Nistala R, Whaley-Connell A, Sowers JR. Redox control of renal function and hypertension. *Antioxid Redox Signal* 2008;10:2047–2089.
- [417] Martin CJ, Goeddeke-Merickel CM. Oxidative stress in chronic kidney disease. *Nephrol Nurs J* 2005;32:683–685.
- [418] Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. *Nephrol Dial Transplant* 2003;18:1272–1280.
- [419] Zoccali C, Mallamaci F, Tripepi G. Novel cardiovascular risk factors in end-stage renal disease. *J Am Soc Nephrol* 2005;15(Suppl 1):77–80.
- [420] Huang KC, Yang CC, Lee KT, Chien CT. Reduced hemodialysis-induced oxidative stress in end-stage renal disease patients by electrolyzed reduced water. *Kidney Int* 2003;64:704–714.
- [421] Siems W, Wiswedel I, Carluccio F, Grune T, Hennig H, Hampl H. Oxidative stress in cardio-renal anemia syndrome. In: *Cardionephrology VIII European Meeting Assisi* (M. Timio, V. Wizemann, S. Venanzi, eds.), Editoriale Bios, Cosenza, 2004, pp. 77–79.
- [422] Levin A. The relationship of haemoglobin level and survival: direct or indirect effects? *Nephrol Dial Transplant* 2002;17(Suppl 5):8–13.
- [423] Kokot F, Wiecek A. Evidence that the anemia of renal failure participates in overall uremic toxicity. *Kidney Int Suppl* 1997;62:83–86.
- [424] Stenvinkel P, Barany P. Anaemia, rHuEPO resistance, and cardiovascular disease in end-stage renal failure; links to inflammation and oxidative stress. *Nephrol Dial Transplant* 2002;17(Suppl 5):32–37.
- [425] Silverberg DS, Iaina A, Wexler D, Blum M. The pathological consequences of anaemia. *Clin Lab Haematol* 2001; 23:1–6.
- [426] Carluccio F, Siems W, Stefanelli G, Sommerburg O, Grune T, Riedel E, Hampl H. Homocysteine in chronic renal failure in relation to renal anemia and to oxidative stress parameters 4-hydroxynonenal and malondialdehyde. *Clin Nephrol* 2002;58(Suppl 1):26–30.
- [427] Siems W, Quast S, Carluccio F, Wiswedel I, Hirsch D, Augustin W, Hampl H, Riehle M, Sommerburg O. Oxidative stress in chronic renal failure as a cardiovascular risk factor. *Clin Nephrol* 2002;58(Suppl 1):12–19.
- [428] Grune T, Sommerburg O, Siems WG. Oxidative stress in anemia. *Clin Nephrol* 2000;53:18–22.
- [429] Silverberg DS, Wexler D, Blum M, Tchebenier J, Sheps D, Keren G, Schwartz D, Baruch R, Yachnin T, Shaked M, Zubkov A, Steinbruch S, Iaina A. The correction of anemia in severe resistant heart failure with erythropoietin and intravenous iron prevents the progression of both the heart and the renal failure and markedly reduces hospitalization. *Clin Nephrol* 2002;58(Suppl 1):37–45.
- [430] Siems W, Quast S, Carluccio F, Wiswedel I, Hirsch D, Augustin W, Kraemer K, Hampl H, Sommerburg O. Oxidative stress in cardio renal anemia syndrome: correlations and therapeutic possibilities. *Clin Nephrol* 2003;60(Suppl 1):22–30.
- [431] Galli F, Ghibelli L, Buoncristiani U, Bordoni V, D'Intini V, Benedetti S, Canestrari F, Ronco C, Floridi A. Mononuclear leukocyte apoptosis in haemodialysis patients: the role of cell thiols and vitamin E. *Nephrol Dial Transplant* 2003;18:1592–1600.
- [432] Noordzij MJ, Lefrandt JD, Smit AJ. Advanced glycation end products in renal failure: an overview. *J Ren Care* 2008;34:207–212.
- [433] Cottone S, Lorito MC, Riccobene R, Nardi E, Mule G, Buscemi S, Geraci C, Guarneri M, Arsena R, Cerasola G. Oxidative stress, inflammation and cardiovascular disease in chronic renal failure. *J Nephrol* 2008;21:175–179.
- [434] Siems W, Carluccio F, Radenkovic S, Grune T, Hampl H. Oxidative stress in renal anemia of hemodialysis patients is mitigated by epoetin treatment. *Kidney Blood Press Res* 2005;28:295–301.
- [435] Wiswedel I, Hirsch D, Carluccio F, Hampl H, Siems W. F2-isoprostanes as biomarkers of lipid peroxidation in patients with chronic renal failure. *Biofactors* 2005;24:201–208.
- [436] Silverberg DS, Wexler D, Blum M, Iaina A. The cardio renal anemia syndrome: correcting anemia in patients with resistant congestive heart failure can improve both cardiac and renal function and reduce hospitalizations. *Clin Nephrol* 2003;60(Suppl 1):93–102.
- [437] Tepel M, van der Giet M, Statz M, Jankowski J, Zidek W. The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a randomized, controlled trial. *Circulation* 2003;107:992–995.
- [438] Beier A, Siems W, Brenke R, Grune T. [Increased formation of free radicals in chronic lymphedema]. *Z Lymphol* 1994;18:8–11.
- [439] Brenke R, Siems W. The role of free radicals in lymphedema-an overview. *Lymphology* 2004;37(suppl):157–167.

- [440] Siems W, Brenke R, Wiswedel I, Sommerburg O, Eckl PM, Bresgen N. In chronic lymphoedema increased oxysterol levels were measured. *LymphForsch* 2009;13:29–33.
- [441] Poli G, Parola M. Oxidative damage and fibrogenesis. *Free Radic Biol Med* 1997;22:287–305.
- [442] Schwartz Y, Dushkin MI, Komarova NI, Vorontsova EV, Kuznetsova IS. Cholesterol-induced stimulation of postinflammatory liver fibrosis. *Bull Exp Biol Med* 2008;145:692–695.
- [443] 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.
- [444] Nemeth E, Nagy E, Lengyel G, Feher J. The preventive role of coenzyme Q10 and other antioxidants in injuries caused by oxidative stress. *Arch Med Sci* 2007;3:1–5.
- [445] Feher J, Lengyel G. A new approach to drug therapy in non-alcoholic steatohepatitis (NASH). *J Int Med Res* 2003;31:537–551.
- [446] Feher J, Nemeth E, Lengyel G. Non-alcoholic steatohepatitis (NASH)- is it a part of the metabolic syndrome? *Arch Med Sci* 2005;1:37–47.
- [447] Wei Y, Rector RS, Thyfault JP, Ibdah JA. Nonalcoholic fatty liver disease and mitochondrial dysfunction. *World J Gastroenterol* 2008;14:193–199.
- [448] Hagymasi K, Lengyel G, Nagy E, Pallai ZS, Kocsis I, Feher J, Tulassay Z, Blazovics A. Impaired antioxidant status in non-organ specific autoantibody positive patients with non-alcoholic liver disease. *Hung Med* 2008;2:563–570.
- [449] Eiszrich A, Feher J. Fatty liver in childhood. *Hung Med* 1999;2:519–531.
- [450] Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999;116:1413–1419.
- [451] Szantho A, Grigorescu M, Dumitra I, Serban A. Steatosis in hepatitis C virusinfection. Response to anti-viral therapy. *J Gastrointestin Liver Dis* 2006;15:117–124.
- [452] Feher J, Lengyel G. Silymarin in the treatment of chronic liver diseases: past and future. *Clin Exp Med* 2009;3:403–413.
- [453] Conde de la Rosa L, Moshage H, Nieto N. [Hepatocyte oxidant stress and alcoholic liver disease]. *Rev Esp Enferm Dig* 2008;100:156–163.
- [454] Cerutti PA. Prooxidant states and tumor promotion. *Science* 1985;227:375–381.
- [455] Szent-Gyorgyi A, Isenberg I, Baird SL. On the electron donating properties of carcinogens. *Proc Natl Acad Sci USA* 1960;46:1444–1449.
- [456] Sullivan PD. Free radicals of benzo(a)pyrene and derivatives. *Environ Health Perspect* 1985;64:283–295.
- [457] Emerit I, Cerutti PA. Tumour promoter phorbol-12-myristate-13-acetate induces chromosomal damage via indirect action. *Nature* 1981;293:144–146.
- [458] Zarkovic N. Antioxidation in der prävention und chemotherapie von tumoren. *Pharmazeutische Zeitung* 2000;145:391–395.
- [459] Zarkovic J. Mechanismus der tumorentstehung. *Pharmazeutische Zeitung* 2000;145:239–245.
- [460] Singhal SS, Awasthi YC, Awasthi S. Regression of melanoma in a murine model by RLIP76 depletion. *Cancer Res* 2006;66:2354–2360.
- [461] 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.
- [462] McKusick VA, Ruddle FH. The status of the gene map of the human chromosomes. *Science* 1977;196:390–405.
- [463] Edwards RH. Inheritance of the Dubin-Johnson-Sprinz syndrome. *Gastroenterology* 1975;68:734–749.
- [464] Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *EMBO J* 1991;10:2247–2258.
- [465] Johnson D, Travis J. The oxidative inactivation of human alpha-1-proteinase inhibitor. Further evidence for methionine at the reactive center. *J Biol Chem* 1979;254:4022–4026.
- [466] Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;420:860–867.
- [467] Seril DN, Liao J, Yang GY, Yang CS. Oxidative stress and ulcerative colitis-associated carcinogenesis: studies in humans and animal models. *Carcinogenesis* 2003;24:353–362.
- [468] Konturek PC, Konturek SJ, Brzozowski T. Gastric cancer and *Helicobacter pylori* infection. *J Physiol Pharmacol* 2006;57(Suppl 3):51–65.
- [469] von Sonntag C. New aspects in the free-radical chemistry of pyrimidine nucleobases. *Free Radic Res Commun* 1987;2:217–224.
- [470] Dizdaroglu M. Oxidative damage to DNA in mammalian chromatin. *Mutat Res* 1992;275:331–342.
- [471] Demple B, Harrison L. Repair of oxidative damage to DNA: enzymology and biology. *Annu Rev Biochem* 1994;63:915–948.
- [472] Marnett LJ. Oxyradicals and DNA damage. *Carcinogenesis* 2000;21:361–370.
- [473] Cooke JP. NO and angiogenesis. *Atheroscler* 2003;4(Suppl 4):53–60.
- [474] Klaunig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol Toxicol* 2004;44:239–267.
- [475] Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 2006;160:1–40.
- [476] Miyake H, Hara I, Kamidono S, Eto H. Oxidative DNA damage in patients with prostate cancer and its response to treatment. *J Urol* 2004;171:1533–1536.
- [477] Weiss JM, Goode EL, Ladiges WC, Ulrich CM. Polymorphic variation in hOGG1 and risk of cancer: a review of the functional and epidemiologic literature. *Mol Carcinog* 2005;42:127–141.
- [478] Diakowska D, Lewandowski A, Kopec W, Diakowski W, Chrzanoska T. Oxidative DNA damage and total antioxidant status in serum of patients with esophageal squamous cell carcinoma. *Hepatogastroenterology* 2007;54:1701–1704.
- [479] Tanaka H, Fujita N, Sugimoto R, Urawa N, Horiike S, Kobayashi Y, Iwasa M, Ma N, Kawanishi S, Watanabe S, Kaito M, Takei Y. Hepatic oxidative DNA damage is associated with increased risk for hepatocellular carcinoma in chronic hepatitis C. *Br J Cancer* 2008;98:580–586.
- [480] Kawanishi S, Hiraku Y. Oxidative and nitrative DNA damage as biomarker for carcinogenesis with special reference to inflammation. *Antioxid Redox Signal* 2006;8:1047–1058.
- [481] Shen HM, Ong CN. Mutations of the p53 tumor suppressor gene and ras oncogenes in aflatoxin hepatocarcinogenesis. *Mutat Res* 1996;366:23–44.
- [482] Nishigori C, Hattori Y, Toyokuni S. Role of reactive oxygen species in skin carcinogenesis. *Antioxid Redox Signal* 2004;6:561–570.
- [483] Tanaka T, Iwasa Y, Kondo S, Hiai H, Toyokuni S. High incidence of allelic loss on chromosome 5 and inactivation of p15INK4B and p16INK4A tumor suppressor genes in oxystress-induced renal cell carcinoma of rats. *Oncogene* 1999;18:3793–3797.
- [484] West JD, Marnett LJ. Endogenous reactive intermediates as modulators of cell signaling and cell death. *Chem Res Toxicol* 2006;19:173–194.
- [485] Ohshima H. Genetic and epigenetic damage induced by reactive nitrogen species: implications in carcinogenesis. *Toxicol Lett* 2003;140–141:99–104.

- [486] Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. *Nat Rev Cancer* 2003;3:276–285.
- [487] Bartsch H, Nair J. Accumulation of lipid peroxidation-derived DNA lesions: potential lead markers for chemoprevention of inflammation-driven malignancies. *Mutat Res* 2005; 591:34–44.
- [488] Irigaray P, Newby JA, Clapp R, Hardell L, Howard V, Montagnier L, Epstein S, Belpomme D. Lifestyle-related factors and environmental agents causing cancer: an overview. *Biomed Pharmacother* 2007;61:640–658.
- [489] Nair J, Gal A, Tamir S, Tannenbaum SR, Wogan GN, Bartsch H. Etheno adducts in spleen DNA of SJL mice stimulated to overproduce nitric oxide. *Carcinogenesis* 1998;19: 2081–2084.
- [490] Zha S, Yegnasubramanian V, Nelson WG, Isaacs WB, De Marzo AM. Cyclooxygenases in cancer: progress and perspective. *Cancer Lett* 2004;215:1–20.
- [491] Schmid K, Nair J, Winde G, Velic I, Bartsch H. Increased levels of promutagenic etheno-DNA adducts in colonic polyps of FAP patients. *Int J Cancer* 2000;87:1–4.
- [492] Lock K, Pomerleau J, Causer L, Altmann DR, McKee M. The global burden of disease attributable to low consumption of fruit and vegetables: implications for the global strategy on diet. *Bull World Health Organ* 2005;83: 100–108.
- [493] Pierre F, Tache S, Gueraud F, Rerole AL, Jourdan ML, Petit C. Apc mutation induces resistance of colonic cells to lipoperoxide-triggered apoptosis induced by faecal water from haem-fed rats. *Carcinogenesis* 2007;28:321–327.
- [494] Zarkovic N, Zarkovic K, Schaur RJ, Stolic S, Schlag G, Redl H, Waeg G, Borovic S, Loncaric I, Juric G, Hlavka V. 4-Hydroxynonenal as a second messenger of free radicals and growth modifying factor. *Life Sci* 1999;65: 1901–1904.
- [495] Uchida K, Shiraiishi M, Naito Y, Torii Y, Nakamura Y, Osawa T. Activation of stress signaling pathways by the end product of lipid peroxidation. 4-hydroxy-2-nonenal is a potential inducer of intracellular peroxide production. *J Biol Chem* 1999;274:2234–2242.
- [496] Masotti L, Casali E, Galeotti T. Lipid peroxidation in tumour cells. *Free Radic Biol Med* 1988;4:377–386.
- [497] Burton GW, Ingold KU, Cheeseman KH, Slater TF. Application of deuterated alpha-tocopherols to the biokinetics and bioavailability of vitamin E. *Free Radic Res Commun* 1990;11:99–107.
- [498] Huang ZZ, Li H, Cai J, Kuhlenkamp J, Kaplowitz N, Lu SC. Changes in glutathione homeostasis during liver regeneration in the rat. *Hepatology* 1998;27:147–153.
- [499] Marquez-Quinones A, Cipak A, Zarkovic K, Fattel-Fazenda S, Villa-Trevino S, Waeg G, Zarkovic N, Gueraud F. HNE-protein adducts formation in different pre-carcinogenic stages of hepatitis in LEC rats. *Free Radic Res* 2010;44:119–127.
- [500] Zivkovic M, Zarkovic K, Ljubomir S, Waeg G, Poljak-Blazi M, Borovic S, Schaur J, Zarkovic N. A new method for detection of HNE-histidine conjugates in rat inflammatory cells. *Croatica Chemica Acta* 2005;78:91–98.
- [501] Yelisyeyeva O, Cherkas A, Zarkovic K, Semen K, Kaminsky D, Waeg G, Zarkovic N. The distribution of 4-hydroxynonenal-modified proteins in gastric mucosa of duodenal peptic ulcer patients. *Free Radic Res* 2008;42:205–211.
- [502] Cherkas A, Yelisyeyeva O, Semen K, Zarkovic K, Kaminsky D, Gasparovic AC, Jaganjac M, Lutsyk A, Waeg G, Zarkovic N. Persistent accumulation of 4-hydroxynonenal-protein adducts in gastric mucosa after *Helicobacter pylori* eradication. *Coll Antropol* 2009;33:815–821.
- [503] Leonarduzzi G, Robbesyn F, Poli G. Signaling kinases modulated by 4-hydroxynonenal. *Free Radic Biol Med* 2004;37:1694–1702.
- [504] Leonarduzzi G, Scavazza A, Biasi F, Chiarotto E, Camandola S, Vogel S, Dargel R, Poli G. The lipid peroxidation end product 4-hydroxy-2,3-nonenal up-regulates transforming growth factor beta1 expression in the macrophage lineage: a link between oxidative injury and fibrosclerosis. *FASEB J* 1997;11:851–857.
- [505] Kreuzer T, Grube R, Wutte A, Zarkovic N, Schaur RJ. 4-Hydroxynonenal modifies the effects of serum growth factors on the expression of the c-fos proto-oncogene and the proliferation of HeLa carcinoma cells. *Free Radic Biol Med* 1998;25:42–49.
- [506] Biasi F, Vizio B, Mascia C, Gaia E, Zarkovic N, Chiarotto E, Leonarduzzi G, Poli G. C-Jun N-terminal kinase upregulation as a key event in the proapoptotic interaction between transforming growth factor-beta1 and 4-hydroxynonenal in colon mucosa. *Free Radic Biol Med* 2006;41:443–454.
- [507] Biasi F, Tessitore L, Zanetti D, Cutrin JC, Zingaro B, Chiarotto E, Zarkovic N, Serviddio G, Poli G. Associated changes of lipid peroxidation and transforming growth factor beta1 levels in human colon cancer during tumour progression. *Gut* 2002;50:361–367.
- [508] Zarkovic K, Uchida K, Kolenc D, Hlupic L, Zarkovic N. Tissue distribution of lipid peroxidation product acrolein in human colon carcinogenesis. *Free Radic Res* 2006;40: 543–552.
- [509] Custovic Z, Zarkovic K, Cindric M, Cipak A, Jurkovic I, Sonicki Z, Uchida K, Zarkovic N. Lipid peroxidation product acrolein as a predictive biomarker of prostate carcinoma relapse after radical surgery. *Free Radic Res* 2010;44: 497–504.
- [510] Abel T, Feher J. [Non-alcoholic fatty liver disease and cardiovascular risk]. *Orv Hetil* 2008;149:1299–1305.
- [511] Karabulut AB, Kafkasli A, Burak F, Gozukara EM. Maternal and fetal plasma adenosine deaminase, xanthine oxidase and malondialdehyde levels in pre-eclampsia. *Cell Biochem Funct* 2005;23:279–283.
- [512] Zeteroglu S, Ustun Y, Ustun YE. Placental and cord malondialdehyde and maternal and perinatal outcomes. *Int J Gynaecol Obstet* 2004;85:47–49.

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