INTRODUCTION

Oxidative stress has been identified to be a crucial component involved in the development and progression of different diseases. Active oxygen, though vital to our body, causes considerable damage to the essential components of cellular system and disturbs the physiologically important functions of proteins, lipids, enzymes and deoxyribonucleic acid (DNA) bearing the genetic code. Protein oxidation contributes to the modification of catalytic and structural integrity of various important proteins, leading to the formation of various diseases, including diabetes, atherosclerosis, cystic fibrosis, and ulcerative colitis [1].

Oxidative stress is implicated in the pathogenesis of numerous disease processes, including diabetes mellitus, atherosclerosis, ischemia reperfusion injury, rheumatoid arthritis, neurodegenerative diseases as well as in the aging process. Chemical modification of amino acids in protein during lipid peroxidation (LPO) results in the formation of lipoxidation products, which may serve as indicators of oxidative stress in vivo. The various types of aldehydes such as 4-hydroxynonenal, malondialdehyde, acrolein and others produced during LPO may serve as potent oxidative stress biomarkers. Their activation in different signaling cascades lead to apoptosis, differentiation, proliferation, etc., Increased amount of these aldehydes in aging or with metabolic complications or in other diseases indicate their pathophysiological significance. Thus, LPO products or other oxidative stress biomarkers may open the way for the development of early detection, prevention, and therapeutic strategies for stress associated human diseases. Now-a-days, antioxidant supplementation has become an increasingly popular practice to restore the redox homeostatic condition of the cell. Disease specific, target directed, bioavailable antioxidants may be beneficial for sustenance of the quality-of-life in future days.

KEY WORDS: Alzheimer, antioxidants, diabetes, disease pathophysiology, hepatic disease, oxidative stress, lipid peroxidation

Oxidative stress was originally defined as the disequilibrium between pro-oxidants and antioxidants in biological systems [3]. Once this imbalance appears, cellular macromolecules may be damaged by the pre-dominant free radicals. This leads to oxidative modifications of the genome, proteins, structural carbohydrates and lipids; in the latter case, lipid peroxidation (LPO) occurs. LPO is a free radical-related process that in biologic systems may occur under enzymatic control, e.g., for the generation of lipid-derived inflammatory mediators, or non-enzymatic process. This later form is
mostly associated with cellular damage as a result of oxidative stress, and a great variety of aldehydes is formed when lipid hydroperoxides break down in biological systems, such as, malondialdehyde (MDA) and 4-hydroxynonenal (HNE) [4]. Involvement of oxidative stress as pathophysiologic mechanisms in diseases or experimental models can be described in several approaches. Oxidative stress may lead to cellular damage. Any marker of cellular disruption may indicate the process, although it may still be necessary to demonstrate directly the intervention of oxidative stress. This may be achieved by the direct detection of activated species in situ, the assay of end products of protein or lipid oxidation, or any other oxidative modification of macromolecules (modified bases of nucleotides, etc.). Further evidence for the role of oxidative stress may be obtained by quantitative estimation of the content and activity of antioxidants. This present review aims to demonstrate the crucial role of oxidative stress in different diseases to identify different stress markers and to report the role of possible antioxidants.

**Identification of Different Oxidative Stress Markers and LPO Products**

Till today, a number of different oxidative stress markers have been explored, which are recognized as a causative agent for DNA or ribonucleic acid (RNA) damage, LPO, protein oxidation or nitration. The markers identified for DNA/RNA damage include 8-hydroxyguanosine, 8-hydroxydeoxyguanosine, benzo(a) pyrene diolepoxide-DNA adduct, double-strand DNA breaks. A number of ROS also have been identified as oxidative stress markers. There exists convincing evidence that oxidative stress and ROS play an important role in the etiology and/or progression of a number of human diseases. Free radicals and other “reactive oxygen/nitrogen/chlorine species” (ROS/RNS/RCS) are widely believed to contribute to the development of several age-related diseases, and perhaps, even to the aging process itself [5] by causing "oxidative stress" and "oxidative damage."

ROS are thought to be the major ones responsible for the alteration of macromolecules, which is often termed oxidative stress. ROS are generated as byproducts of cellular metabolism, primarily in the mitochondria [6] and include free radicals such as superoxide anion (O$_2^−$), hydroperoxyl (perhydroxyl) radical (HO$_2^•$), hydroxyl radical (●OH), nitric oxide (NO), and other species such as hydrogen peroxide (H$_2$O$_2$), singlet oxygen (‘O$_2$), hypochlorous acid, and peroxynitrite (ONOO$^−$) [7]. ROS are toxic and are known to be involved in the etiology of age-related disease such as Alzheimer [8]. Mammalian cells have developed elaborate defense mechanisms to detoxify ROS. Oxidative stress occurs in the cells as a consequence of an imbalance between the pro-/anti-oxidant systems [3]. The generation of ROS may occur by a large number of physiological and non-physiological processes, which include their generation as byproducts of normal cellular metabolism, primarily in the mitochondria. ROS may damage all types of biological molecules. Oxidative damages to proteins, lipids or DNA may all be seriously deleterious and may be concomitant. However, proteins are possibly the most immediate vehicle for inflicting oxidative damage on cells because they are often catalysts rather than stoichiometric mediators; hence, the effect of damage to one molecule is greater than stoichiometric. ROS leading to protein oxidation include radical species such as superoxide, hydroxyl, peroxyl (RO$_2^•$), alkoxyl (RO$^−$), hydroperoxy, and non-radical species such as H$_2$O$_2$, hypochlorous acid, ozone (O$_3$), singlet oxygen, and ONOO$^−$ [9].

There are reports of different stress markers produced by oxidation of protein or nitrogen and the most important compound among them have been designated as “lipoxidation products.” Thus, lipoxidation products are lipid-derived chemical modifications of protein formed during LPO reactions. The lipid components are formed from oxidized polyunsaturated fatty acids (PUFA) in triglycerides, glycerophospholipids and cholesterol esters. Abstraction of a hydrogen atom from the PUFA moiety of membrane phospholipids initiates the process of LPO. The resulting alkyl radical may rearrange to a more stable conjugated diene, which enters the autocatalytic LPO cascade. Phospholipid hydroperoxides and fatty acid hydroperoxides constitute the major portion of the LPO and can propagate LPO chain reactions. The fatty acid carbon chain may also be spontaneously cleaved (scission) during LPO, yielding a variety of highly reactive compounds, including pentane and ethane radicals, and the, unsaturated aldehydes. The oxidation of PUFA generates reactive carbonyl compounds (RCCs). LPO also produces highly reactive α, β-unsaturated hydroxylkenals, such as 4-HNE and 4-hydroxyhexenal (4-HHE) [Figure 1] [10]. HNE (MW 156 Da) derives from the oxidation of membrane n-6-PUFA, essentially arachidonic acid and linoleic acid, i.e., the two most represented fatty acids in biomembranes [11,12]. The enzymes 12- and 15-lipoxygenase (LO) (12- and 15-LO) metabolize arachidonic acid to the corresponding hydroperoxyecosatetraenoic acids (12- and 15-HpETE), which are further converted by glutathione peroxidase (GPx) to 12- or 15-hydroxyecosatetraenoic acid [13]. Radical induced inactivation of GPx diverts 12- and 15-HpETE to the peroxidation pathway to ultimately generate the respective 4-hydroxodecadienal and 4-HNE [Figure 2].

**Figure 1:** Chemical formulae of some aldehydic compounds derived from lipid peroxidation, including saturated aldehydes, unsaturated aldehydes, 4-hydroxy-2-alkenals, and dicarbonyls [10]
Oxidation of n-3 PUFA's (docosahexaenoic acid [DHA], eicosapentaenoic acid and linolenic acid) generates 4-HHE [14]. These aldehydes vary in chain length, depending on the site of oxidation and the location of the double bonds in the starting lipid, ranging from 9-carbon compounds, such as HNE, to small 2- and 3-carbon reactive compounds, such as MDA, acrolein, and glyoxal [Figure 1]. Each of these compounds may react with amino groups in protein and even simple aldehydes, such as hexanal, are potent protein cross-linking reagents. Prostaglandins (PGs) and isoprostanes (IsoPs) may also be produced from free radical catalyzed peroxidation of arachidonic acid [15].

Increased amounts of LPO end products can probably be detected in almost any disease state because cells and tissues damaged by any mechanism may peroxidize more rapidly than normal [16] [Figure 3]. Although discrete products of the reaction of lipid peroxides (LOOH) with protein have not been identified, a variety of aldehydes formed during LPO reactions are known to react with nucleophilic groups in proteins like AGE, AOPP, 3-nitrotyrosine, protein carbonyl content, etc.. These compounds react not only with lysine residues but also with amino-terminal amino acids, and histidine and cysteine residues in proteins. Mechanisms of the reaction include Schiff base formation, Amadori and Cannizzaro re-arrangements [17] and Michael addition reactions [18], as well as secondary oxidation reactions involving protein-bound intermediates. Because a broad spectrum of aldehydes are formed and may react with protein by multiple mechanisms at multiple sites, it is essential to focus on the measurement of specific lipid oxidation products in order to gain insight into the quantitative role of lipoxidation reactions in biological systems. AOPP and pentosidine are two important markers of oxidative stress implicated with the pathogenesis in diabetic complications [2].

LPO induced by oxidants and oxidative stress, generates a huge variety of LPO products, including RCCs and more stable products such as ketones and alkanes. RCCs, formed endogenously during LPO, are precursors of advanced lipid peroxidation end products (ALEs) [Figure 4] [10,19-21]. LPO also involve the glycoxidation of carbohydrates as precursors of AGEs, which form cross-links on tissular proteins (carbonyl stress), and accumulate during aging and in chronic diseases [22]. Carbonyl stress induces progressively protein dysfunctions and damages in all tissues, with pathological consequences such as inflammation and apoptosis contributing to the progression of diseases [23]. Therefore, inhibiting the chemical modification of tissue proteins may prevent the pathological consequences of carbonyl stress and may represent a new therapeutic strategy for patients. Most of the carbonyl stress inhibitors used so far have been developed to prevent the accumulation of AGEs in diabetes and its complications, including accelerated atherosclerosis, nephropathy, cataract, and neuropathies [24,25]. In contrast, apart from antioxidants that inhibit indirectly the generation of LPO products, few carbonyl scavenger agents known to reduce the accumulation of ALEs precursors in vitro have been tested in vivo on the progression of ALE-related diseases.

**TYPES OF DIFFERENT LPO PRODUCTS**

4-hydroxy-2-alkenals (HNE)

LPO leads to the formation of a broad array of different products with diverse and powerful biological activities. Among them are a variety of different aldehydes [26]. The primary products

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**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 radical induced inactivation of GPx diverts 12- and 15-HpETE to the peroxidation pathway to ultimately generate the respective 4-hydroxdodecadienal and 4-hydroxynonenal [13].

**Figure 3:** Interrelationship of oxidant damaging mechanisms [16]
of LPO, lipid hydroperoxides, can undergo carbon-carbon bond cleavage through alkoxy radicals in the presence of transition metals, giving rise to the formation of short chain, un-esterified aldehydes of 3-9 carbons in length and a second class of aldehydes still esterified to the parent lipid [4]. The important agents that give rise to the modification of a protein may be represented by reactive aldehyde intermediates, such as 2-alkenals and 4 (HNE) [27].

HNE is a reactive, polar lipid which is cytotoxic to cells in culture. HNE reacts with protein by both Schiff base formation with amino groups and by Michael addition reactions with cysteine, histidine and lysine residues. Like MDA, HNE can cause cross-linking of proteins, but a variety of adducts and cross-links may be formed; for example, by Michael addition to cysteine, histidine or lysine, followed by Schiff base formation with an amino group [Figure 5] [10,28,29]. HNE-lysine is a chemically stable product and a permanent chemical modification of protein. Thus, measurements of Michael adduct of HNE to lysine may provide an index of long-term damage to proteins as a result of LPO reactions. Because cysteine and histidine residues are strong nucleophiles in proteins, there are likely to be higher levels of HNE adducts to these amino acids than to lysine in oxidized low density lipoprotein (LDL) [18] and tissue proteins; other products of reaction between HNE and protein are yet to be explored.

**MDA**

MDA is one of the most readily assayed end-products of both enzymatic and non-enzymatic LPO reactions. The measurement of MDA as an index of LPO was introduced by Sato et al. [30] in the form of the thiobarbituric acid (TBA) assay. Due to its high reactivity, most MDA in plasma is protein-bound, and the TBA-MDA assay actually measures the quantity of MDA released from plasma proteins under the acidic conditions of the assay. Two adducts of MDA to protein are formed in both the LDL and ribonuclease systems: (i) The MDA Schiff base adduct to the Ne-amino group of lysine residues in protein; and (ii) the bis-Schiff base diamine cross-link formed by reaction of MDA with two lysine residues in protein, resulting in either intra- or inter-molecular cross-linking of the protein. The Schiff base adducts of MDA to lysine is unusually resistant to reduction by sodium borohydride. Since MDA is a potent protein cross-linking agent [31], it is possible that the aging of lipoxidized proteins may lead to the gradual conversion of MDA adduct, 3-(Ne-lysino) propanol (LM) adducts to 1,3-bis-(Ne-lysino) propane (LML) cross-links in protein.

**F2-IsopPs, 4-HHE and Other Aldehydes as LPO Products**

Free radicals produced during LPO have some very local effects, because of their short life, but the breakdown products of LOOH may serve as “oxidative stress second messengers,” due to their prolonged half-life and their ability to diffuse from their site of formation, compared to free radicals. The free radical mediated non-enzymatic peroxidation of PUFAs has been extensively studied. The F2-IsopPs, especially 8-iso-PG F2, are products of free radical-catalyzed LPO of arachidonic acid [15]. They are formed in situ, esterified to phospholipids, and subsequently released by phospholipases into the plasma,
Acrolein is much more electrophilic than HNE and reacts with glutathione (GSH) much faster [4]. Conjugation with GSH, followed by mercapturic acid transformation of the GSH moiety, is the main pathway for its elimination. Mercapturic acids of acrolein can be reduced or oxidized, as is the case for HNE [35]. It seems that glutathione S-transferase A4-4 (GSTA4-4) can also catalyse the conjugation of GSH to acrolein [39]. Reduced mercapturic acid of acrolein is the major metabolite in urine, as is the case for HNE. Acrolein is also formed during LPO and is a strong electrophile exhibiting high reactivity with cysteine, histidine and lysine nucleophile residues [40]. In the series of aldehydes studied at early times, the rank order of carbonyl incorporation was acrolein > ONE > HNE > DDE > MDA. Mainly acrolein and HNE, but also other LPO by-products including ONE and HHE, are characterized by an α, β-unsaturated carbonyl structure that is a conjugated system and contains mobile π (π)-electrons. The carbonyl oxygen atom is electronegative and can cause regional electron deficiency, electron polarizability. Both acrolein and HNE are considered to be soft electrophiles that form 1,4-Michael type adducts with soft nucleophilic sulfhydryl thiolate groups of cysteine [41].

**ROLE OF LPO PRODUCTS IN CELL SIGNALLING**

**Role of Lipoproteins (Lps) in Cell Signaling**

ROS and the LOOH formed due to oxidative stress are cytotoxic. Recent studies suggest that both, ROS and LOOH, are also involved in the intracellular signaling mechanisms which determine the cell’s final fate. Oxidative alteration of biomembranes and circulating Lps has also many potential consequences in human pathophysiology. Oxidation of LDL also increases its sensitivity to aggregation and to modification by sphingomyelinase, promoting further the likelihood of its intimal modification and uptake by macrophages. Oxidized LDL (ox-LDL) attenuates endothelium dependent relaxation of blood vessels and can cause injury, apoptosis and necrosis of vascular cells. This may lead to the release of lipids and lysosomal enzymes into the intima and promoting the progression of atherosclerotic lesions, such as the development of the acellular lipid core [42].

In particular, the pro-inflammatory and pro-atherosclerotic effects of ox-LDLs are increasingly supported by a multitude of independent, but consistent experimental studies [43]. The biological responses triggered by ox-LDLs are associated with LPO derivatives, which are able to induce various pathogenic intracellular signals leading to cellular dysfunction. Ox-LDLs have been shown to interfere with various signaling pathways involving calcium, trimeric G-proteins and cyclic adenosine monophosphate (cAMP), phospholipase C and D, protein kinase C, ceramide, and mitogen activated protein kinase (MAPK) cascade [43].

**Role of Advanced Lipid Oxidation Products and Lipid Derived Aldehydes in Cell Signaling**

ALEs play an active role in signal transduction by altering progressively the structure and function of circulating and tissular proteins, with consequences on the inflammatory status, cell proliferation and viability [44]. Elevated concentrations of 4-HNE or acrolein (420 mM) are highly toxic for most cell types. Role of advanced LPO products or lipid oxidation derived aldehydes, particularly 4-HNE in cell cycle signaling is becoming increasingly clear. The mechanism of apoptosis elicited by ALE precursors involves various effects, including signaling or protein modification. In this article, recent studies suggesting an important role of 4-HNE in stress mediated signaling for apoptosis are critically evaluated.

Basal HNE concentration in human blood and serum is ~0.05-0.15 μM, and it increases with age [45]. Nutrition influences these values since HNE is a lipoperoxidation product derived from linoleic acid n-6 PUFA. Under conditions of oxidative stress (rheumatologic diseases for example), HNE concentrations increase up to 3-10-fold of physiological concentrations [46]. Multidrug resistance protein 1 (MRP1) and Ral interacting protein (RLIP76) are kind of adenosine triphosphate (ATP)-binding cassette transporters which are involved in the influx of GSH-conjugated HNE. 4-HNE, a
relatively stable end product of LPO, is a potent alkylating agent which can react with a variety of nucleophilic sites in DNA and protein, generating various types of adducts [4]. Its role in signaling mechanisms has been suggested earlier [47] but lately, numerous studies from different laboratories using a variety of cell lines have shown that 4-HNE activates stress-activated protein kinases (SAPK)/c-jun N-terminal-kinases (JNK) [48], a member of MAPK family which is involved in apoptosis. 4-HNE or its homologs can be reduced to corresponding alcohols by aldose reductase or oxidized to corresponding acids by aldehyde dehydrogenase [49]. However, these two pathways account for only a minor fraction of 4-HNE metabolism, and the majority of 4-HNE is conjugated to GSH through the reaction catalyzed by GSTs to form the GSH-conjugate (GS-HNE) [50]. Even though, most of the major classes of GSTs present in mammalian tissues have some detectable activity toward 4-HNE, a subgroup of the α-class GST isozymes have higher catalytic efficiency for 4-HNE [51]. Two GST isozymes, human GST A4-4 [52] and hGST5.8 [53], belonging to this subgroup have been characterized in humans. In vitro studies with cell lines strongly suggest that GSTA4-4 and hGST5.8 are the major determinants of the intracellular concentrations of 4-HNE [50]. Even though 4-HNE can be reduced by aldehyde dehydrogenase and aldose reductase [54,55], majority of cellular 4-HNE is metabolized through its conjugation by reaction catalyzed by GSTs [56] as evident from the mouse model; over-expression of a subclass GSTA4-4 (mGSTA4-4) leads to a dramatic decrease in the levels of 4-HNE in human erythroleukemia cells due to its conjugation to GSH. GS-HNE must be transported out of cells to sustain GST-mediated conjugation of 4-HNE because the conjugate inhibits GSTs. GS-HNE is transported from cells by ATP-dependent primary active transport similar to other GSH-conjugates [57]. Studies with various cell lines and erythrocytes in humans indicate, that the majority (about 2/3) of GS-HNE transport is catalyzed by RLIP76 [47,58-60], a previously described Ral binding protein [Figure 6]. Studies [61] have shown that the MRp1 accounts for only about 1/3 of GS-HNE transport which is consistent with reports that MRp1 also mediates transport of GS-HNE. Studies have also shown that a coordinated action of GSTs and RLIP76 [Figure 7] is the major determinant of 4-HNE concentration in cells [47]. During oxidative stress, heat shock or UV irradiation, which cause increased 4-HNE levels in cells, a rapid but transient induction of hGST5.8 and RLIP76 occurs, which strongly suggests that both these proteins play an important role in the regulation of the intracellular levels of 4-HNE [50]. In cells with induced hGST5.8 and RLIP76, transport of GS-HNE occurs at a several fold higher rate as compared to the controls which further confirms the role of these proteins in regulations of cellular concentrations of 4-HNE. The role of 4-HNE in signaling mechanisms has been suggested for quite some time [62] and lately, numerous studies from different laboratories using a variety of cell lines have shown that 4-HNE activates SAPK/JNK [63], a member of MAPK family which is involved in apoptosis. The activation of JNK has been particularly investigated in the anti-proliferative and apoptotic effect of 4-HNE. This JNK pathway plays a major role in the cooperative apoptotic effect of transforming growth factor β-1 and 4-HNE on colon cancer cell lines [64]. Both Acrolein and 4-HNE increase the levels of the phosphorylated form of transcription factors c-jun (which promotes apoptosis) and cAMP response element binding protein (CREB) (involved in survival), but decrease the activity of the CREB-responsive promoters (while increasing c-jun responsive promoter), which contributes to neuron degeneration and apoptosis [65]. Methylglyoxal and glyoxal are pro-apoptotic through mechanisms involving calcium deregulation [66], GSH depletion, oxidative stress, and activation of stress kinases p38 and JNK [67]. 4-HNE increases the mRNA and protein expression of the pro-apoptotic

Figure 6: Regulation of 4-hydroxynonenal concentration by coordinated action of glutathione S-transferases (GSTs) and Ral interacting protein-76 (RLIP76) [47]. (1) Reaction catalyzed by human GST5.8 [51], human GSTA4-4 [52], mouse GSTA4-4 [59] and rat GSTA4-4 [60]. (2) adenosine triphosphate-dependent transport catalyzed by RLIP76

Figure 7: Lipid peroxidation and stress-mediated signaling [47]
Adaptors/regulators Fas receptor (FasR), Fas ligand (FasL), Bax, and caspases-1, -2, -3, and -8 [68]. In human lens, cultured cells (human lens epithelial B-3), 4-HNE adducts are correlated with the induction of Fas, the activation of JNK and caspase 3 while the transfection of the α-class GST mGSTA4α (which neutralizes 4-HNE) inhibits Fas expression. The mechanism of cell death evoked by these aldehydes could also involve the generation of ONOO-, as reported for 4-HHE and for methylglyoxal [69]. 4-HNE impairs the mitochondrial function, through the alteration of GSH metabolism and the induction of massive mitochondrial oxidative stress [70]. More specifically, moderately elevated concentrations of 4-HNE or very low doses of 4-HHE trigger a calcium-mediated induction of the mitochondrial transition pore [71]. In addition, in vitro experiments on isolated mitochondria or reconstituted models for the adenine nucleotide translocator (ANT) pre-treated with 4-HNE or 4-HHE indicate that the modification of ANT by these aldehydes impairs its function and activity. Lastly, 4-HNE alters mitochondrial calcium uptake and cytosolic calcium homeostasis, which results in necrosis or apoptosis. This mechanism is involved in neuronal cell death [72].

**PATHOLOGICAL ASPECTS OF LIPID PEROXIDATION IN DISEASES**

**Diabetes: An Endemic Threat of Metabolism**

Oxidative stress and inflammation resulting tissue damage are hallmarks of chronic diseases like diabetes. Increased production of oxygen free radicals or ineffective scavenging of ROS, AOPP, and accumulation of AGE play crucial role in diabetes pathogenesis [2]. One of the most prominent LOOH, MDA levels are increased in Type 2 diabetes mellitus (T2DM) patients with complications compared with those non-complicated [73], a fact which is also present in comparison with non-DM individuals [74]. A number of biomarkers of oxidative stress have been studied in Type 1 DM (T1DM) patients such as MDA, F2-IsoPs, AGEs, and nytrotyrosine [75]. Interaction between hyperglycemia and LPO also includes the fact that MDA increases in vivo modification by glycation, as reported in chronic renal failure patients [76]. Other lipoxidation markers, as the isoketal F2-IsoPs, oxidized cholesterol [77] and increased conjugated linolenic acid [78] also increases in DM patients. In T2DM patients, plasma free fatty acid (FFA) levels are significantly and negatively correlated with mitochondrial function, leading to impaired ATP production [79]. TBA adducts (thiobarbituric acid reactive substances [TBARS]) also increase with the degree of metabolic impairment, from healthy patients to T2DM in serum [80]. Mitochondrial ROS a 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 are prone to ROS-induced oxidative damage, resulting in the formation of LOOH. 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.

Diabetes mellitus is a major public health concern where 400 million individuals are expected to be affected within 2050. This is mainly due to the clash between genes and environment—consumption of high energy diets and lack of physical activity sometimes unmask the prevalent gene causing the disease. Both chronic hyperglycemia and hyperglycemic peaks during post-prandial periods constitute a factor for increased oxidative stress in diabetes. Increasing evidence in both experimental and clinical studies suggest that oxidative stress plays a central role in the onset of diabetes mellitus as well as in the development of vascular and neurologic complications of the disease [81].

**Alzheimer’s Disease (AD): A Disease of the Century**

AD represents a highly common form of dementia, but early diagnosis is crucial for successful therapeutic interventions. Specific fluorescent intermediates of brain LPO have been identified to act as blood biomarkers for AD [82]. AD is characterized clinically by early memory dysfunction, later progression of disorders with dysphasia and dyspraxia to mute and immobility. AD is morphologically present by amyloid-containing neuritic (senile) plaques (SP) and neurofibrillary tangles (NFT), pre-dominantly in the hippocampus and frontal cortex. Many studies of AD show increased oxidation of brain lipids, carbohydrates, proteins, and DNA in NFT and SP. Oxidative modification of macromolecules decreases or eliminate their function, and activate inflammatory processes in the brain of patients with AD. In oxidative pathogenesis of AD, a particular role is played by LPO, formed from PUFA, which build the brain phospholipids. Phosphorus nuclear magnetic resonance of central nervous system (CNS) shows a decrease in phosphatidylethanolamine and stearic, oleic, arachidonic and docosahexaenoic acid in the hippocampus and inferior parietal lobule of AD patients compared with age-matched controls [83]. On the other hand, IsoPs are formed non-enzymatically by free radical induced oxidation of arachidonic acid in these patients [84]. Namely, oxidation of DHA leads to the formation of F4-Isop-like compounds, so-called F4-neuroprostanes (F4-NP); their concentration in colony stimulating factor (CSF) of AD patients is significantly elevated if compared with controls [85].

**Parkinson’s Disease (PD): The Progressive Nervous System Disorder**

PD is a neurodegenerative disorder clinically characterized by extrapyramidal movement disturbance: Rigidity, bradykinesia, resting tremor and sometimes dysphagia, autonomic dysfunction, or dementia. The plasma of PD patients, both sporadic and familial form, has elevated levels of LOOH and is furthermore susceptible for LPO more than normal healthy human plasma [86].

**CVD: Curse of Modern Unhealthy Lifestyle**

Inflammatory reactions in the arterial wall triggered and sustained mainly by lipid oxidation products are concerned with
the initiation and promotion of atherosclerosis and CVD [87]. Among the most potent LPO products formed during the LDL oxidation process, oxidized phospholipids (OxPLs), 4-HNE and oxidized cholesterol derivatives (oxysterols) are accumulated 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 [88]. OxPLs exhibit a high affinity for Lp(a), which could represent a detoxification mechanism for low Lp(a) concentrations, since Lp(a) is associated with the platelet activating factor acetyl hydrolase that degrades OxPLs. However, high levels of OxPLs in Lp(a) are highly pro-atherogenic as they bind the vessel wall, where they exert pro-inflammatory and prothrombotic properties [89]. Such elevated circulating levels of Lp(a), associated with increased OxPLs, are observed in patients affected with CVDs.

**Aging: The Ultimate Sport**

In general, an increase of LPO products with age is found. Tissue specificity, cellular involvement or linearity of such an accumulation remains obscure. A wealth of studies investigated the steady state concentration increase of various LPO products in a number of different tissues in humans and model organisms. This includes measurements of MDA by TBARS formation, the measurement of conjugated dienes, 4-HNE, and protein adducts of LPO products and various other products.

LPO was investigated by several authors in various brain regions; numerous studies show an increase in LPO during aging as a change in the membrane transition temperature in human myelin from white matter of patients over the age of 50, in the liver and brain homogenates and individual regions of the brain. A number of investigations demonstrated not only an age-association of LPO, but also an increase of LPO in several age-related diseases, such as AD [90] or in obesity and metabolic syndrome [91]. LPO products accumulate with aging, in particular in oxidative stress-related neurological disorders such as ischemic, inflammatory, metabolic, developmental, and degenerative diseases [92]. 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. 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 nicotinamide adenine dinucleotide phosphate-oxidase (NAD(P)H) oxidase, endothelial NO synthase, xanthine oxidase, myeloperoxidase, superoxide dismutase (SOD), catalase (CAT), and GPx [93]. ROS and 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 [94].

**Liver Diseases: Hepatic Hazards Due to Stress**

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). Among the various kinds of liver diseases, 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. Researchers have identified the factors that can play a causative role: Oxidative stress, LPO, abnormal cytokine production, fatty acid metabolic disturbance, and insulin resistance. The pathophysiology of NAFLD involves insulin resistance and production of ROS, which stimulate the synthesis of several cytokines through the up-regulation of their transcription by nuclear factor-kappa B. The combination of these events causes hepatocyte injury through direct oxidative injury, tumor necrosis factor-alpha-induced apoptosis or inflammation [95]. HCC can develop in all kind of chronic liver diseases. In NAFLD, the possible follow-up of the pathogenetic trends are increased fatty acid fluxes, the increased fatty acid content in the liver, 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 carcinogenesis. 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. Antioxidants are often acting as anti-promoters and anti-carcinogens [96], in agreement with their fundamental role of biological response modifiers of oxidative homeostasis [97]. 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. Lowered activities of primary antioxidant enzymes, such as the cytoplasmic CuZnSOD (SOD1), are often, but not always, seen in tumors, 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.

**Renal Diseases**

It is generally accepted that renal failure is associated with drastic LPO [98,99]. LPO contributes to pathogenesis and progression of renal failure. Cardiovascular injury has been shown to be the most critical factor affecting quality-of-life and mortality in patients suffering from end-stage renal disease undergoing hemodialysis. LPO and oxidative stress have been thought to be significant risk factors for cardiovascular disorders in renal patients. HNE, MDA, protein carbonyls, F$_2$-IsOPs, and cholesterol oxidation products are increased in plasma of patients with renal failure [13].

**PROTECTION AGAINST LPO PRODUCTS**

**Endogenous Defense in the Human Body System**

Uncontrolled generation of ROS can lead to their accumulation causing oxidative stress in the cells. Thus, cells have evolved...
The antioxidant enzymes constitute the second-line of defenses which provide a variety of primary and secondary defenses against oxidative stress. Primary antioxidant enzymes are mainly preventive, and these enzymes such as SOD, CAT, and GPx can decompose ROS and prevent damage to cellular constituents and initiation of LPO. Reduced GSH is an important scavenger of free radicals and a potent endogenous antioxidant, which helps to protect cells from oxidative injury. Besides, its role in the maintaining the redox potential within the cell, it is also a key component of the enzymatic antioxidant system. The reduced GSH concentration was observed to be significantly lower in β-thalassemic and E/β-thalassemic red blood cells (RBC) compared with the carrier and control subjects, suggesting that the erythrocyte is in a pro-oxidant condition, which may be a partial cause of the increased hemolysis and shortened RBC survival observed in β-thalassemic and E/β-thalassemic RBC [103].

Secondary defenses typically involve excision or repair of any lesions caused by ROS. In the event of ROS-induced LPO, secondary defense enzymes are involved in the removal of LOOH to terminate the autocatalytic chain of LPO and protect membranes. GPx and GST, which catalyze GSH-dependent reduction of LOOH through their peroxidase activity, are the major secondary defenses to guard against ROS-induced LPO. In addition to selenium-dependent GPx, the selenium-independent GPx activity of the-α-class GST is also involved in the reduction of LOOH.

**Dietary Compounds as Potential Antioxidants**

The external source of defense against LPO products are performed by different antioxidants or flavonoids. A variety of dietary plants including grains, legumes, fruits, vegetables, tea, wine, etc., contain antioxidants. The prophylactic properties of dietary plants have been attributed to the antioxidants/ polyphenols present in them. Polyphenols with over 8000 structural variants are secondary metabolites of plants and represent a huge amount of substances having aromatic ring(s) bearing one or more hydroxyl moieties. Polyphenols are effective ROS scavengers and metal chelators due to the presence of multiple hydroxyl groups. Examples of polyphenolic natural antioxidants derived from plant sources include vitamin E, flavonoids, cinnamic acid derivatives, curcumin (CUR), caffeine, catechins, gallic acid derivatives, salicylic acid derivatives, chlorogenic acid, resveratrol, folate, anthocyanins, and tannins [104]. Apart from polyphenols there are also some plant derived non-phenolic secondary metabolites such as vitamin E, flavonoids, cinnamic acid derivatives, CUR, lignins and lignans, anthocyanins, and tannins that show excellent antioxidant activity [105]. Vitamin C, the water soluble natural vitamin, plays a crucial role in regenerating lipid soluble antioxidants like vitamin E6. Both vitamin E and C are used as standards for evaluating the antioxidant capacity of new molecules.

A growing amount of evidence indicates that free radicals play an important role as mediators of skeletal muscle damage and inflammation after strenuous exercise. It has been postulated that the generation of oxygen free radicals is increased during exercise as a result of increases in mitochondrial oxygen consumption and electron transport flux, inducing LPO. The literature suggests that dietary antioxidants are able to detoxify the peroxides produced during exercise, which could otherwise result in LPO and that they are capable of scavenging peroxyl radicals and therefore may prevent muscle damage. Endogenous antioxidant enzymes also play a protective role in the process of LPO [106].

Foods and beverages, rich in flavonoids and other types of antioxidants, have been associated with decreased risk of CVDs in several epidemiologic studies [107]. Flavonoids have powerful antioxidant activities in vitro, being able to scavenge a wide range of ROS, RNS and RCS, such as superoxide, hydroxyl and peroxyl radicals, hypochlorous and peroxynitrous acid. They can also chelate metal ions, often decreasing metal ion pro-oxidant activity [108-110]. Considerable evidence indicates that increased oxidative damage is associated with and may contribute to the development of all major age-related diseases, it has been logical to attribute the alleged protective...
effects of flavonoids to their antioxidant ability. Moreover, flavonoids and other phenols are complex molecules and are likely to have multiple potential biological activities, such as inhibiting telomerase [111], affecting signal transduction pathways [112,113], inhibiting cyclooxygenases (COXs) and LOs [114], decreasing xanthine oxidase [115], matrix metalloproteinase [116], angiotensin-converting enzyme [117] and sulfotransferase [118] activities, and interacting with sirtuins [119]. Flavonoids may also interact with cellular drug transport systems [120], compete with glucose for transmembrane transport [121], interfere with cyclin-dependent regulation of the cell cycle [122], and affect platelet function [123].

In other words, flavonoids stabilize the ROS by reacting with the reactive compound of the radical. Siblin is a flavonoid, which is directly reported to inhibit the NO scavenging 

Based on these studies, it is clear that a need exists for antioxidant agents, which are efficient in removing ROS, inexpensive to manufacture, stable and possess advantageous pharmacokinetic properties, such as the ability to cross the blood-brain barrier and penetrate tissues. Such versatile antioxidants would find use as pharmaceuticals and possibly as nutraceuticals.

**Therapeutic Potential of Antioxidants Against LPO Products**

Antioxidants are assumed to counteract the harmful effects of ROS and therefore prevent or treat oxidative stress-related diseases. Recently human studies are being conducted exploring the efficiency of antioxidants in prevention and treatment of various diseases. However, despite much enthusiasm in the 1980s and 1990s, many well-known agents such as antioxidant vitamins have not successfully passed the scrutiny of clinical trials for prevention and treatment of various diseases. This has given rise to a pessimistic view of antioxidant therapy; however, the evidence from human epidemiological studies about the beneficial effects of dietary antioxidants and preclinical in vitro and animal data are compelling.

Many people take antioxidant supplements believing to improve their health. Whether antioxidant supplements are beneficial or harmful is uncertain [131,132]. Antioxidant supplementation in rats found to be more protective such as in a recent review of Alshatwi et al. [133] where tomato powder was given as antioxidant supplementation. It has also found effective against tuberculosis as evident from the study of 60 patients in Nigeria, where vitamin C and E supplements elevated their level in the body, after 6 weeks of anti-tuberculosis treatment [134]. Experimental evidence suggests that daily supplementation for 2 months with vitamin C alone significantly decreased the plasma levels of F2-IsoPs in smokers with body mass index above the median when compared with those who received placebo [135]. Animal models have demonstrated that dietary supplementation with antioxidant vitamins can prevent or reverse the age-related changes in antioxidant defenses in CNS and decrease oxidative stress [136]. Viña et al. [137] confirm the idea that vitamin E may be considered as an effective treatment of AD. A major limitation present in most of the intervention studies exploring the effects of antioxidant supplementation (e.g., vitamin E) on AD outcomes is that they have been conducted on subjects who already have been diagnosed with this clinical condition. Moreover, antioxidants are often tested as single agents, while it is becoming clearer that combinations of antioxidants are more effective. As for evidence related to CVD, a large part of studies on the topic (mostly from epidemiologic reports) has shown that individuals, who consume higher amounts of fruits and vegetables, as well as a vitamin supplement users, have lower rates of AD [138]. Recently, the supplementation of vitamins and mineral antioxidants (SU. VI.MAX; “Supplémentation en Vitamines et Minéraux...
Anti-oXydants”) study [139], a randomized, double-blind, placebo-controlled primary prevention trial, tested the efficacy of supplementation with a combination of antioxidant vitamins and minerals, at nutritional doses, in reducing the incidence of cancer in a general population not selected for risk factors. After a 7.5-year follow-up, antioxidant supplementation was associated with a reduction in cancer incidence in men only. However, authors discussed that antioxidant supplementation may have beneficial effects on cancer incidence only in healthy subjects, who are not exposed to cancer risk and with particularly low baseline antioxidant levels [140]. In 2006, Flores-Mateo et al. [141] reported that the use of supplements containing selenium did not reduce the risk of coronary heart disease in the meta-analysis of six trials. More recently, Lee et al. [142] found that folic acid supplementation with B vitamins had potential small benefits in the prevention of stroke, and Qin et al. [143] indicated that folic acid treatment decreased the risk of CVD by 15% in patients with end-stage renal disease or advanced chronic kidney disease.

Excessive antioxidants can adversely affect key physiological processes. Antioxidants cannot neutralize the effects of ALE precursors once adducts are formed. For instance, trolox and phenolic acids cannot protect against the modification of tyrosine kinase receptors induced by 4-HNE [144]. The results of recent reviews and meta-analyses of the role of antioxidant supplements for prevention of gastrointestinal cancers were unforeseen; antioxidant supplements significantly increased mortality in the antioxidant group with the fixed-effect model meta-analysis, but not with the random effects meta-analysis [132]. Several meta-analyses have reported conflicting evidences from randomized controlled trials. In 2003, a meta-analysis of 12 trials indicated that vitamin E supplements did not provide benefit in cardiovascular death or cerebrovascular events [131]. Instead, it showed that β-carotene supplementation led to a small increase in all-cause mortality and cardiovascular death. Polyphenols reduce hyperlipidemia and inhibit LPO and atherosclerosis development in diabetic LDL receptor knockout mice [145]. Resveratrol, a red wine polyphenol, exhibits protective properties against LPO and ALE formation in experimental models for numerous diseases including atherosclerosis, diabetes, and AD [146]. However, most agents were not tested in human patients, and any correlation between their protective effect on ALE generation and the progression of the disease remains speculative. Further randomized controlled trials are required to determine whether vitamin and antioxidant supplementation would be beneficial against different disease for people who are deficient in vitamins or antioxidants at baseline. Regarding the assessment of methodological quality of each trial, further validated tools that could assess the actual performance or biases of each trial should be developed.

CONCLUSION

Last two decades have witnessed great progress in the development of biomarkers of oxidative stress that may eventually be useful in disease prediction and diagnosis. The challenges for the future are to validate the available biomarkers for oxidative damage based on their specificity, stability for storage, reproducibility, causal relation with disease, and response to antioxidant intervention. LPO products can be regarded as potent biomarkers for oxidative stress in regard to its pathological aspects and advanced lipid oxidation products in a large variety of diseases representing suitable markers. The two main types of LPO, i.e., HNE and MDA contribute to initiate several diseases or aggravate their severity. HNE and other LPO products also enhance different signaling cascades leading to disease pathogenesis. The preventive role of antioxidants revealed its efficiency in animal models, whereas most of antioxidants in therapeutic human trials fail to protect on more advanced states. Thus, the biomarkers of oxidative damage, if validated, may open the way for the development of prediction, early detection and prevention strategies associated with human diseases.

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REFERENCES

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