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Review

Biomarkers of oxidative and nitrosative damage in Alzheimer's disease and mild cognitive impairment

Francesca Mangialasche [a,b], M. Cristina Polidori [c], Roberto Monastero [d], Sara Ercolani [a], Cecilia Camarda [d], Roberta Cecchetti [a], Patrizia Mecocci [a,*]

[a] Section of Gerontology and Geriatrics, Department of Clinical and Experimental Medicine, University of Perugia, Perugia, Italy
[b] Aging Research Center, Karolinska Institutet, Stockholm, Sweden
[c] Institut für Biochemie und Molekularbiologie I, Heinrich-Heine Universität Düsseldorf, Düsseldorf, Germany
[d] Laboratory of Epidemiology and Psychology of Aging and Dementia, Section of Neurology, Department of Clinical Neuroscience, University of Palermo, Palermo, Italy

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ABSTRACT

Alzheimer's disease (AD) is the most common type of dementia in the elderly. Products of oxidative and nitrosative stress (OS and NS, respectively) accumulate with aging, which is the main risk factor for AD. This provides the basis for the involvement of OS and NS in AD pathogenesis. OS and NS occur in biological systems due to the dysregulation of the redox balance, caused by a deficiency of antioxidants and/or the overproduction of free radicals. Free radical attack against lipids, proteins, sugars and nucleic acids leads to the formation of bioproducts whose detection in fluids and tissues represents the currently available method for assessing oxidative/nitrosative damage. Post-mortem and in-vivo studies have demonstrated an accumulation of products of free radical damage in the central nervous system and in the peripheral tissues of subjects with AD or mild cognitive impairment (MCI). In addition to their individual role, biomarkers for OS and NS in AD are associated with altered bioenergetics and amyloid-beta (Aβ) metabolism. In this review we discuss the main results obtained in the field of biomarkers of oxidative/nitrosative stress in AD and MCI in humans, in addition to their potential role as a tool for diagnosis, prognosis and treatment efficacy in AD.

1. Introduction

Alzheimer's disease (AD) is the most common type of dementia in the elderly, aging being the main risk factor. Due to the dramatic increase in the elderly population in developed countries, the already high prevalence of AD is expected to further rise to up to 13 million cases in USA and over 4 million cases in the EU in 2050 (Small et al., 1997; Hebert et al., 2003). Of the several age-related diseases, AD is a major socioeconomical and medical challenge because there is still no significant treatment. Therefore, much research has been undertaken in recent decades to decode the main pathophysiological changes responsible for AD development. The main biochemical pathways shown to be associated with the histophatological and clinical hallmarks of AD appear to be related to the production and metabolism of amyloid-beta (Aβ) fragments and neurofibrillary tangles (NFT) (Lambert et al., 1998; Deshpande et al., 2006; Cappai and Barnham, 2008). Furthermore, impaired bioenergetics (Beal, 2005), oxidative stress (Mariani et al., 2005; Valko et al., 2007) and inflammation (Chung et al., 2009) have been suggested as additional hallmarks. In addition to their individual role, biomarkers of oxidative and nitrosative stress in AD have been shown to be associated with altered bioenergetics and Aβ metabolism. For this reason, oxidative and nitrosative damage are generally accepted as a central process in AD pathophysiology. The aim of this work is to offer the reader an overview regarding the main results obtained in the field of oxidative/nitrosative stress in AD in humans. Furthermore, recent data concerning the role of oxidative/nitrosative stress in mild cognitive impairment (MCI) will also be assessed. In the following paragraphs, after a brief introduction on the theory of oxidative stress, we report the main results of studies on subjects with diagnosis of AD and MCI assessing the most widely investigated biomarkers of OS and NS in the brain, cerebrospinal fluid (CSF), blood and urine. We systematically searched the PubMed, National Library of Medicine database for English-language articles published from 1990 to January 2009 (last accessed on January 31, 2009). Furthermore, we found additional papers by performing a manual search of the reference lists of relevant retrieved articles.

* Corresponding author at: Institute of Gerontology and Geriatrics, Department of Clinical and Experimental Medicine, Ospedale Santa Maria della Misericordia, Piazzale Menghini 1, S. Andrea delle Fratte, 06156 Perugia, Italy.
Tel.: +39 075 578 3270; fax: +39 075 578 3878.
E-mail address: mecocci@unipg.it (P. Mecocci).

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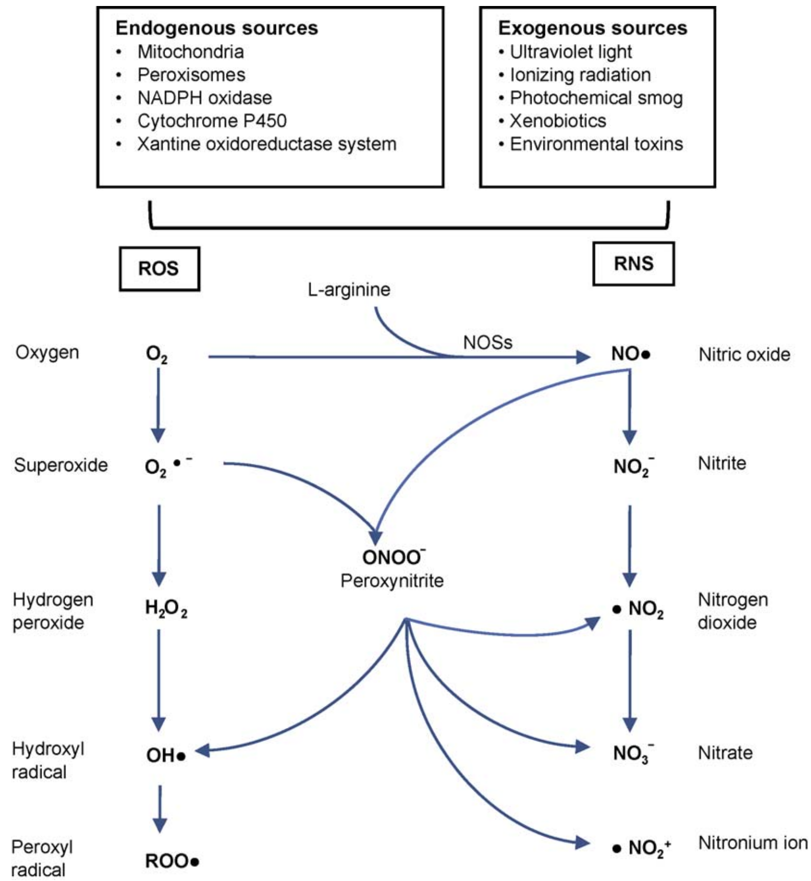

Fig. 1. Main ROS and RNS responsible for biomolecules damage in the human body, and their main sources. The most reactive ROS are the superoxide anion and the hydroxyl radical. Peroxynitrite is the main RNS, but also nitrogen dioxide and nitronium ion exhibit significant oxidant properties. ROO•: peroxyl radical; the simplest peroxyl radical is HOO•, termed hydroperoxyl radical or perhydroxyl radical. Another important peroxyl radical is LOO•, called lipid peroxyl radical. NOSs: nitric oxide synthases.

2. Oxidative and nitrosative stress

Free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals, which characterises free radicals with high reactivity (Halliwell and Gutteridge, 1999). Exogenous agents (such as photochemical smog, ozone, pesticides, xenobiotics and ionizing radiation) and a variety of endogenous processes (for example, mitochondrial respiration, cytochrome P-450 detoxification reactions, phagocytic oxidative bursts, and peroxisomal leakage) can generate significant amounts of ROS and RNS in the human body (Chakravarti and Chakravarti, 2007) (Fig. 1). Indeed, both species of free radicals are products of normal cellular metabolism. Mitochondrial oxidative phosphorylation generates the majority of free radicals in the cell. There is a wide variance in the literature regarding the percentage of basal mitochondrial oxygen consumption leading to ROS generation, which can be partially accounted for by the fact that various studies have been conducted in isolated mitochondria, thus in non-physiological conditions. It has been proposed that 0.2–2% of the total oxygen consumption is converted into free radicals in mitochondria (Balaban et al., 2005). During energy transduction, a small number of electrons "leak" out from oxygen prematurely, thereby forming ROS, which is mainly the oxygen free radical superoxide ($O_2^{\bullet-}$) (Chance et al., 1979). This easily reacts with nitric oxide ($NO^{\bullet}$) and forms peroxynitrite ($ONOO^-$), an RNS with very high reactivity (Vatassery, 2004). ROS and RNS are also normally generated by tightly-regulated enzymes, such as nitric oxide synthases (NOSs) and nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase isoforms, since free radicals take part in many physiological processes. These are involved in cellular signalling, reactions to stress/noxia (for example, a defence against infectious agents) and the induction of mitogenic and apoptotic responses. In addition, $NO^{\bullet}$ has effects on neuronal transmission and synaptic plasticity in the central nervous system (CNS) (Calabrese et al., 2007; Valko et al., 2007).

However, ROS and RNS can be harmful as they can damage cellular lipids, sugars, proteins and nucleic acids, thus inhibiting the latter's normal function. This damage can compromise cell viability or induce cellular responses leading to cell death by necrosis or apoptosis (Valko et al., 2007). Free radical attack against lipids, proteins, sugars and nucleic acids leads to the formation of respective bioproducts, which will be discussed in detail below, and whose detection in fluids and tissues represents the currently available method of assessing oxidative/nitrosative damage in several systems (Polidori et al., 2001b).

Living systems have developed mechanisms with which to control the harmful effects of ROS/RNS. These systems are mainly based on: (i) the presence of antioxidants (enzymatic and non-enzymatic); (ii) the repair of injured molecules; and (iii) the removal of damaged molecules. The wide variety of antioxidant defence systems helps to prevent and repair ROS and RNS-induced damage. These antioxidants, however, might themselves become a target of ROS/RNS damage, their efficiency to counteract free radical hyperproduction thereby being impaired (Beckman and Ames, 1998). As in the case of the measurable bioproducts of free radical damage, several compounds of the antioxidant defence system of the organism can be measured and therefore used as additional important information regarding the oxidant/antioxidant balance of the organism. In ideal circumstances, the rate of production of an oxidatively modified cellular component should be comparable to that of its removal or repair. A fine regulation of ROS/RNS production and neutralization is essential for avoiding their detrimental effects, and different mechanisms cooperate to preserve this equilibrium, termed "redox balance" or "redox homeostasis" (Droge, 2002).

The damage promoted by ROS and RNS is termed oxidative stress (OS) and nitrosative stress (NS) respectively. These occur in biological systems when there is a dysregulation of the redox balance, caused by a deficiency of enzymatic and non-enzymatic antioxidants, and/or an overproduction, or altered spatiotemporal distribution, of ROS/RNS. On the basis of the most recent discoveries in this field, oxidative and nitrosative stress might be defined as the disturbance in the balance between ROS/RNS acting as oxidants and levels of protecting antioxidant defence systems in favour of the former, potentially leading to damage (Sies, 1985) and linked to a disruption of redox signalling and control (Jones, 2006).

A large body of experimental research suggests an important pathophysiological role of increased OS and NS in the ageing process (Sies, 1985; Polidori et al., 2001a; Valko et al., 2007). There are definitely certain drawbacks in the defence/repair mechanisms, since there is evidence of progressive accumulation of oxidative and nitrosative damage to lipids, proteins, carbohydrates, DNA and RNA with aging, even in the healthiest individuals, suggesting that aging is due to a shift from redox regulation to oxidative and nitrosative damage (Mariani et al., 2005; Chakravarti and Chakravarti, 2007). Several studies have demonstrated an increase in OS and NS in several central nervous system diseases, like AD, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease and cerebrovascular diseases (Beal, 2000; Calabrese et al., 2001; Mariani et al., 2005; Migliore et al., 2005a; Valko et al., 2007).

The brain is particularly vulnerable to oxidative/nitrosative damage since (i) it contains a high proportion of polyunsaturated fatty acids, which are highly susceptible to lipid peroxidation, and catecholamines prone to autoxidation; (ii) it has a high metabolic activity that requires large amounts of oxygen (the brain uses approximately 20% of the oxygen consumed by the resting body); (iii) it is relatively deficient in antioxidant systems with a lower activity of glutathione peroxidase (GPx) and catalase (Cat) compared to other organs; and (iv) it contains redox-active metals (copper, iron) that can promote the production of free radicals (Kedar, 2003; Nunomura et al., 2006b). In neurodegenerative diseases, increased levels of OS/NS within specific brain regions which undergo selective neurodegeneration have been reported, thereby suggesting that ROS/RNS can contribute to the development of disease through primary or secondary pathophysiologic mechanisms.

3. Oxidative and nitrosative damage in AD and MCI

AD is the most common neurodegenerative disorder worldwide. Neurophatologically, it is characterized by regionalized neuronal death, synaptic loss, accumulation of intraneuronal NFT and extracellular senile plaques (SP), and proliferation of reactive astrocytes in the entorhinal cortex, hippocampus, amygdala and association areas of frontal, temporal, parietal and occipital cortex. NFT are formed by intracellular deposits of paired helical filaments composed of hyperphosphorylated tau. SP can be present as diffuse plaques, composed of amorphous extracellular deposits of Aβ that lack neuritis, and as neuritic plaques, which consist of extracellular deposits of insoluble Aβ surrounded by dystrophic neurites, reactive astrocytes, and activated microglia. Aβ is a 39–43 amino acid peptide derived from the larger Aβ precursor protein (APP) by proteolytic cleavage. Aβ 1–40 is the most frequent form of Aβ even if the minor species (i.e., Aβ 1–42) has a higher propensity to aggregate and is greatly enriched in amyloid deposits. Furthermore, recent studies suggest that soluble Aβ oligomers are present in the AD brain and they may represent the main toxic form of Aβ (Klein et al., 2001; Walsh et al., 2002; Glabe, 2006).

The fact that age is the main risk factor for AD has provided the basis for the involvement of oxidative and nitrosative imbalance in the disease, since products of oxidative and nitrosative damage do accumulate during aging (Mariani et al., 2005; Valko et al., 2007). Free radicals promoting OS/NS are thought to play an early pathophysiological role in AD, and oxidative/nitrosative modification to virtually all classes of biomacromolecules has been described in brain regions susceptible to degeneration in this disorder, and also in the peripheral tissues of AD subjects (Table 1). More recently there have been multiple studies showing increased levels of oxidative and nitrosative damage in MCI (Markesbery and Lovell, 2007). This is a condition in which memory or other cognitive abilities are slightly abnormal but they coexist with predominantly normal functions in the activities of daily living and absence of dementia (Winblad et al., 2004; Artero et al., 2006). MCI is related to an increased risk of conversion to dementia and it may be considered, in most of the cases, a prodromal AD (Mariani et al., 2007). Since intervention in preclinical conditions would have the greatest public health impact, there is increasing attention regarding MCI, in order to identify biomarkers that can predict the risk of conversion to dementia, which could be used to monitor responses to therapeutic interventions aimed at reducing the progression of cognitive decline.

Several post-mortem and in-vivo studies have demonstrated an accumulation of the products of ROS/RNS damage in AD and MCI subjects; these substances can be considered biomarkers of oxidative and nitrosative damage respectively. Biomarkers are defined as characteristics that can be objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention (Dalle-Donne et al., 2006). The role of OS/NS biomarkers in AD is not yet totally understood, but it is becoming clear that several biomolecules could help in clarifying the pathogenesis, diagnosis and prognosis of AD. In the following paragraphs we summarize the main findings regarding the biomarkers of OS and NS in AD and MCI.

4. Lipids

ROS can attack lipids and extract a hydrogen atom from a methylene carbon in their side chain. The greater the number of double bonds in the lipid molecule, the easier will be the removal of the hydrogen atom. This explains why the polyunsaturated fatty acid residues of phospholipids are very sensitive to ROS. Lipid peroxidation, which refers to the oxidative degradation of lipids, is one of the major outcomes of free radical-mediated injury. The peroxidation of lipids in plasmalemma or sub-cellular membranes can be very damaging because it can promote alterations in their biological properties (such as the degree of membrane fluidity), and lead to the inactivation of membrane-bound receptors or enzymes, which in turn may impair normal cellular function and increase cell permeability (Anzai et al., 1999; Yehuda et al., 2002).

Lipid peroxidation is a self-propagating process that can proceed until the substrate is consumed or termination occurs, thus promoting extensive tissue injury (Niki et al., 1993; Porter et al., 1995). Moreover, various products of lipid peroxidation are chemically reactive and they covalently modify critical biomolecules like proteins and DNA, thus increasing cellular damage (Uchida, 2003a). Lipid peroxidation generates a variety of relatively stable end products, mainly aldehydic by-products, such as malondialdehyde, and more reactive α,β-unsaturated reactive aldehydes, such as trans-4-hydroxy-2-nonenal, and 2-propenal (acrolein) (Pryor and Porter, 1990; Esterbauer et al., 1991; Loidl-Stahlhofen et al., 1994; Uchida, 2003b; Carini et al., 2004). Carbonyl-crotonaldehyde is another highly reactive aldehyde recently studied in AD (Ichihashi et al., 2001; Kawaguchi-Niida et al., 2006). Other products, derived from the endocyclization of lipid hydroperoxyl radicals, are isoprostanes and neuroprostanes; more recently another class of compounds, named neurofurans, has been identified (Roberts et al., 1998; Cracowski et al., 2002; Song et al., 2008). All these substances have been extensively assessed in brain and biological fluids (CSF, plasma, urine), as an index of oxidative damage, in subjects with AD and MCI.

4.1. Malondialdehyde, trans-4-hydroxy-2-nonenal, acrolein, and carbonyl-crotonaldehyde

Malondialdehyde (MDA) is a product that can be generated by thromboxane synthase. However, a report from the Biomarkers of Oxidative Stress Study showed that peripheral levels of MDA

Table 1
Summary of the main findings relating to biomarkers of oxidative/nitrosative stress in subjects with AD and MCI.

Biomarkers of lipid peroxidation	Main findings
Malondialdehyde (MDA) and thiobarbituric acid-reactive substances (TBARS)	AD: increased levels in the brain (Balazs and Leon, 1994; Palmer and Burns, 1994; Lovell et al., 1995; Marcus et al., 1998; Keller et al., 2005). Results on plasma and peripheral blood cells are inconsistent (Ahlskog et al., 1995; Ceballos-Picot et al., 1996; Sinclair et al., 1998; Polidori and Mecocci, 2002; Kawamoto et al., 2005; Aybek et al., 2007; Casado et al., 2008) MCI: increased levels in the brain (Keller et al., 2005)
Trans-4-hydroxy-2-nonenal (HNE)	AD: increased levels in the brain (Montine et al., 1997; Sayre et al., 1997b; Markesbery and Lovell, 1998; Volkel et al., 2006; Williams et al., 2006). Results on plasma and peripheral blood cells are inconsistent (Cecchi et al., 2002; Calabrese et al., 2006) MCI: increased levels in the brain (Butterfield et al., 2006b; Williams et al., 2006)
Acrolein	AD: increased levels in the brain (Calingasan et al., 1999; Lovell et al., 2001; Williams et al., 2006). MCI: increased levels in the brain (Williams et al., 2006)
Carbonyl-crotonaldehyde (CRA)	AD: increased levels in the brain (Kawaguchi-Niida et al., 2006)
F_2-isoprostanes (F_2-IsoPs)	AD: increased levels in the brain (Praticò et al., 1998; Yao et al., 2003; Markesbery et al., 2005; Casadesus et al., 2007; Forman et al., 2007) and CSF (Montine et al., 1998, 1999a; Praticò et al., 1998, 2000). Results on plasma and urine are inconsistent (Feillet-Coudray et al., 1999; Praticò et al., 2000, 2002; Montine et al., 2002; Bohnstedt et al., 2003; Kim et al., 2004; Irizarry et al., 2007) MCI: increased levels in the brain (Markesbery et al., 2005) and CSF (Praticò et al., 2002; de Leon et al., 2004). Results on plasma and urine are inconsistent (Praticò et al., 2002; Irizarry et al., 2007)
F_4-neuroprostanes (F_4-NPs)	AD: increased levels in the brain (Nourooz-Zadeh et al., 1999; Reich et al., 2001; Markesbery et al., 2005) MCI: increased levels in the brain (Markesbery et al., 2005)
Biomarkers of DNA/RNA oxidation	**Main findings**
DNA strand breaks	AD: increased levels in the brain (Mullaart et al., 1990; Anderson et al., 1996; Li et al., 1997; Lucassen et al., 1997; Sugaya et al., 1997; Stadelmann et al., 1998; Adamec et al., 1999)
8-Hydroxy-2′-deoxyguanosine (8-OHdG)	AD: increased levels in the brain (Mecocci et al., 1994; Nunomura et al., 1999; Wang et al., 2005), in the CSF (Lovell et al., 1999; Lovell and Markesbery, 2001), and in peripheral blood cells (Mecocci et al., 1998, 2002; Morocz et al., 2002; Migliore et al., 2005b) MCI: increased levels in the brain (Wang et al., 2006) and in peripheral blood cells. (Migliore et al., 2005b)
Other biomarkers of DNA oxidation: 8-hydroxyadenine (8-OHA); 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy-guanine); 4,6-diamino-5-formamidopyrimidine (Fapy-adenine); 5-hydroxycytosine (5-OHC); 5-hydroxyuracil (5-OHU); thymine glycol	AD: increased levels in the brain (Lyras et al., 1997; Gabbita et al., 1998; Wang et al., 2005) MCI: increased levels in the brain (Wang et al., 2006)
8-Hydroxyguanosine (8-OHG)	AD: increased levels in the brain (Nunomura et al., 1999, 2001, 2004; Shan et al., 2003; Ding et al., 2005, 2006; Honda et al., 2005; Shan and Lin, 2006; Lovell and Markesbery, 2008) and in the CSF (Abe et al., 2002) MCI: increased levels in the brain (Ding et al., 2005; Lovell and Markesbery, 2008)
Biomarkers of protein oxidation/ nitrosylation	**Main findings**
Protein carbonyls	AD: increased levels in the brain (Hensley et al., 1995; Lyras et al., 1997; Aksenova et al., 1999; Lauderback et al., 2001; Castegna et al., 2002a, 2002b; Choi et al., 2004; Keller et al., 2005; Pamplona et al., 2005; Sultana et al., 2006a, 2006b), plasma and peripheral blood cells (Calabrese et al., 2006; Bermejo et al., 2008) MCI: increased levels in the brain (Keller et al., 2005; Butterfield et al., 2006a) and plasma (Bermejo et al., 2008)
3-Nitrotyrosine (3-NT) and dityrosine	AD: increased levels in the brain (Smith et al., 1997b; Su et al., 1997; Hensley et al., 1998; Luth et al., 2002; Castegna et al., 2003; Sultana et al., 2006e), in the CSF (Hensley et al., 1998; Tohgi et al., 1999; Ahmed et al., 2005), plasma and peripheral blood (Polidori et al., 2004; Calabrese et al., 2006) MCI: increased levels in the brain (Butterfield et al., 2007)
Other biomarkers of protein oxidation: glutamic semialdehyde; aminoadipic semialdehyde; carboxyethyl-lysine; carboxymethyl-lysine	AD: increased levels in the brain (Pamplona et al., 2005)

derive primarily from the non-enzymatic peroxidative degradation of unsaturated lipids (Kadiiska et al., 2005). Thiobarbituric acid-reactive substances (TBARS) mainly measure the level of MDA since MDA reacts with thiobarbituric acid (TBA) to form the MDA–TBA adduct (Gutteridge, 1982). TBARS assessment by spectrophotometric assay has been widely used to quantify lipid peroxidation, but it has been criticized due to its lack of specificity, sensitivity, and reproducibility (Pincemail et al., 1996). The direct assessment of MDA by high-performance liquid chromatography (HPLC) is more specific but this approach does not deduce all the limitations of this biomarker (Gutteridge and Halliwell, 1990; Gotz et al., 1993; Sultana et al., 2006d); HPLC is also used to assess the MDA–TBA complex (Lepage et al., 1991).

Another product of free radical damage to lipids is *trans-4-hydroxy-2-nonenal* (HNE), which is one of the more toxic products of lipid peroxidation. MDA and HNE are able to covalently modify proteins, thus affecting their function, and MDA can lead to the formation of immunogenic MDA-modified peptide adducts (Stocker and Keaney, 2004; Butterfield et al., 2006b). MDA can impair mithocondrial respiration, inhibiting the activity of several mithocondrial enzymes (complexes I, II, V, pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, superoxidodismutase) and promoting ROS generation (Long et al., 2008). This lead to neuronal mitochondrial damage, which is believed to be a major contributor to aging and neurodegenerative diseases (Reddy and Beal, 2008). HNE can interfere with the synthesis of DNA, RNA and proteins, alter cell metabolism and signalling, and mediate Aβ-induced oxidative damage. Some studies suggest that MDA and HNE can promote the degeneration of cholinergic neurons and Aβ aggregation (Mark et al., 1995; Keller et al., 1997; Pedersen et al., 1999; Lauderback et al., 2001; Butterfield et al., 2006b; Chen et al., 2007; Siegel et al., 2007), and there is also evidence that increased lipid peroxidation can promote amyloidogenesis through the up-regulation of β-site APP cleavage enzyme 1 (BACE1) (Chen et al., 2008). *Acrolein* is the most reactive aldehydic product derived from the metal-catalyzed oxidation of polyunsaturated fatty acids, including arachidonic and docosahexanoic acids. Due to its high reactivity, acrolein can promote the formation of protein adducts, thus inhibiting protein functions, and this can promote presynaptic damage (LoPachin et al., 2008). Acrolein preferentially reacts with lysine residues, which are prominent components of tau protein, and the presence of acroilein adducts has been described in neurofibrillary tangles (Calingasan et al., 1999). In vitro data has shown that acrolein can induce tau oligomerization, thus promoting the production of aggregates of paired helical filaments. The process is accelerated if tau is phosphorylated, suggesting that acrolein can promote neurofibrillary tangles formation (Kuhla et al., 2007). Furthermore, acrolein can modify DNA and promote lipid peroxidation (Esterbauer et al., 1991; Uchida et al., 1998; Lovell et al., 2000a).

Different studies have demonstrated significantly increased levels of MDA and TBARS in AD brains compared to controls, especially in regions where NFT and SP are more prominent (e.g., temporal lobe, hippocampus, pyriform cortex, and amygdala) (Subbarao et al., 1990; Balazs and Leon, 1994; Palmer and Burns, 1994; Lovell et al., 1995; Marcus et al., 1998; Keller et al., 2005). Two studies failed to find differences in levels of MDA-TBA adducts, assessed by HPLC, in different brain regions of AD subjects, as compared to controls (Hayn et al., 1996; Lyras et al., 1997). Other studies have revealed that, even if basal brain levels of TBARS were not different between AD brains and controls, there was an increased TBARS production in the AD brain after incubation with pro-oxidant substances, suggesting that AD subjects are more vulnerable to lipid oxidation (Hajimohammadreza and Brammer, 1990; McIntosh et al., 1997). Although the concentration of TBARS in the hippocampus of AD patients was not increased compared to controls, in one study it was related to APOE genotype, since in AD patients the highest TBARS levels were found in APOE ε4 carriers, thereby suggesting that APOE genotype affects the extent of the oxidative stress-induced damage and that the increased risk to develop AD in ε4 carriers could be, at least in part, mediated through an increased susceptibility to lipoperoxidation (Ramassamy et al., 2000).

Increased levels of free HNE (Markesbery and Lovell, 1998) and HNE–protein adducts (Montine et al., 1997; Sayre et al., 1997b) have been described in the brains of AD patients compared with control subjects; HNE particularly accumulated in regions affected by neurodegenerative processes, including the amygdala, hippocampus and parahippocampal gyrus. Volkel et al. (2005, 2006) have developed a sensitive method, based on mass spectrometry (MS) analysis, with which they demonstrated increased levels of HNE–glutathione conjugates in different brain regions affected by neurodegeneration in AD patients, compared to control samples. Another research group used MS analysis to show increased HNE and acrolein levels in the hippocampus, parahippocampal gyrus, superior and middle temporal gyrus of subjects with early AD, compared to control subjects (Williams et al., 2005, 2006). The presence of increased levels of acrolein-protein adducts in the AD brain has also been demonstrated in earlier studies (Calingasan et al., 1999; Lovell et al., 2001). The levels of these biomarkers may be altered by increased production and decreased metabolism: indeed the activity of some of the major metabolizing enzymes for these products of lipid peroxidation is altered in the diseased brain regions of subjects with AD. These data suggest that the detoxification of lipid-aldehydes could be impaired in AD, but conclusive data are not yet available (Picklo and Montine, 2007). Free HNE has also been assessed in ventricular CSF from patients with AD, and its levels were found to be significantly elevated compared to age-matched controls, while no differences were detected in HNE–protein adducts (Lovell et al., 1997). Free *carbonyl-crotonaldehyde* (CRA) is an highly reactive aldehydic intermediate, able to cause carbonylation, and then dysfunction, of a wide range of cellular proteins; CRA also promotes glutathione (GSH) depletion and DNA damage (Esterbauer et al., 1991; Reddy et al., 2002; Luczaj and Skrzydlewska, 2003). CRA has been recently described as a product of lipid peroxidation which is increased in the hippocampal region of AD brain, compared to control subjects; CRA mainly accumulates in glial cells, where it promotes the formation of CRA-protein adducts (Kawaguchi-Niida et al., 2006).

Biomarkers of lipid peroxidation have also been explored in blood with conflicting results. Different authors have demonstrated increased levels of TBARS in serum/plasma (Ozcankaya and Delibas, 2002; Polidori and Mecocci, 2002; Aybek et al., 2007), and in blood cells (erythrocyte) (Serra et al., 2001; Kawamoto et al., 2005) from AD subjects, compared to controls, whereas other studies did not confirm these findings (Ahlskog et al., 1995; Ceballos-Picot et al., 1996; Fernandes et al., 1999; Baldeiras et al., 2008). Polidori and Mecocci (2002) showed that not only was the TBARS plasma level higher in AD subjects than in age-matched controls, but plasma exposure to a free radical generator promoted a greater production of TBARS in AD patients. This was also associated with an increased rate of consumption of plasma antioxidants (e.g. ascorbate), thereby suggesting an enhanced plasma susceptibility to oxidative damage in AD patients.

Free MDA has been reported in various studies to be higher in plasma and erythrocyte from subjects with AD, compared to controls (Bourdel-Marchasson et al., 2001; Casado et al., 2008; Martin-Aragon et al., 2009), while other investigations have failed to show any differences in serum MDA in AD subjects (Sinclair et al., 1998; McGrath et al., 2001). Cecchi et al. (2002) found increased levels of MDA and HNE in peripheral cells (skin fibroblasts and lymphoblasts) derived from patients with familial AD, carrying APP and presenilin-1 (PS-1) gene mutations; in the same study no differences in MDA and HNE levels were identified between sporadic AD and control subjects. There are also conflicting results regarding HNE: some authors have demonstrated elevated plasma levels of HNE in AD subjects, compared to controls (McGrath et al., 2001; Calabrese et al., 2006), while others did not observe any difference (Sinclair et al., 1998).

The presence of lipid peroxidation has also been investigated in subjects with MCI. Keller et al. (2005) have shown increased levels of TBARS and MDA in the superior and middle temporal gyri of subjects with amnestic MCI, and other studies have demonstrated increased HNE levels in the hippocampus, inferior parietal lobule, temporal lobe and cerebellum of patients with amnestic MCI (Butterfield et al., 2006b; Williams et al., 2006). Williams et al. (2006) have revealed that acrolein levels were also significantly elevated in the superior and middle temporal gyri in MCI subjects; both HNE and acrolein were increased compared with healthy controls, and there was no difference between MCI and early AD subjects, which were analyzed in the same study (Williams et al., 2006).

A few of the above cited studies documenting the presence of increased levels of lipid oxidative damage in AD and MCI, both in the CNS and peripheral fluids, may have shortcomings that need to be taken into account. First, the amount of lipid peroxidation products is affected by several physiological and non-physiological conditions, such as lifestyle: acrolein and crotonaldehyde are largely present in cigarette smoke (Nath et al., 1998), and lipid hydroperoxides and aldehydes can also be absorbed from the diet and excreted in urine, in turn influencing MDA and HNE assessment in plasma (Wilson et al., 2002). Secondly, comorbidity is another element that needs to be considered: levels of TBARS, MDA and HNE are increased in the presence of diabetes (Ahlskog et al., 1995; Martin-Gallan et al., 2003), chronic renal failure (Siems et al., 2002; Dirican et al., 2007), cardiovascular diseases (Uchida, 2000; Nakamura et al., 2005; Sathiyapriya et al., 2007), and cancer (Bartsch and Nair, 2005; Kosova et al., 2007; Nayak and Pinto, 2007). In conclusion, the value of these biomarkers when studying MCI and AD, is strictly linked to the possibility of taking into account potential confounders (e.g. diet, smoking, comorbidity). Furthermore, it is also important to consider more than one biomarker in order to improve the identification of changes in the status of lipid peroxidation as related to neurodegeneration.

4.2. Isoprostanes, neuroprostanes and neurofurans

Of the *isoprostanes*, F_2-Isoprostanes (F_2-IsoPs) have been extensively studied as biomarkers of lipid oxidative damage. They are considered as ideal candidates for the accurate quantitative assessment of OS status and lipid peroxidation due to their chemical stability in-vivo and ex-vivo, and minimal metabolism in-situ (Roberts and Morrow, 2002; Basu, 2004; Halliwell and Whiteman, 2004; Montuschi et al., 2004; Morrow, 2005). F_2-IsoPs include 64 different products containing an F-type prostane ring, generated in-vivo by the non-enzymatic free radical-mediated peroxidation of esterified arachidonic acid (AA), which is distributed throughout the white and grey matter of CNS; F_2-IsoPs are thereafter cleaved and released into the circulation by phospholipases before excretion in the urine as free isoprostanes (Morrow et al., 1992; Montine et al., 2007). F_2-IsoPs can be measured by different techniques: MS methods [gas chromatography–MS (GC–MS); GC–tandem-MS (GS–MS/MS), liquid chromatography MS (LC–MS), LC–MS/MS] which are considered the gold standard, and immunological methods [radioimmunoassay (RIA) and enzyme immunoassay (EIA)]. There is still little information regarding the precision and accuracy of these immunological techniques, but this area of research is expanding since these methods are more easily available than MS (Dalle-Donne et al., 2006; Montine et al., 2007).

Increased levels of F_2-IsoPs have been detected in different brain regions of patients with AD compared to controls: the frontal, parietal and temporal lobes and hippocampus are involved, while the level of F_2-IsoPs in the cerebellum (a region rarely affected by AD) was not found to increase (Praticò et al., 1998; Reich et al., 2001; Yao et al., 2003; Markesbery et al., 2005; Forman et al., 2007). One study has demonstrated the specific cellular localization of F_2-IsoPs which mainly accumulate in neuronal cells. Other cellular types like glia are involved in the build up of F_2-IsoPs but the main localization in neurons suggests that lipid peroxidation takes part in the pathogenetic process leading to neurodegeneration, being not merely a simple marker of reactive gliosis occurring late on in the disease (Casadesus et al., 2007).

Average levels of F_2-IsoPs were also found increased in post-mortem ventricular CSF obtained from AD subjects, compared to age-matched controls (Montine et al., 1998; Praticò et al., 1998). Montine et al. (1999b) observed that the concentration of ventricular CSF F_2-IsoPs in patients with AD significantly correlated with indices of neurodegeneration, such as a reduction in brain weight and degree of cortical atrophy, suggesting that brain lipid peroxidation is associated with the progression of AD. Studies in-vivo have confirmed the presence of increased levels in CSF F_2-IsoPs in AD subjects, compared to age-matched controls. In these studies F_2-IsoPs were measured in lumbar CSF, where they were lower compared to ventricular CSF, probably due to a rostrocaudal gradient and an earlier stage of the disease in patients enrolled in the in-vivo CSF assessment, while post-mortem studies were conducted on patients with an advanced stage of the disease (Montine et al., 1999a,c; Praticò et al., 2002). The concentration of CSF F_2-IsoPs seems to correlate with clinical severity (Praticò et al., 2000) and other biomarkers of the disease, like Aβ42 and the concentration of tau in CSF (Praticò et al., 2000; Montine et al., 2001). This suggests that, even if the role of biochemical markers in diagnosing AD has not yet been well-defined, the quantification of CSF F_2-IsoPs together with other biomarkers may enhance the accuracy of the laboratory identification of AD (Montine et al., 2001). CSF isoprostanes are potentially useful for detecting early AD, representing an index of brain oxidative damage which could be helpful for monitoring the course of neurodegeneration and quantifying the effectiveness of experimental therapeutics, like antioxidant treatments. Indeed, Quinn et al. (2004) demonstrated a longitudinal increased in CSF F_2-IsoPs in AD patients and, in the same study, they found that α-tocopherol supplementation, only if associated with vitamin C intake, was able to reduce the progressive raise in CSF F_2-IsoPs in those subjects. However, more studies are required to confirm these data and to evaluate if a reduction in the levels of CSF isoprostanes is related to a delay in the neuropathological and clinical progression of AD.

The quantification of isoprostanes in the plasma and urine of AD patients has yielded conflicting results. Praticò et al. (2000, 2002) have found elevated levels of F_2-IsoPs in the urine and blood of AD patients, that were correlated with the concentration of CSF F_2-IsoPs. Other groups of researchers have confirmed the presence of increased isoprostanes urinary levels in AD (Tuppo et al., 2001; Kim et al., 2004), but further studies by other researchers did not (Waddington et al., 1999; Bohnstedt et al., 2003). Another laboratory showed an increase in the levels of plasma isoprostanes in AD (Waddington et al., 1999), unconfirmed by other researchers (Feillet-Coudray et al., 1999; Irizarry et al., 2007). Finally, Montine et al. (2000, 2002) observed no difference in levels of plasma and urinary F_2-IsoPs in AD compared to controls; methodological differences could explain these conflicting results. Furthermore, multiple physiological and pathological processes influence the concentration of isoprostanes in blood and urine (e.g. diet, physical activity, smoker or non-smoker, cardiovascular diseases, diabetes). Accordingly, it is necessary to control for confounding when analysing the level of isoprostanes in blood and urine (Morrow et al., 1995; Dalle-Donne et al., 2006).

F_2-IsoPs have also been investigated in MCI and increased levels of F_2-IsoPs have been documented in different brain regions of MCI subjects in comparison to cognitively normal individuals (Markesbery et al., 2005). These results were not confirmed in a recent study by Forman et al. (2007), who have shown higher levels of F_2-IsoPs in the brains of AD patients in comparison to controls, but no difference between MCI and, respectively, AD or cognitively normal subjects. Interestingly, in this study F_2-IsoPs levels were related to both senile plaque and the burden of NFT, but not to clinical diagnosis. The association of isoprostanes level with neuropathological hallmarks, like plaques and tangles, suggests that lipid peroxidation accurately reflects the burden of AD pathology. Another study has revealed no difference in brain levels of F_2-IsoPs among fronto-temporal dementia cases and controls (Yao et al., 2003). Since MCI is a heterogeneous clinical entity that may include not only prodromal AD but also the preclinical stage of other types of dementia (Winblad et al., 2004), these results suggest that lipid peroxidation could be a key feature in AD. Thus, it is possible that a significant increase in lipid peroxidation could be detected only in MCI cases which represent prodromal AD.

This hypothesis is supported by studies which identified increased CSF F_2-IsoPs in subjects with MCI, compared to age-matched controls (Praticò et al., 2002; de Leon et al., 2004, 2007). Particularly, a small longitudinal study showed that not only isoprostanes were increased in CSF of MCI subjects, but there was also a significant raise in levels of F_2-IsoPs in this group after 12 months, compared to cognitively normal subjects (de Leon et al., 2004). Another study found that the rate of increase of CSF F_2-IsoPs was higher in MCI subjects who converted to AD compared to healthy controls and stable MCI (Brys et al., 2009). de Leon et al. (2006, 2007) confirmed that a longitudinal evaluation of CSF F_2-IsoPs could predict future AD development in amnestic MCI, and the decline to MCI in cognitively normal subjects: in both cases there was a higher rate of increase in CSF F_2-IsoPs compared to stable subjects. In these studies it has also been also shown that isoprostane assessment in CSF, together with other clinical markers [neuropsychological evaluation; hippocampal atrophy detected with Magnetic Resonance Imaging; CSF levels of phosphorylated tau (P-tau231)], increases the accuracy of diagnosing MCI. The plasma or urinary assessment of F_2-IsoPs would be easier for the longitudinal monitoring of MCI subjects, but again, as demonstrated in AD subjects, the data on plasma and urinary F_2-IsoPs in MCI are inconsistent. One study showed an increase in the levels of F_2-IsoPs in plasma and urine in MCI patients compared with control subjects (Praticò et al., 2002), but another study did not confirm these findings (Irizarry et al., 2007).

Other compounds studied in AD and MCI as biomarkers of lipid peroxidation are *neuroprostanes*, particularly F_4-neuroprostanes (F_4-NPs). Neuroprostanes are F_2-IsoPs-like molecules, and they are stable products of free radical damage to docosahexanoic acid (DHA). Their name derives from the high levels of DHA in the brain, particularly in the grey matter where it comprises approximately 25–35% of the total amount of fatty acids in aminophospholipids (Skinner et al., 1993). DHA is synthesized mainly by astrocytes, after which it is secreted and taken up by neurons (Moore et al., 1991). F_4-NPs increase in different brain regions of subjects with AD and MCI compared to controls (Nourooz-Zadeh et al., 1999; Reich et al., 2001; Markesbery et al., 2005), and there is also evidence that neuroprostanes could inhibit the proteasome function in AD, thus impairing the clearance of oxidized proteins (Cecarini et al., 2007). Once formed, F_4-NPs can undergo hydrolysis thereafter being released into biological fluids. A small study found that CSF F_4-NPs levels in patients with AD were significantly higher than age-matched control subjects (Roberts et al., 1998).

Finally, *neurofurans* (nFs), are a novel family of lipid peroxidation biomarkers, comprising DHA-derived peroxidation products containing a substituted tetrahydrofuran ring (Arneson and Roberts, 2007). Preliminary studies showed increased levels of nFs in the brain cortex of a mouse model of Alzheimer's disease, thus suggesting that nFs may be useful biomarkers for this neurodegenerative disorder (Song et al., 2008).

5. Nucleic acids

Nucleic acids [nuclear DNA (nDNA), mitochondrial DNA (mtDNA), and RNA] are one of the cellular macromolecules damaged by free radicals. Mitochondrial DNA is more susceptible to OS/NS compared to nDNA (Barja, 2004). This is due to: (i) its lack of protective histones; (ii) its high information density, due to the absence of introns; (iii) its close proximity to the inner mitochondrial membrane, where ROS are generated; and (iv) the presence of limited repair mechanisms (Clayton et al., 1974; Linnane et al., 1989; Ames et al., 1993; Mecocci et al., 1994; Wallace, 2005; LeDoux et al., 2007).

Neurons are highly differentiated, post-mitotic cells, and they survive as long as the organism does. Thus, the oxidative/nitrosative modifications of RNA, DNA and, particularly, mitochondrial DNA, are thought to play a key role in the selective neuronal loss associated with aging and neurodegeneration (Barzilai, 2007; Moreira et al., 2008). In AD and in MCI, an accumulation of oxidative/nitrosative damage in nucleic acid is observed in the brain and in peripheral tissues, indicating an increased level of oxidative/nitrosative stress and/or a decreased capacity to repair the damage. Several groups of researchers have investigated the DNA repair mechanisms in AD patients, with conflicting results. Numerous studies have confirmed a decrease in DNA repair efficiency in AD (Parshad et al., 1996; Lovell et al., 2000b; Iida et al., 2002; Morocz et al., 2002; Jacobsen et al., 2004; Weissman et al., 2007; Shao et al., 2008) and also in subjects with MCI (Shao et al., 2008), whereas others did not (Kinsella et al., 1987; Edwards et al., 1989).

5.1. DNA damage

ROS, and mainly the hydroxyl radical ($^{\bullet}$OH), can react with all components of the DNA molecule (the purine and pyrimidine bases and the deoxyribose backbone), causing different kinds of damage, such as base or sugar lesions, single-strand breaks, abasic sites formation, and DNA–DNA or DNA–protein cross-links (Halliwell and Gutteridge, 1999; Dizdaroglu et al., 2002). DNA can also undergo nitrosative/nitroxidative damage by RNS, which may promote nitration and deamination of purines (Malinski, 2007). DNA oxidation/nitroxidation can produce mutations and impair transcriptional and post-transcriptional pathways, thus compromising protein synthesis (Colurso et al., 2003). Damage to nDNA and mtDNA may promote neuron death through defects in oxidative phosphorylation and cell metabolism (Moreira et al., 2008). There is also evidence that in neurons DNA damage could induce an abortive re-entry in the cell cycle, leading to apoptosis (Becker and Bonni, 2004; Kruman, 2004; Fishel et al., 2007).

DNA injury has been investigated in AD and MCI subjects, starting with the analysis of DNA strand breaks, which may be an expression of DNA oxidation. Subsequent studies have assessed the presence of specific oxidized DNA bases and at least 20 different bases adducts derived from DNA oxidation have been described (Dizdaroglu et al., 2002). The most investigated DNA adduct is 8-hydroxy-2′-deoxyguanosine (8-OHdG) which derives from guanine which, being the DNA base with the lowest oxidation potential, is readily oxidized (Lovell and Markesbery, 2007). DNA damage has been evaluated with several techniques: immunoassay; Comet assay; capillary electrophoresis; HPLC; GC–MS; and LC–MS. MS methods permit the identification of a wide range of base adducts in a single run, thus giving a more complete picture of DNA oxidation, but this techniques can be skewed by the artefactual oxidation of DNA (Lyras et al., 1997; Lovell and Markesbery, 2007). Methods to assess purine nitration and deamination have not yet been completely validated and they are not extensively used in studies on humans (Halliwell and Whiteman, 2004; Dalle-Donne et al., 2006).

Several post-mortem studies have revealed significant DNA fragmentation (strand breaks) in the brain of AD subjects compared to non-demented controls. An at least two-fold increase in DNA strand breaks has been detected in the brain cortex of patients with AD (Mullaart et al., 1990) and many investigators have confirmed a higher presence of neuronal DNA fragmentation in those areas which are more vulnerable to neurodegeneration, such as the temporal isocortex, hippocampus, midfrontal gyrus, and entorhinal cortex (Su et al., 1994; Anderson et al., 1996; Li et al., 1997; Lucassen et al., 1997; Sugaya et al., 1997; Stadelmann et al., 1998; Adamec et al., 1999; Colurso et al., 2003). Su et al. (1997) have demonstrated that the build-up of DNA strand breaks in AD brain was associated with increased levels of nitrotyrosine, a marker of protein nitroxidative damage, and they suggested that peroxynitrite could promote DNA damage.

Mecocci et al. (1993) were the first group to demonstrate a progressive age-related accumulation of 8-OHdG in the cerebral cortex of cognitively normal subjects, aged 42–97 years. The amount of 8-OHdG was higher in mtDNA, as compared with nDNA, and the author proposed that such damage could contribute to an age-dependent increase in the incidence of neurodegeneration. This hypothesis was reinforced in a subsequent study, in which Mecocci et al. (1994) provided preliminary proof regarding increased DNA oxidation in AD by assessing 8-OHdG. Using HPLC assay, the research group detected elevated levels of 8-OHdG in post-mortem brain tissue from AD subjects, compared to age-matched controls. The 8-OHdG build-up was significantly higher in mtDNA compared to nDNA, both in cases and controls. There was a threefold increase in oxidative damage to mtDNA in the AD samples, compared to controls, and the most marked changes were in the parietal cortex; the levels of 8-OHdG in nDNA were slightly increased in AD in comparison with normal subjects, but the difference was not significant.

Nunomura et al. (1999), by means of an immunocytochemical assay, have confirmed the presence of elevated 8-OHdG levels in neurons from AD subjects, compared to healthy controls. This increase involved brain areas typically affected by AD neurodegeneration: the hippocampus, subiculum, entorhinal cortex, frontal, temporal and occipital neocortex, while the level of 8-OHdG in the cerebellum displayed no differences between AD and controls. Further, the presence of oxidized nucleosides was inversely related to NFT content, thereby suggesting that DNA oxidation could precede lesion formation. There exist only two studies which detected unaltered levels of 8-OHdG in AD brain, compared to controls using an HPLC assay (Te Koppele et al., 1996; Seidl et al., 1997). Increased levels of 8-OHdG have also been demonstrated in intact DNA which was extracted from the ventricular CSF of patients with AD, compared to controls. This was concurrent with a depletion of free 8-OHdG, a marker of DNA repair activity, thus suggesting that AD is characterized by increased ROS production and decreased DNA repair capacities (Lovell et al., 1999; Lovell and Markesbery, 2001).

The higher level of DNA oxidation in the AD brain has been confirmed by other research groups who, using MS methods, assessed different products of DNA damage, arising from all four DNA bases: 8-OHdG, that has been confirmed to be the main product of DNA oxidation (Gabbita et al., 1998; Wang et al., 2005); 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy-guanine), a degradation product of 8-OHdG; 8-hydroxyadenine (8-OHA); 4,6-diamino-5-formamidopyrimidine (Fapy-adenine), a degradation product of 8-OHA; 5-hydroxycytosine (5-OHC); and 5-hydroxyuracil (5-OHU), a degradation product of cytosine, and thymine glycol (Lyras et al., 1997; Gabbita et al., 1998; Wang et al., 2005). Lyras et al. (1997) used GC–MS to demonstrate a generalized increase in DNA oxidative damage in the AD brain. They found increased levels of various (8-OHdG, 8-OHA, thymine glycol, Fapy-guanine, 5-OHC, 5-OHU, and Fapy-adenine), but not all, oxidized DNA bases, in the parietal, temporal, occipital, and frontal lobes, superior temporal gyrus, and hippocampus. The damage was higher in the parietal lobe, where a significant statistical difference between AD and controls on 8-OHdG, 8-OHA and 5-OHC was identified, but the level of DNA oxidative damage in the temporal lobe was higher than in other brain regions in controls and the AD brain. Lovell and co-workers have confirmed these findings: using GC–MS they evaluated nDNA damage, showing increased levels of 8-OHdG, 8-OHA, 5-OHC, and 5-OHU in the neocortex of AD brain. They also demonstrated that the cerebellum, which is usually spared in AD, was fairly unaffected by DNA oxidation. The increase in each biomarker of nDNA oxidative damage was dissimilar in different brain regions, but overall the number of oxidatively modified bases was remarkably higher in AD temporal, parietal and frontal lobes, compared to age-matched controls. As with Lyras' study, the temporal lobe was found to be the principal target of OS; in this region the ratio of oxidatively modified bases per number of bases was 1:600 in AD and 1:2100 in controls. Specifically, it was 1:900 in AD *vs* 1:2800 in controls, and 1:700 in AD *vs* 1:2400 in controls in parietal and frontal lobe respectively (Gabbita et al., 1998).

More recently, Lovell's group corroborated these results in a study, where they also demonstrated that mtDNA had approximately 10-fold higher levels of oxidized bases than nDNA, thus suggesting higher levels of OS in mitochondria (Wang et al., 2005). Furthermore, the author performed the same analysis in subjects with amnestic MCI, revealing statistically significant elevations of 8-OHdG in the nDNA of frontal and parietal lobes, and the mtDNA from temporal lobes, compared to normal control subjects. They also observed higher levels of 8-OHA, 5-OHC and Fapy-adenine in nDNA, and Fapy-adenine in mtDNA from all three neocortical regions of MCI compared to controls. DNA oxidation was higher in mtDNA than in nDNA, and no differences were observed between MCI and controls in the amount of DNA oxidation in the cerebellum. Interestingly, levels of DNA base adducts in MCI were not significantly different from those observed in AD subjects, suggesting that DNA oxidation is an early event in the pathogenesis of neurodegeneration in AD (Wang et al., 2006).

In subjects with AD, the biomarkers of DNA oxidative damage derived from the interaction between nucleic acid and products of lipid peroxidation, such as acrolein and HNE, have been also investigated. An attack on DNA by these aldehydic compounds leads to the formation of bulky exocyclic adducts, which can promote DNA–DNA and DNA–protein cross linking and impair transcription factors binding (Kozekov et al., 2003; Liu et al., 2006; Lovell and Markesbery, 2007). Liu et al. (2005) have shown a statistically significant two-fold increase in the mean level of acrolein-deoxyguanosine adduct in nDNA, isolated from the hippocampus of eight AD subjects, compared to age-matched controls. In contrast, levels of the HNE–deoxyguanosine adduct in nDNA from the inferior parietal lobule and hippocampus of AD did not significantly differ compared to control subjects (Liu et al., 2006). These findings are consistent with earlier studies of Gotz et al. (1993), who showed no significant differences in the HNE–deoxyguanosine adduct in nDNA isolated from the hippocampus, parietal cortex and cerebellum of AD subjects, in comparison with controls.

Increased levels of DNA oxidation have been demonstrated not only in the CNS, but also in the peripheral tissues of AD individuals, suggesting the systemic nature of oxidative damage in AD, even though most of the pathological changes are in the brain. Using HPLC analysis, Mecocci et al. (1998, 2002) have observed in AD a significantly higher lymphocyte concentration of 8-OHdG at DNA level, compared to age-matched controls. Moreover, there was a significant inverse relationship in AD patients between lymphocyte 8-OHdG content and the plasma level of several non-enzymatic antioxidants (mainly carotenoids), which were significantly depleted in comparison to control subjects. The increased presence of markers of DNA oxidation (in addition to CNS) has been confirmed by different authors, who applied a modified version of the Comet assay (single-cell gel electrophoresis) for the detection of oxidised purines and pyrimidines in the peripheral lymphocytes and leukocytes of AD patients, and the same findings have also been demonstrated in MCI subjects (Morocz et al., 2002; Kadioglu et al., 2004; Migliore et al., 2005b). One study investigated the presence of urinary oxidized nucleosides in AD subjects, showing increased levels compared to control individuals (Lee et al., 2007). Many authors agree that oxidized purines and pyrimidines in lymphocyte DNA, whilst useful, cannot be used as a unique diagnostic biomarker for AD, since there is an overlap in the levels of purine and pyrimidine oxidation between AD and controls; increased DNA oxidation is not specific to AD but it is also present in other neurodegenerative conditions, such as Parkinson's disease and amyotrophic lateral sclerosis (Migliore et al., 2005a).

5.2. RNA damage

RNA is more vulnerable to oxidative damage than DNA, probably because (unlike DNA) RNA is mostly single-stranded and its bases are not protected by hydrogen bonding; furthermore, RNA is not covered with protective histones (Fiala et al., 1989; Wamer and Wei, 1997; Bregeon and Sarasin, 2005). RNA molecules are intermediaries in the transfer of genetic information from DNA to proteins, and they are of importance in regulating gene expression. Oxidative injury to RNA may interfere with correct base pairing, compromise the accuracy of transcription and translation, thus prejudicing normal protein synthesis; it could also promote protein aggregation (Nunomura et al., 1999; Shan et al., 2003; Bregeon and Sarasin, 2005; Szymanski et al., 2005). Studies on neuronal cultures have indicated that messenger RNA (mRNA) oxidation may be an important factor initiating the cascade of neurodegeneration (Shan et al., 2007). An analysis of RNA oxidative damage has been performed on the CNS (brain tissue, CSF) and serum of patients with AD. RNA species are easily attacked by the hydroxyl radical (•OH) and several studies have evaluated 8-hydroxyguanosine (8-OHG) as a marker of hydroxyl radical damage to RNA (Fiala et al., 1989).

Nunomura et al. (1999) demonstrated a marked accumulation of 8-OHG from cytoplasmic RNA using a semiquantitative immunohistochemical analysis in the neurons of patients with AD, particularly in vulnerable brain areas, such as the hippocampus, and frontal, temporal and occipital neocortex. Neuronal 8-OHG immunoreactivity displayed a significant negative correlation with the duration of the illness and the extent of Aβ deposition, thereby suggesting that RNA oxidative damage preceded pathological changes in AD (Nunomura et al., 2001). The same research team also demonstrated increased levels of 8-OHG in the frontal cortex of subjects with familial AD, due to a mutation in the PS-1 or the APP gene (Nunomura et al., 2004). Increased 8-OHG levels of RNA in AD brains have been confirmed in other studies, in which different assessment methods were used. Shan et al. detected that approximately 30–70% of mRNA was oxidized in the frontal cortex of subjects with AD, compared to 2% of the mRNA in age-matched controls, while no differences were observed in the cerebellum (Shan et al., 2003; Shan and Lin, 2006). Oxidation appeared not to be a random but a selective process: of the mRNA species, the most susceptible to OS were identified as transcripts of genes involved in free radical modulation, detoxification, cell death pathway, signal transduction, synaptic plasticity, long-term potentiation, and cell proliferation. Several oxidized mRNA coded for proteins which are believed to be implicated in AD pathogenesis, such as PS-1, peptides involved in free radicals regulation and detoxification (e.g. Cu/Zn Superoxide dismutase; Carbonyl reductase 1), cell metabolism (e.g. Calpain), and signal transduction (e.g. MAPK kinase 1) (Shan et al., 2003; Shan and Lin, 2006).

Ribosomal RNA (rRNA) in neurons is more abundant compared to mRNA, and rRNA easily binds to redox-active iron, which promotes rRNA oxidation through the Fenton reaction (Honda et al., 2005). In hippocampal neurons from AD cases, Honda et al. have demonstrated a significant increase in redox active iron which in cytoplasm was mainly associated with rRNA, thus suggesting that redox active iron plays a pivotal role in rRNA oxidation. Furthermore, increased levels of 8-OHG were detected in the same cells and, of the RNA species, the greatest amount of 8-OHG was detected in rRNA (Honda et al., 2005). Ding et al. (2005, 2006) found increased RNA oxidation in brains from AD and MCI subjects, mainly in the superior middle temporal gyri and inferior parietal lobule, while no alteration in 8-OHG levels was detected in the cerebellum. Increased RNA oxidative damage mainly affected rRNA and it was associated, both in AD and MCI, with a significant depletion of rRNA and RNA transfer (tRNA), impairment in ribosome function and decreased protein synthesis. On the basis of these results, the authors have hypothesized that an impairment in protein production, mediated in part by OS, could promote the onset and development of AD. Another research group investigated RNA oxidation by assessing 8-OHG and 1-N2-propanodeoxyguanosine (NPrG) (an adduct formed between guanine and acrolein) in the hippocampus and parahippocampal gyrus from amnestic MCI, late stage AD, and control subjects. Increased levels of 8-OHG and NPrG were demonstrated in MCI and AD parahippocampal gyrus, compared to controls, and they were accumulated primarily in neurons with conformational alterations of tau; the latter typically precedes neurofibrillary tangles formation. These findings, together with the comparable intensity of RNA oxidation in MCI and AD, led the authors to conclude that RNA oxidative damage may be an early event in AD (Lovell and Markesbery, 2008).

Abe et al. (2002) have measured 8-OHG levels in the CSF and serum of patients with AD, investigating their relationships with the duration and severity of dementia. The concentration of 8-OHG in CSF from AD patients was approximately fivefold compared with controls, and it decreased significantly with the duration of the illness and the progression of cognitive dysfunctions, suggesting that RNA oxidation is an early event in AD. The level of 8-OHG in serum was not significantly altered in AD patients compared to controls, and there was no correlation between CSF and serum levels of 8-OHG in both AD cases and controls.

In conclusion and drawing on the above-mentioned studies, it appears that 8-OHG is increased in the CNS of AD subjects, even if RNA oxidation has not been extensively investigated in AD, compared to other biomolecules. Post-mortem studies on AD brains have demonstrated that RNA oxidation is more prominent in cases with a smaller amount of Aβ plaque deposition or a shorter duration of the disease (Nunomura et al., 2001, 2004; Abe et al., 2002); 8-OHG accumulation is more marked in hippocampal neurons free of NFT, compared to neurons with NFT (Nunomura et al., 1999, 2001). Furthermore, 8-OHG is increased in MCI brains, which, at least in part, represent a prodromal stage of AD (Ding et al., 2006; Lovell and Markesbery, 2008). All this data point to 8-OHG as an effective candidate as a biomarker of early AD, or a marker predicting conversion from a prodromal stage to an early stage of AD (Nunomura et al., 2006a).

6. Proteins

Within proteins, all amino acids can be attacked by ROS and RNS, but sulphur-containing and aromatic amino acids are the most susceptible (Stadtman and Levine, 2003). The oxidation of amino acids leads mainly to the formation of *carbonyl* derivates, while peroxynitrite ($ONOO^-$) can nitrate tyrosine groups of proteins and form the stable compound *3-nitrotyrosine*. Another product of protein oxidation is *dityrosine*. Intracellular proteins might also be oxidatively modified via secondary mechanisms resulting from the reactions of free radicals with other cellular constituents, such as lipids, carbohydrates, and nucleic acids (Breusing and Grune, 2008). In addition to the side chain modifications, protein fragmentation and protein cross-linking can also occur. Furthermore, protein oxidation can give rise to other radical species, which can cause damage to other biomolecules. Unlike DNA oxidation products, oxidized amino acids are rarely repaired: mildly oxidized proteins are usually degraded by the 20S proteasome, an intracellular protease present in all cells of the CNS. It is responsible for the degradation of oxidized, aggregated, and misfolded proteins, thus playing an important role in maintaining neuronal homeostasis (Voss and Grune, 2007). Only the easily oxidizable amino acids, cysteine and methionine, can be reduced to the initial form, and their repair is promoted by different enzymes, including the thioredoxin/thioredoxin reductase system and methionine sulphoxide reductase (Breusing and Grune, 2008).

Specifically, it has been suggested that methionine acts as an antioxidant in proteins and peptides, such as Aβ, by scavenging oxidizing species and forming methionine sulphoxide (Hou et al., 2002). This oxidized amino acid is specifically reduced to its native form by methionine sulphoxide reductase (Stadtman et al., 2003). However, this enzyme activity has been reported to decline in the superior and middle temporal gyri and hippocampus of patients with AD, resulting in a loss of antioxidant defence and increase in oxidized methionine residues (Gabbita et al., 1999). Cysteine sulfhydryl groups can be generated from disulfides by cellular reducing agents, such as GSH and protein disulfide isomerase, that reverse the formation of non-native disulfide bridges (Laboissiere et al., 1995). Furthermore, it has been suggested that nitrotyrosine may be repaired by a denitrase enzyme, although this has not been substantiated (Irie et al., 2003). Protein oxidation/nitration results in functional disruption and the cross-linking of proteins by oxidative processes may lead to resistance to intracellular and extracellular removal, even though damaged peptides are extensively ubiquinated. Carbohydrates and oxidized lipids are able to react with oxidized proteins contributing to the process of protein aggregation and inhibiting proteasome degradation (Friguet and Szweda, 1997; Shringarpure et al., 2000; Breusing and Grune, 2008).

Several studies have shown that proteasome activity is impaired in AD and MCI. It has been also demonstrated that proteasome oxidative modifications are increased in AD, and this could contribute to reducing proteasome ability to clearance intracellular protein aggregates (Ding and Keller, 2001; Song and Jung, 2004; Cecarini et al., 2007). Therefore, the accumulation of oxidized/nitrated proteins in AD is a probable consequence of imbalance in any one of a number of different systems, including free radical generation, antioxidant defences or the efficiency of oxidized/nitrated protein repair or removal.

The use of proteins as markers of oxidative/nitrosative stress can offer several advantages as compared with measuring lipid peroxidation or the oxidative base modification of DNA. This is due to the following reasons: (i) proteins play a key role in maintaining cellular structure and functions, thus alterations of the protein structure due to OS/NS may be reflected at functional level, and the change of activity can be assayed; (ii) the products of oxidative/nitrosative modifications of proteins are relatively stable; and (iii) sensitive assays are available for their detection (Chakravarti and Chakravarti, 2007).

Protein carbonyls have been assessed in several investigations. They increase exponentially with age and have been shown to play a role in the pathophysiology of AD. Protein carbonyls may be generated by backbone fragmentation, hydrogen atom extraction at α carbon or an attack on several amino acid side-chains, and by the formation of adducts between some amino acid residues and the products of lipid peroxidation. Protein carbonyls are also produced by glycation/glycoxidation of lysine amino groups, which end with the synthesis of "advanced glycation end products" (AGEs) (Sultana et al., 2006d). Protein carbonyls can be detected with 2,4-dinitrophenylhydrazine (DNPH), and it appears that most of the DNPH-detectable carbonyls found on proteins result from modification by bi-functional reactive aldehyde products of lipid and sugar oxidation.

Most of the studies conducted in the aging brain have concluded that oxidized proteins do accumulate with age. In a study by Smith et al. (1991) the amount of protein oxidation was measured by a general DNPH assay. The results revealed a general logarithmic increase in protein damage with age in the human cerebral cortex, and no significant differences were observed between controls and subjects with AD. In this study not only protein carbonyls levels, but also the activities of creatine kinase and glutamine synthase were measured in the frontal and occipital lobe regions as a function of age. While protein carbonyl content increased with age, the activities of creatine kinase and glutamine synthase decreased, and glutamine synthase activity appeared to be selectively lost in AD brains compared with age-matched controls. In a later study, Aksenova et al. (1999) demonstrated a significant increase in protein carbonyl content in the frontal cortex of subjects with AD; protein carbonyls levels were negatively correlated with creatine kinase activity, which was lower in AD than in controls.

Hensley et al. (1995) evaluated the content of four biomarkers of neuronal protein oxidation, including DNPH-reactive protein carbonyls, in three brain regions (the cerebellum, inferior parietal lobule and hippocampus) of AD and age-matched control subjects. Protein carbonyls were significantly increased in both the hippocampus and the inferior parietal lobule, but unchanged in the cerebellum, a finding which is consistent with the regional pattern of histopathology of AD. Similar results have been found by Lyras et al. (1997), who assessed protein carbonyls in different brain regions of AD subjects and controls. Overall, samples levels of protein carbonyls in AD tended to be increased in the frontal, occipital, parietal, and temporal lobes, middle temporal gyrus, and hippocampus, but a significant difference was found only in the parietal lobe. In another study, increased levels of protein carbonyls were detected in the superior and middle temporal gyri in patients with early-stage AD and MCI compared with normal control subjects, while no significant alteration in protein carbonyl content was detected in the cerebellum of both early AD and MCI subjects, in comparison with controls (Keller et al., 2005).

Pamplona et al. have used GC–MS to analyze the presence of *glutamic semialdehyde*, which is derived from the metal-catalyzed oxidation of proline and arginine, and *aminoadipic semialdehyde*, in turn resulting from lysine oxidation, in the frontal cerebral cortex of brains from AD subjects. Both biomarkers were significantly higher in AD samples than in age-matched controls and the author also demonstrated increased levels of other biomarkers of protein oxidation. The latter include *MDA-adducts*, which are considered lipoxidation products, and *carboxyethyl-lysine/carboxymethyl-lysine*, which have been termed mixed *AGEs-advanced lipoxidation products* (Pamplona et al., 2005). CRA, generated during lipid peroxidation, is highly reactive and it promotes protein carbonylation. One study showed an increased presence of CRA-protein adducts in the hippocampus of AD subjects, compared to age-matched controls, which were preferentially localized in reactive glial cells (Kawaguchi-Niida et al., 2006).

Ortho-tyrosine is another index of protein oxidation, formed by a hydroxyl radical reaction with tyrosine. It has been investigated in AD brain, but no difference in the level of ortho-tyrosine was found in the frontal cortex from AD, compared to controls (Hayn et al., 1996). Peroxynitrite is a source of hydroxyl radical-like reactivity that directly oxidizes proteins, with resultant carbonyls formation from the side-chain and peptide-bond cleavage. Peroxynitrite also causes the nitration of tyrosine residues, thus forming 3-nitrotyrosine (3-NT), which can be used as an index of peroxynitrite action (Crow and Beckman, 1995). Tyrosine nitration has been shown to alter protein functioning, with a change in catalytic activity, cell signalling, and cytoskeletal organization (Schopfer et al., 2003); 3-NT proteins cannot be phosphorylated by tyrosine kinases (Martin et al., 1990). Subsequently, signal transduction mediated by tyrosine kinases – such as trophic factors, nerve growth factors and brain-derived neurotrophic factors – is disturbed and cell apoptosis may occur. In two reports increased 3-NT was detected in the neuronal cytoplasm of AD brains, both in cells containing NFT and in those lacking NFT (Smith et al., 1997b; Su et al., 1997). In these studies nitrotyrosine immunoreactivity was increased in regions of the cerebral cortex affected by neurodegeneration, whereas it was undetectable in the same brain regions of controls. The distribution of nitrotyrosine was essentially identical to the distribution of free carbonyls (Smith et al., 1996).

Data obtained by immunoprecipitation analysis has shown a significant increased level of carbonylation and nitration of the proapoptotic protein p53 in the AD brain. A higher degree of carbonylation of p53 was observed also in the MCI brain, in comparison to controls. The authors also found higher p53 levels in the AD and MCI brain, and increased p53 oxidation by the lipid peroxidation product HNE (Cenini et al., 2008b). p53 plays a key role in oxidative-dependent apoptosis and neuronal death, thus p53 oxidative/nitrosative modifications could be involved in the neuronal loss observed in AD and MCI (Cenini et al., 2008a). This hyphotesis has been reinforced by recent findings from the same research group, showing increased levels of the monomeric and dimeric form of p53 in the AD brain; patients having increased brain level of p53 showed higher level of S-glutathionylation of both p53 monomer and dimer, compared to controls (Di Domenico et al., 2009). Proteins associated with neurodegeneration which are redox regulated through S-glutathionylation have been also showed to be significantly more S-glutathionylated in the brain of subjects with AD, in comparison to controls (Newman et al., 2007). GSH is one of the most important antioxidant in the brain, and S-glutathionylation is a reversible post-translational modification due to GSH reversible binding to protein thiol groups. The role of S-glutathionylation has not been completely clarified, but it seems that this process plays a critical role in sulfhydryl homeostasis and signal transduction. Some studies report S-glutathionylation as a protective mechanisms against permanent oxidative damage of protein, while others describe the role of this post-translational modification in modulating protein functioning. Protein S-glutathionylation increases under condition of oxidative stress, thus contributing to the disturbance of normal cell signalling and potentially promoting neurodegeneration (Mieyal et al., 2008).

Biomarkes of protein oxidative and nitrosative damage have been searched for not only in the brain, but also in CSF. Deploying sensitive HPLC methods, Hensley et al., have demonstrated that 3-NT and dityrosine levels were five to eight fold higher in the hippocampus, neocortex and ventricular CSF of patients with AD, when compared with cognitively normal controls (Hensley et al., 1998). 3-NT concentration and 3-NT/tyrosine ratio were increased six-fold in the lumbar CSF of AD compared with controls of similar age, and both parameters were raised significantly with decreasing cognitive functions (Tohgi et al., 1999). These results were not confirmed when the GC–MS/MS method was applied to analyzing human CSF. Indeed, the investigators demonstrated that the concentration of free 3-NT CSF was considerably lower than that previously reported, and only a few subjects with AD showed increased levels, while the majority of patients had 3-NT levels in the same range as the controls (Ryberg et al., 2004). Any discrepancy between these results is probably due to different methods of sample preparation and analysis. Furthermore, human CSF contains free tyrosine, nitrites and nitrates, thus the in vitro production of 3-NT in CSF samples is possible; regarding HPLC and MS techniques, the results may be altered by the artificial formation of 3-NT.

More recently, the concentration of 3-NT, carboxymethyl-lysine and the oxidized tryptophan moiety, *N-formyl kynurenine*, were found to be significantly elevated in CSF from AD, where the Mini Mental State Examination (MMSE) score revealed an inverse correlation with 3-NT levels. Moreover, after removal of the CSF proteins by means of ultra-filtration techniques, increased levels of free 3-NT were detected, indicating that the degradation of nitrated proteins had occurred (Ahmed et al., 2005).

Protein oxidative and nitrosative damage has also been explored in peripheral tissues. Polidori et al. (2004) have evaluated the carbonyl and dityrosine content in plasma immunoglobulins from patients with AD and control subjects. Immunoglobulin levels of dityrosine but not of carbonyls were shown to be significantly higher in AD as compared to controls, in contrast to a previous report of an increased total amount of oxidatively modified proteins (Conrad et al., 2000); no significant difference in total plasma carbonyls between AD and normal subjects has been identified by other researchers (McGrath et al., 2001; Baldeiras et al., 2008). Calabrese et al. (2006) have demonstrated increased levels of OS and NS in AD subjects in plasma and peripheral lymphocytes, detecting higher levels of protein carbonyls, 3-NT and HNE in AD plasma and lymphocytes, compared to controls. Interestingly, these values were related to decreased levels in the free radical scavenger GSH, an increased level of its oxidized moiety, disulfide oxidized glutathione (GSSG), and lower levels of the GSH/GSSG ratio in lymphocytes from AD patients, in comparison to controls. In lymphocytes from AD there was observed an increased expression of stress-induced proteins, such as the heat shock proteins (HSPs) Hsp72 and Hsp60. Furthermore, the enzymes heme oxygenase (HO-1) and thioredoxin reductase (TRXr), which, have been shown to play a critical role in protecting against oxidative injury, were increased in AD plasma and lymphocytes, in comparison with controls. Similar findings have also been described in another recent study, showing increased plasma concentrations of protein carbonyls in AD and amnestic MCI patients, in comparison with cognitively normal subjects. The degree of protein oxidation was comparable in MCI and AD, and in both groups the GSH/GSSG ratio was significantly decreased, compared to controls (Bermejo et al., 2008).

The oxidative inactivation of enzymes is another index of oxidative damage to proteins, and indeed various studies have demonstrated that glutamine synthase and creatine kinase activities decrease with age and do so more markedly in the AD brain (Smith et al., 1991; Aksenova et al., 1999). Sohal and co-workers, who had initially verified that protein carbonyl content in houseflies was associated with life expectancy, also found that in the same model mitochondrial aconitase, an enzyme involved in the citric acid cycle, is a specific target of OS together with age (Sohal et al., 1993; Yan et al., 1997). The oxidative damage was paralleled by a loss in the catalytic activity of aconitase, which contains an iron-sulphur cluster, thereby rendering it very susceptible to oxidative stress. While the potential serious implications of an impaired activity of aconitase have been elegantly summarized (Shadel, 2005), we recently observed that aconitase activity decreases in lymphocytes and purified mitochondria from AD compared to controls (Mecocci et al., unpublished data). This is of interest not only in light of the possibility of peripherally assessing aconitase as a biomarker of oxidative stress in AD, but also because the decline of aconitase activity in patients with AD markedly resembles that of aconitase activity in subjects with MCI, thus suggesting that mitochondrial dysfunction and oxidative stress have chronological primacy in AD.

A further step in understanding the relationship between the oxidative/nitroxidative modifications of proteins, their functional impairment, and neuronal death in AD has been made by means of the redox proteomic analysis. Protein oxidation/nitroxidation appears not to be a random process but rather it involves specific proteins, which could be more susceptible because of sequence motifs, environmentally-exposed residues, bound ligands, and interactions with redox-metal group. Proteomic approach is an emerging method for the identification of proteins, possibly allowing the screening of a subset of peptides within the brain proteome, which might reflect the extent of OS/NS in AD. The use of proteomics to specifically identify ROS/RNS-modified proteins in the AD brain permits the determination of which peptides are more affected by oxidation and nitration, providing insights into the potential mechanisms of neurodegeneration. Redox proteomics has been used by different laboratories to detect and identify carbonylated and nitrated proteins in the AD brain, and for several peptides there is evidence that they may play an important role in neurodegeneration (Castegna et al., 2002a,b, 2003; Butterfield, 2004; Korolainen et al., 2006; Sultana et al., 2006a,b,c,e).

Butterfield et al. performed the first proteomic analysis in detecting protein oxidative damage on AD brains, by identifying three main targets of protein carbonylation: creatine kinase BB (CK BB), ubiquitin carboxy-terminal hydrolase L-1 (UCH L-1), and glutamine synthetase (GS) (Castegna et al., 2002a). Thereafter, many studies from the same laboratory and other research groups identified different proteins [α-enolase, γ-enolase, β-actin, lactate dehydrogenase (LDH), triose phosphate isomerase (TPI), carbonic anhydrase II (CAH II), γ-soluble *N*-ethylmaleimide-sensitive factor-attachment protein (γ-SNAP), neuropolypeptide h3, phosphoglycerate mutase 1 (PGM1), dihydropyrimidinase related protein-2 (DRP-2), glutamate transporter-1 (GLUT-1), heat shock cognate 71 (HSC 71), peptidyl prolyl cis–trans isomerase (Pin 1), glutathione-S-transferase, neurofilament protein L, α-tubulin, glial fibrillary acidic protein], which were oxidized and functionally impaired in AD brains, further supporting the hypothesis of oxidative stress as a mediator of synaptic loss and a presumed factor in the formation of tangles and plaques (Lauderback et al., 2001; Castegna et al., 2002a,b; Choi et al., 2004; Pamplona et al., 2005; Sultana et al., 2006a; Sultana et al., 2006b; Butterfield and Sultana, 2007).

Deploying a redox proteomics approach, Butterfield et al. observed in the AD brain the nitration of specific proteins: α-enolase, γ-enolase, L-LDH, triose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), ATP synthase α chain, voltage-dependent anion channel protein-1 (VDAC-1), and carbonic anhydrase II. These results support the role of protein nitration in promoting the perturbations, observed in AD, of mitochondrial functions, energy metabolism and pH regulation (Castegna et al., 2003; Sultana et al., 2006e). UCH L-1, one of the components of the proteosomal pathway, was found to be oxidized and dysfunctional in the inferior parietal lobule and hippocampus of AD subjects (Castegna et al., 2002a), and this could be one of the reasons for the observed increase in the nitrated proteins associated with this disorder. A proteomic analysis allowed Butterfield's research group to identify deoxyhemoglobin, α-crystallin B, GAPDH, and α-enolase as targets of increased S-glutathionylation in the brain of patients with AD, in comparison to age-matched controls. GAPDH and α-enolase were also shown to have reduced activity in the AD brain, with possible negative implications for brain glucose metabolism (Newman et al., 2007).

Increased levels of protein carbonyls and HNE-modified proteins were detected in the hippocampus of subjects with amnestic MCI, which is considered a transition between normal aging and AD. Using proteomic analysis, it has been shown that protein oxidation concerned mainly energy-related enzymes (α-enolase, pyruvate kinase M2), proteins involved in neurotransmission (glutamine synthetase), in the cell cycle and in tau phosphorylation (Pin 1). The oxidized proteins also displayed decreased activity in MCI, when compared to controls, and since those peptides are important in cell metabolism, synaptic plasticity and mitogenesis/proliferation, their oxidative inhibition could play a key role in the development of AD (Butterfield et al., 2006a; Reed et al., 2008). It was also observed in the MCI brain that protein nitration was 25% higher in the inferior parietal lobule and 41% higher in the hippocampus, in comparison to the same brain regions from control subjects (Butterfield et al., 2007). Few investigations have been published regarding on the proteomic analysis in the peripheral tissues of patients with AD: one study found that α-1-antitrypsin and fibrinogen γ-chain precursor proteins exhibited a two-six fold specific oxidation index in plasma from AD subjects when compared to controls, and both these proteins have been suggested to be involved in AD pathophysiology (Choi et al., 2002).

In conclusion, redox proteomics studies have identified several damaged proteins in AD and MCI. These proteins deal with energy metabolism, glutamate reuptake, the recycling of damaged or aggregated proteins through the proteasome, the maintenance of membrane structure and function and directing dendrites to adjacent neurons. All these functions are compromised in AD and proteomic analysis has identified peptides whose decreased function is consistent with the pathophysiology of AD, thereby providing new insights into the potential mechanisms of neurodegeneration in this disorder. Specifically, data from proteomic studies support the notion that protein carbonylation and nitration alter energy metabolism, pH regulation, and mitochondrial functions. The imbalance of these processes could promote the onset of AD and its progression. Furthermore, modified proteins identified by proteomics could be a diagnostic and prognostic marker of the disease. The proteomic-mediated identification of oxidized/nitrosylated peptides in the MCI brains is ongoing in several studies which could assist the discovery of novel biomarkers of AD-related neurodegeneration. Thus, mechanisms of disease progression would be clarified, thereby providing insights into the development of pharmacological strategies in preventing the conversion from MCI to AD.

7. Oxidative and nitrosative stress: cause or consequence in Alzheimer's disease?

The mechanisms responsible for the selective dysfunction and neuronal death in the AD brain remain unclear. Aging is the major risk factor for AD, and, since it has been largely proved that an elevation in oxidative/nitrosative damage is one of the most ubiquitous alterations observed in aging cells and tissues, it is likely that increased OS/NS contributes to the development of age-related disorders, such as AD. However, it has not been totally clarified whether the increased oxidative/nitrosative damage in MCI and AD subjects constitutes an acceleration of the "normal" age related raise in oxidative/nitrosative injury, or whether alternative pathways of ROS/RNS production are responsible for the increased levels of OS/NS, which are observed in MCI and AD subjects (Ding et al., 2007).

Several sources/conditions causing ROS/RNS hyperproduction have been suggested as playing a role in AD pathogenesis (Zhu et al., 2007b):

(i) The loss of metal homeostasis, with a subsequent accumulation of iron and/or copper in the brain (Lovell et al., 1998; Crouch et al., 2007), which catalyzes the formation of $^{\bullet}OH$ from H_2O_2, as well as the synthesis of AGEs. The latter, in the presence of transition metals, can undergo redox cycling with consequent ROS production (Yan et al., 1994, 1995). Furthermore, AGEs and Aβ activate specific receptors, such as that for advanced glycation end products (RAGE) and the class A scavenger-receptor, which increase ROS production (El Khoury et al., 1996; Yan et al., 1996).

(ii) Activated microglia/astrocytes, such as those surrounding most senile plaques (Cras et al., 1990), are a source of $NO^{\bullet}$ and $O_2^{\bullet-}$ (Colton and Gilbert, 1987) and they can react to form peroxynitrite.

(iii) Impairment in mitochondrial metabolism, and subsequent deficiency in key enzyme functions. Mitochondrial dysfunction, due in part to damage of the mitochondrial genome, can increase free radical production, suggesting that these organelles may be the major and possible initiating source of ROS (Davis et al., 1997; Castellani et al., 2002; Moreira et al., 2008).

(iv) Dysfunction in cellular proteolysis, including altered lysosomal functions and proteasome inhibition, induces protein oxidation and impairs the activity of mitochondrial complexes I and II, thus increasing ROS production (Zhu et al., 2007b).

(v) Aβ itself has been directly implicated in ROS formation through peptidyl radicals (Hensley et al., 1994; Sayre et al., 1997a). Specifically, Aβ oligomers seem to be more efficient in promoting OS, compared to the fibrilized form of Aβ (Tamagno et al., 2006). APP and Aβ can be detected in mitochondrial membranes, where they can damage the electron-transport chain, promote mitochondria dysfunction and increase ROS production (Reddy and Beal, 2008). In addition to the direct induction of oxidative stress, Aβ can also indirectly generate an oxidative microenvironment, for example, via the stimulation of a local immune response. Indeed, the cellular and soluble mediators of inflammation have been identified in post-mortem AD tissues (McGeer et al., 2000). Oxidative damage may, in turn, play a role in the amyloid deposition observed in AD; the complex reciprocal relationships between Aβ deposition, excitotoxicity, calcium dysregulation and ROS production in AD have been elegantly summarized (Barnham et al., 2004; Mattson, 2004; Valko et al., 2007). A recent hypothesis which considers Aβ production as a protective consequence to an underlying disease mechanism merits consideration: it views the known lesions of AD as a compensatory response which is adaptive and protective (Joseph et al., 2001; Lee et al., 2004). Indeed, Aβ possesses many physiological roles, including redox-active metal sequestration (Smith et al., 1997a) and superoxide dismutase (SOD)-like activity (Curtain et al., 2001). Furthermore, it has been shown to be inversely correlated with oxidative stress markers (Nunomura et al., 2001), thereby suggesting that it may have antioxidant effects. It has been proposed that oxidative damage could elicit compensatory mechanisms, such as Aβ deposition and hyperphosphorylated tau, in an attempt to restore the redox balance thereby avoiding neuronal death. However, the antioxidant activity of both agents evolves into a pro-oxidant during the progression of the disease, representing a "gain-of-function" transformation, which can result in an increase in free radical production and a decrease in clearance mechanisms (Zhu et al., 2007b).

Not only free radical hyperproduction, but also an impaired response to oxidative and nitrosative injury may predispose to an accumulation of oxidative and nitrosative damage in AD. Rinaldi et al. (2003) have evaluated the peripheral levels and activities of a broad spectrum of non-enzymatic and enzymatic antioxidants in elderly subjects with MCI and AD, in comparison with controls. MCI and AD subjects displayed a similar depletion in non-enzymatic antioxidants (Vitamin A, Vitamin C, Vitamin E, uric acid and carotenoids) and the in activities of plasma and erythrocyte SOD, in addition to plasma GPx, when compared to controls. These findings suggest that subjects developing MCI and AD may have an antioxidant network which is inadequate at counteracting the hyper-production of free radicals during a recently established condition of oxidative stress.

Several other studies have confirmed decreased levels of non-enzymatic antioxidants in subjects with AD (Bourdel-Marchasson et al., 2001; Mecocci et al., 2002; Glaso et al., 2004; Polidori et al., 2004; Praticò and Sung, 2004), while the overall data regarding levels of enzymatic antioxidants in plasma and/or CNS in AD remain conflicting. Indeed, while some investigations have demonstrated a decreased level in the activity of enzymatic antioxidants in AD patients (Pappolla et al., 1992; Marcus et al., 1998; Ihara et al., 2000; Rinaldi et al., 2003; Casado et al., 2008), others did not confirm these findings. This fact either revealed no differences (Bourdel-Marchasson et al., 2001) or increased levels of enzymes, such as GPx, SOD, glutathione reductase, and Cat, which in turn suggest a compensatory rise in antioxidant activity in response to increased free radical formation (Balazs and Leon, 1994; Lovell et al., 1995; Repetto et al., 1999; Serra et al., 2001; Schuessel et al., 2004; Martin-Aragon et al., 2009). A recent study has demonstrated an alteration in the levels and activity of several antioxidant enzymes in the MCI brain, compared to age-matched controls, suggesting that MCI subjects could have an inadequate antioxidant enzymatic activity, that might contribute to oxidative damage accumulation (Sultana et al., 2008).

Whilst there is a large body of evidence pointing to higher levels of OS and NS in AD in CNS and in peripheral tissues, it is not yet clear whether the increase in oxidative/nitrosative damage in the AD brain plays a role in promoting AD pathogenesis or whether these phenomena are simply by-products of neurodegeneration. It is conceivable that OS/NS are both early, "upstream", events which contribute to AD pathogenesis but they could also be secondary, "downstream", consequences of other pathogenetic mechanisms, which amplify oxidative/nitrosative damage, thus generating a vicious circle promoting neurodegeneration (Sayre et al., 2008).

Several considerations allow us to consider OS/NS as key factors in AD pathogenesis: primarily, not only age, but also other risk factors for AD are able to promote alterations in the redox homeostasis, including apolipoprotein E genotype (Miyata and

Smith, 1996; Mazur-Kolecka et al., 2002), APP and presenilin mutations (Cecchi et al., 2002; Rottkamp et al., 2002; Marques et al., 2003; Li et al., 2004). The temporal and spatial occurrence of oxidative/nitrosative injury are also important in supporting causal relationship of neurodegeneration in AD. Indeed, growing evidence suggests that OS/NS occur before the onset of symptoms, and that oxidative/nitrosative changes mainly accumulate in those cerebral regions vulnerable to AD pathology, while areas like the cerebellum, usually spared in AD, display levels of OS/NS which are similar to age-matched controls. An accumulation of markers of OS/NS appears to precede the formation of senile plaques, possibly independent of NFT accumulation (Zhu et al., 2000; Nunomura et al., 2001; Praticò et al., 2001; Gomez-Ramos et al., 2003); increasing evidence for the potential pathogenetic role of soluble Aβ oligomers rather than Aβ fibrils merits further research to assess the temporal relationship between OS/NS and formation/release of soluble Aβ oligomers (Zhu et al., 2007a).

More recently, multiple studies have demonstrated that lipid peroxidation, protein oxidation/nitrosylation, and nucleic acid oxidation occur in MCI subjects, in the same selected brain regions damaged in AD and, as with AD, they can also be detected in peripheral tissues. In the numerous studies summarized in this paper, the degree of OS/NS was comparable between AD and MCI subjects, and, since MCI may represent a prodromal stage of AD, these findings support the functional importance of an oxidative/nitrosative imbalance as an early crucial event in mediating AD pathogenesis.

The evidence that higher antioxidant plasma levels are associated with improved cognitive functions and a lower risk of developing dementia/AD further supports a pathogenetic role of OS/NS in AD. Several clinical and epidemiological studies regarding healthy adults/elderly subjects demonstrated a positive correlation between various circulating antioxidants and cognitive performance, and an inverse relationship with the risk of developing dementia/AD (Gale et al., 1996; La Rue et al., 1997; Perrig et al., 1997; Riviere et al., 1998; Schmidt et al., 1998; Berr et al., 2000; Helmer et al., 2003; Cherubini et al., 2005). Mecocci et al. (2000) have detected high levels of vitamin A and vitamin E in the plasma of mentally healthy centenarians, thereby suggesting a protective role of these micronutrients in the oldest old.

How could increased oxidative/nitrosative damage promote cellular dysfunction and neurodegeneration? The nature of the relationship between OS/NS and cell death has not yet been completely elucidated but it is clear that a controlled production of ROS and RNS is important in cell activation, proliferation or programmed cell death. A redox imbalance represents a regulatory sensor for several nuclear transcription factors, and there is increasing evidence that ROS and RNS are important mediators of signal transduction via several pathways. However, under pathological conditions abnormally large concentrations of ROS/RNS may lead to permanent changes in signal transduction and gene expression (thus impairing cell metabolism and homeostasis) and contribute to cell failure in adapting to stressful stimuli or even surviving (Polidori et al., 2007; Valko et al., 2007). The ability of oxidative/nitrosative damage in promoting toxicity probably relies on the capacity of oxidative/nitrosative modifications to impair the function of a given macromolecule, or alternatively promote a potentially deleterious gain of function event for a specific macromolecule (Ding et al., 2007).

Many products of OS/NS, regardless of whether they represent causes or consequences of tissue injury, have the potential for promoting neurodegeneration. Several human, animal, and in vitro studies have implicated products of lipid peroxidation in the pathogenesis of neuronal degeneration in AD (Keller and Mattson, 1998; Mattson, 1998; Markesbery and Carney, 1999; Cenini et al., 2008b; Valko et al., 2007; LoPachin et al., 2008). Nucleic acid oxidation is also important since the oxidative modifications of RNA, nDNA and mtDNA are thought to play a key role in the selective neuronal loss associated with mammalian aging and neurodegeneration (Nunomura et al., 1999; Lu et al., 2004; Melov, 2004; Beal, 2005). Specifically, there is evidence that RNA oxidation is not a harmless epiphenomenon, but it may be directly associated with neuronal deterioration (Shan et al., 2003, 2007). Finally, different studies have demonstrated how protein oxidative/nitrosative damage, especially if associated with impaired protein synthesis and the altered removal of injured peptides, can have deleterious consequences for the cell: it can promote metabolism dysregulation and increase ROS/RNS production, thus being an important step in progressive neuronal injury, leading to clinically-evident disease (Butterfield and Sultana, 2007; Ding et al., 2007; Polidori et al., 2007).

As it is not yet clear what category of biomolecules is first affected by OS/NS in the course of AD, studies regarding the temporal profile of biomolecule oxidative/nitrosative modifications are, therefore, required, to clarify which class of molecule is initially damaged, and how this may influence the metabolism and activity of other cellular macromolecules.

Another important question is why only certain neuronal populations are affected by the disease. A model of multi-step pathogenesis, the so-called "two-hits hypothesis", considers AD as result of serial insults that alone are insufficient to cause the disease, but when they occur together in the same cell, they are able to promote the degenerative process (Zhu et al., 2004). Based on an analysis of oxidative stress signalling and mitotic signalling pathways in vulnerable neuronal populations in AD, it has been hypothesized that both oxidative stress and aberrant mitotic stimuli can independently initiate AD neurodegeneration, but both are necessary in propagating AD pathogenesis and progression (Zhu et al., 2007a). Accordingly, whilst they may not constitute primary initiating events, oxidative/nitrosative stress is an early phenomenon associated with neurodegeneration. It is required for the propagation of an integrated series of cellular events, including excitotoxic stimulation, the dysfunction of critical proteins, and loss of metal homeostasis, all contributing to neuronal death. A shift from redox regulation to a condition of oxidative/nitrosative stress could, therefore, play a key role in AD pathogenesis as OS/NS could be main actor in a cycle of events leading to neurodegeneration; inhibiting OS and NS could break this cycle (Andersen, 2004; Sayre et al., 2008).

Studies involving MCI subjects may provide an opportunity for clarifying what specific neurochemical alterations are important in initiating AD pathogenesis, and which are secondary phenomena. The study of MCI can assist us in explaining the role of many of the pathways which could contribute to ROS/RNS hyperproduction in MCI and AD, bearing in mind that these sources of free radicals interact with each other in a complex manner, and most sources have a positive feedback (Zhu et al., 2007b).

8. Conclusions

While AD affects patients later in life, abundant evidence suggests the existence of a "preclinical" stage, commencing years before the clinical diagnosis, when an individual appears cognitively normal while he/she is undergoing extensive pathological changes in the brain. Biomarkers should serve as early diagnostic indicators or as markers of preclinical pathological change. Regarding the development and implementation of neuroprotective and disease-modifying therapies, the presence of robust biomarkers will be fundamental in screening for an increased susceptibility to AD, as an aid to diagnosis, identifying subsets of patients who are more responsive to treatment, and objectively monitoring the progression and response to treatments.

Expert consensus guidelines specify that a biomarker should reflect a neuropathological characteristic of AD. It should be validated in patients with a neuropathological diagnosis (Growdon, 1998; Frank et al., 2003) and several large-scale studies are on-going in different countries to develop and validate these markers. Biomarkers of OS/NS could have an important impact on the ability to test hypotheses concerning oxidative/nitrosative damage in AD pathogenesis. The key role of OS/NS in AD implies that biomarkers for oxidative/nitrosative damage could become an expression of risk factors for AD since they are effectively an indirect index of ROS/RNS levels, albeit more difficult to quantify. The primacy of OS/NS in AD pathogenesis implies that therapeutic strategies aiming at decreasing the level of oxidative/nitrosative damage are one of the main routes to preventing the onset of AD or delay its progression. In this regard, it is necessary to identify the reason for the lack of convincing, beneficial effects of antioxidant therapies in lowering the incidence of AD or delaying the progression of the disease (Sano et al., 1997; Morris et al., 1998; Luchsinger et al., 2003; Zandi et al., 2004; Fillenbaum et al., 2005; Maxwell et al., 2005; Petersen et al., 2005).

The influence that ROS and RNS has in modulating many different signalling pathways might explain the limited efficacy of antioxidant supplements in preventing or treating AD, although it cannot be excluded that this is due instead to the use of high doses of a single antioxidant. Indeed, epidemiological studies regarding the dietary intake of antioxidants revealed a higher efficacy than antioxidant supplements in preventing AD, thereby suggesting that the antioxidant mixture contained in food is more effective (Engelhart et al., 2002; Morris et al., 2002; Laurin et al., 2004) (other studies did not confirm these findings (Laurin et al., 2004)).

It is also important to note that all clinical trials regarding antioxidants to date share a common weakness, such as the monitoring of drug circulating levels and/or markers for monitoring the ability of an in-vivo drug to reducing the magnitude of oxidative and nitrosative damage (Praticò, 2008). Indeed, a central issue to be addressed in the field of the oxidant/antioxidant balance of the organism in AD and the evaluation of the efficacy of antioxidant therapies is the use of an appropriate index of oxidative/nitrosative damage capable of identifying subjects who could benefit from treatment and which could monitor the in-vivo antioxidant effect. In order to function as appropriate indicators of disease and constitute useful diagnostic/prognostic tools, biomarkers for oxidative/nitrosative stress have to be stable, accumulate to detectable concentrations, reflect specific oxidation/nitroxidation pathways, and possibly correlate with disease severity (Roberts and Morrow, 1997). The choice of appropriate biomarkers is extremely important in this field, especially given the impossibility of directly evaluating OS/NS qualitatively and quantitatively in the brains of living subjects. A blood biomarker would be widely applicable and reduce the need for invasive, expensive, or time-consuming testing.

Peripheral assessment for some biomarkers provides conflicting results and this is probably due to different assessment methods, sample preparation and treatment. The standardization of assessment methods and data reporting are critical to reducing any inconsistencies between studies. Furthermore, several parameters (e.g. diet, lifestyle, comorbidity, drug intake), which affect OS/NS levels, have not always been taken into consideration. In any biomarker study it is important in increasing diagnostic specificity to consider the stage of the disease and a comparison not only with healthy controls but also with other neurodegenerative diseases. Future studies are required to overcome these problems since the identification of the valuable reliable peripheral markers of OS/NS, used in diagnosing and monitoring the progression of the disease and drug efficacy in AD patients, is of fundamental importance for researchers and clinicians. Increased specificity and sensitivity can also be enhanced by using a panel of different biochemical indices (including β-amyloid), and integrating these measures with neuro-imaging techniques in assessing regional structure, function and biochemistry of the brain. There currently exists no single biomarker of excellence for oxidative/nitrosative stress and a set of such biomarkers, or at least a combination of two of these, appropriately chosen, together with the antioxidant profile, may provide the most accurate information regarding the oxidant/antioxidant balance of the organism, the nutritional needs of the patient and possible antioxidant strategies (Mariani et al., 2005; Migliore et al., 2005a).

References

Abe, T., Tohgi, H., Isobe, C., Murata, T., Sato, C., 2002. Remarkable increase in the concentration of 8-hydroxyguanosine in cerebrospinal fluid from patients with Alzheimer's disease. J. Neurosci. Res. 3, 447–450.

Adamec, E., Vonsattel, J.P., Nixon, R.A., 1999. DNA strand breaks in Alzheimer's disease. Brain Res. 1-2, 67–77.

Ahlskog, J.E., Uitti, R.J., Low, P.A., Tyce, G.M., Nickander, K.K., Petersen, R.C., Kokmen, E., 1995. No evidence for systemic oxidant stress in Parkinson's or Alzheimer's disease. Mov. Disord. 5, 566–573.

Ahmed, N., Ahmed, U., Thornalley, P.J., Hager, K., Fleischer, G., Munch, G., 2005. Protein glycation, oxidation and nitration adduct residues and free adducts of cerebrospinal fluid in Alzheimer's disease and link to cognitive impairment. J. Neurochem. 2, 255–263.

Aksenova, M.V., Aksenov, M.Y., Payne, R.M., Trojanowski, J.Q., Schmidt, M.L., Carney, J.M., Butterfield, D.A., Markesbery, W.R., 1999. Oxidation of cytosolic proteins and expression of creatine kinase BB in frontal lobe in different neurodegenerative disorders. Dement. Geriatr. Cogn. Disord. 2, 158–165.

Ames, B.N., Shigenaga, M.K., Hagen, T.M., 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. U.S.A. 17, 7915–7922.

Andersen, J.K., 2004. Oxidative stress in neurodegeneration: cause or consequence? Nat. Med. S18–S25.

Anderson, A.J., Su, J.H., Cotman, C.W., 1996. DNA damage and apoptosis in Alzheimer's disease: colocalization with c-Jun immunoreactivity, relationship to brain area, and effect of postmortem delay. J. Neurosci. 5, 1710–1719.

Anzai, K., Ogawa, K., Goto, Y., Senzaki, Y., Ozawa, T., Yamamoto, H., 1999. Oxidation-dependent changes in the stability and permeability of lipid bilayers. Antioxid. Redox Signal. 3, 339–347.

Arneson, K.O., Roberts 2nd, L.J., 2007. Measurement of products of docosahexaenoic acid peroxidation, neuroprostanes, and neurofurans. Methods Enzymol. 127–143.

Artero, S., Petersen, R., Touchon, J., Ritchie, K., 2006. Revised criteria for mild cognitive impairment: validation within a longitudinal population study. Dement. Geriatr. Cogn. Disord. 5–6, 465–470.

Aybek, H., Ercan, F., Aslan, D., Sahiner, T., 2007. Determination of malondialdehyde, reduced glutathione levels and APOE4 allele frequency in late-onset Alzheimer's disease in Denizli, Turkey. Clin. Biochem. 3–4, 172–176.

Balaban, R.S., Nemoto, S., Finkel, T., 2005. Mitochondria, oxidants, and aging. Cell 4, 483–495.

Balazs, L., Leon, M., 1994. Evidence of an oxidative challenge in the Alzheimer's brain. Neurochem. Res. 9, 1131–1137.

Baldeiras, I., Santana, I., Proenca, M.T., Garrucho, M.H., Pascoal, R., Rodrigues, A., Duro, D., Oliveira, C.R., 2008. Peripheral oxidative damage in mild cognitive impairment and mild Alzheimer's disease. J. Alzheimers Dis. 1, 117–128.

Barja, G., 2004. Free radicals and aging. Trends Neurosci. 10, 595–600.

Barnham, K.J., Masters, C.L., Bush, A.I., 2004. Neurodegenerative diseases and oxidative stress. Nat. Rev. Drug Discov. 3, 205–214.

Bartsch, H., Nair, J., 2005. Accumulation of lipid peroxidation-derived DNA lesions: potential lead markers for chemoprevention of inflammation-driven malignancies. Mutat. Res. 1–2, 34–44.

Barzilai, A., 2007. The contribution of the DNA damage response to neuronal viability. Antioxid. Redox Signal. 2, 211–218.

Basu, S., 2004. Isoprostanes: novel bioactive products of lipid peroxidation. Free Radic. Res. 2, 105–122.

Beal, M.F., 2000. Oxidative metabolism. Ann. N. Y. Acad. Sci. 164–169.

Beal, M.F., 2005. Mitochondria take center stage in aging and neurodegeneration. Ann. Neurol. 4, 495–505.

Becker, E.B., Bonni, A., 2004. Cell cycle regulation of neuronal apoptosis in development and disease. Prog. Neurobiol. 1, 1–25.

Beckman, K.B., Ames, B.N., 1998. The free radical theory of aging matures. Physiol. Rev. 2, 547–581.

Bermejo, P., Martin-Aragon, S., Benedi, J., Susin, C., Felici, E., Gil, P., Ribera, J.M., Villar, A.M., 2008. Peripheral levels of glutathione and protein oxidation as markers in the development of Alzheimer's disease from mild cognitive impairment. Free Radic. Res. 2, 162–170.

Berr, C., Balansard, B., Arnaud, J., Roussel, A.M., Alperovitch, A., 2000. Cognitive decline is associated with systemic oxidative stress: the EVA study. Etude du Vieillissement Arteriel. J. Am. Geriatr. Soc. 10, 1285–1291.

Bohnstedt, K.C., Karlberg, B., Wahlund, L.O., Jonhagen, M.E., Basun, H., Schmidt, S., 2003. Determination of isoprostanes in urine samples from Alzheimer patients using porous graphitic carbon liquid chromatography-tandem mass spectrometry. J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 1, 11–19.

Bourdel-Marchasson, I., Delmas-Beauvieux, M.C., Peuchant, E., Richard-Harston, S., Decamps, A., Reignier, B., Emeriau, J.P., Rainfray, M., 2001. Antioxidant defences and oxidative stress markers in erythrocytes and plasma from normally nourished elderly Alzheimer patients. Age Ageing 3, 235–241.

Bregeon, D., Sarasin, A., 2005. Hypothetical role of RNA damage avoidance in preventing human disease. Mutat. Res. 1–2, 293–302.

Breusing, N., Grune, T., 2008. Regulation of proteasome-mediated protein degradation during oxidative stress and aging. Biol. Chem. 3, 203–209.

Brys, M., Pirraglia, E., Rich, K., Rolstad, S., Mosconi, L., Switalski, R., Glodzik-Sobanska, L., De Santi, S., Zinkowski, R., Mehta, P., Pratico, D., Saint Louis, L.A., Wallin, A., Blennow, K., de Leon, M.J., 2009. Prediction and longitudinal study of CSF biomarkers in mild cognitive impairment. Neurobiol. Aging 5, 682–690.

Butterfield, D.A., 2004. Proteomics: a new approach to investigate oxidative stress in Alzheimer's disease brain. Brain Res. 1–2, 1–7.

Butterfield, D.A., Sultana, R., 2007. Redox proteomics identification of oxidatively modified brain proteins in Alzheimer's disease and mild cognitive impairment: insights into the progression of this dementing disorder. J. Alzheimers Dis. 1, 61–72.

Butterfield, D.A., Poon, H.F., St Clair, D., Keller, J.N., Pierce, W.M., Klein, J.B., Markesbery, W.R., 2006a. Redox proteomics identification of oxidatively modified hippocampal proteins in mild cognitive impairment: insights into the development of Alzheimer's disease. Neurobiol. Dis. 2, 223–232.

Butterfield, D.A., Reed, T., Perluigi, M., De Marco, C., Coccia, R., Cini, C., Sultana, R., 2006b. Elevated protein-bound levels of the lipid peroxidation product, 4-hydroxy-2-nonenal, in brain from persons with mild cognitive impairment. Neurosci. Lett. 3, 170–173.

Butterfield, D.A., Reed, T.T., Perluigi, M., De Marco, C., Coccia, R., Keller, J.N., Markesbery, W.R., Sultana, R., 2007. Elevated levels of 3-nitrotyrosine in brain from subjects with amnestic mild cognitive impairment: implications for the role of nitration in the progression of Alzheimer's disease. Brain Res. 243–248.

Calabrese, V., Scapagnini, G., Giuffrida Stella, A.M., Bates, T.E., Clark, J.B., 2001. Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity. Neurochem. Res. 6, 739–764.

Calabrese, V., Sultana, R., Scapagnini, G., Guagliano, E., Sapienza, M., Bella, R., Kanski, J., Pennisi, G., Mancuso, C., Stella, A.M., Butterfield, D.A., 2006. Nitrosative stress, cellular stress response, and thiol homeostasis in patients with Alzheimer's disease. Antioxid. Redox Signal. 11–12, 1975–1986.

Calabrese, V., Guagliano, E., Sapienza, M., Panebianco, M., Calafato, S., Puleo, E., Pennisi, G., Mancuso, C., Butterfield, D.A., Stella, A.G., 2007. Redox regulation of cellular stress response in aging and neurodegenerative disorders: role of vitagenes. Neurochem Res. 4–5, 757–773.

Calingasan, N.Y., Uchida, K., Gibson, G.E., 1999. Protein-bound acrolein: a novel marker of oxidative stress in Alzheimer's disease. J. Neurochem. 2, 751–756.

Cappai, R., Barnham, K.J., 2008. Delineating the mechanism of Alzheimer's disease A beta peptide neurotoxicity. Neurochem. Res. 3, 526–532.

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

Casadesus, G., Smith, M.A., Basu, S., Hua, J., Capobianco, D.E., Siedlak, S.L., Zhu, X., Perry, G., 2007. Increased isoprostane and prostaglandin are prominent in neurons in Alzheimer disease. Mol. Neurodegener. 2, 2.

Casado, A., Encarnacion Lopez-Fernandez, M., Concepcion Casado, M., de La Torre, R., 2008. Lipid peroxidation and antioxidant enzyme activities in vascular and Alzheimer dementias. Neurochem. Res. 3, 450–458.

Castegna, A., Aksenov, M., Aksenova, M., Thongboonkerd, V., Klein, J.B., Pierce, W.M., Booze, R., Markesbery, W.R., Butterfield, D.A., 2002a. Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part I. Creatine kinase BB, glutamine synthase, and ubiquitin carboxy-terminal hydrolase L-1. Free Radic. Biol. Med. 4, 562–571.

Castegna, A., Aksenov, M., Thongboonkerd, V., Klein, J.B., Pierce, W.M., Booze, R., Markesbery, W.R., Butterfield, D.A., 2002b. Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part II. Dihydropyrimidinase-related protein 2, alpha-enolase and heat shock cognate 71. J. Neurochem. 6, 1524–1532.

Castegna, A., Thongboonkerd, V., Klein, J.B., Lynn, B., Markesbery, W.R., Butterfield, D.A., 2003. Proteomic identification of nitrated proteins in Alzheimer's disease brain. J. Neurochem. 6, 1394–1401.

Castellani, R., Hirai, K., Aliev, G., Drew, K.L., Nunomura, A., Takeda, A., Cash, A.D., Obrenovich, M.E., Perry, G., Smith, M.A., 2002. Role of mitochondrial dysfunction in Alzheimer's disease. J. Neurosci. Res. 3, 357–360.

Ceballos-Picot, I., Merad-Boudia, M., Nicole, A., Thevenin, M., Hellier, G., Legrain, S., Berr, C., 1996. Peripheral antioxidant enzyme activities and selenium in elderly subjects and in dementia of Alzheimer's type—place of the extracellular glutathione peroxidase. Free Radic. Biol. Med. 4, 579–587.

Cecarini, V., Ding, Q., Keller, J.N., 2007. Oxidative inactivation of the proteasome in Alzheimer's disease. Free Radic. Res. 6, 673–680.

Cecchi, C., Fiorillo, C., Sorbi, S., Latorraca, S., Nacmias, B., Bagnoli, S., Nassi, P., Liguri, G., 2002. Oxidative stress and reduced antioxidant defenses in peripheral cells from familial Alzheimer's patients. Free Radic. Biol. Med. 10, 1372–1379.

Cenini, G., Sultana, R., Memo, M., Butterfield, D.A., 2008a. Effects of oxidative and nitrosative stress in brain on p53 proapoptotic protein in amnestic mild cognitive impairment and Alzheimer disease. Free Radic. Biol. Med. 1, 81–85.

Cenini, G., Sultana, R., Memo, M., Butterfield, D.A., 2008b. Elevated levels of pro-apoptotic p53 and its oxidative modification by the lipid peroxidation product, HNE, in brain from subjects with amnestic mild cognitive impairment and Alzheimer's disease. J. Cell Mol. Med. 12, 987–994.

Chakravarti, B., Chakravarti, D.N., 2007. Oxidative modification of proteins: age-related changes. Gerontology 3, 128–139.

Chance, B., Sies, H., Boveris, A., 1979. Hydroperoxide metabolism in mammalian organs. Physiol. Rev. 3, 527–605.

Chen, K., Kazachkov, M., Yu, P.H., 2007. Effect of aldehydes derived from oxidative deamination and oxidative stress on beta-amyloid aggregation; pathological implications to Alzheimer's disease. J. Neural. Transm. 6, 835–839.

Chen, L., Na, R., Gu, M., Richardson, A., Ran, Q., 2008. Lipid peroxidation up-regulates BACE1 expression in vivo: a possible early event of amyloidogenesis in Alzheimer's disease. J. Neurochem. 1, 197–207.

Cherubini, A., Martin, A., Andres-Lacueva, C., Di Iorio, A., Lamponi, M., Mecocci, P., Bartali, B., Corsi, A., Senin, U., Ferrucci, L., 2005. Vitamin E levels, cognitive impairment and dementia in older persons: the InCHIANTI study. Neurobiol. Aging 7, 987–994.

Choi, J., Malakowsky, C.A., Talent, J.M., Conrad, C.C., Gracy, R.W., 2002. Identification of oxidized plasma proteins in Alzheimer's disease. Biochem. Biophys. Res. Commun. 5, 1566–1570.

Choi, J., Levey, A.I., Weintraub, S.T., Rees, H.D., Gearing, M., Chin, L.S., Li, L., 2004. Oxidative modifications and down-regulation of ubiquitin carboxyl-terminal hydrolase L1 associated with idiopathic Parkinson's and Alzheimer's diseases. J. Biol. Chem. 13, 13256–13264.

Chung, H.Y., Cesari, M., Anton, S., Marzetti, E., Giovannini, S., Seo, A.Y., Carter, C., Yu, B.P., Leeuwenburgh, C., 2009. Molecular inflammation: underpinnings of aging and age-related diseases. Ageing Res. Rev. 1, 18–30.

Clayton, D.A., Doda, J.N., Friedberg, E.C., 1974. The absence of a pyrimidine dimer repair mechanism in mammalian mitochondria. Proc. Natl. Acad. Sci. U.S.A. 7, 2777–2781.

Colton, C.A., Gilbert, D.L., 1987. Production of superoxide anions by a CNS macrophage, the microglia. FEBS Lett. 2, 284–288.

Colurso, G.J., Nilson, J.E., Vervoort, L.G., 2003. Quantitative assessment of DNA fragmentation and beta-amyloid deposition in insular cortex and midfrontal gyrus from patients with Alzheimer's disease. Life Sci. 14, 1795–1803.

Conrad, C.C., Marshall, P.L., Talent, J.M., Malakowsky, C.A., Choi, J., Gracy, R.W., 2000. Oxidized proteins in Alzheimer's plasma. Biochem. Biophys. Res. Commun. 2, 678–681.

Cracowski, J.L., Durand, T., Bessard, G., 2002. Isoprostanes as a biomarker of lipid peroxidation in humans: physiology, pharmacology and clinical implications. Trends Pharmacol. Sci. 8, 360–366.

Cras, P., Kawai, M., Siedlak, S., Mulvihill, P., Gambetti, P., Lowery, D., Gonzalez-DeWhitt, P., Greenberg, B., Perry, G., 1990. Neuronal and microglial involvement in beta-amyloid protein deposition in Alzheimer's disease. Am. J. Pathol. 2, 241–246.

Crouch, P.J., White, A.R., Bush, A.I., 2007. The modulation of metal bio-availability as a therapeutic strategy for the treatment of Alzheimer's disease. FEBS J. 15, 3775–3783.

Crow, J.P., Beckman, J.S., 1995. The role of peroxynitrite in nitric oxide-mediated toxicity. Curr. Top. Microbiol. Immunol. 57–73.

Curtain, C.C., Ali, F., Volitakis, I., Cherny, R.A., Norton, R.S., Beyreuther, K., Barrow, C.J., Masters, C.L., Bush, A.I., Barnham, K.J., 2001. Alzheimer's disease amyloid-beta binds copper and zinc to generate an allosterically ordered membrane-penetrating structure containing superoxide dismutase-like subunits. J. Biol. Chem. 23, 20466–20473.

Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D., Milzani, A., 2006. Biomarkers of oxidative damage in human disease. Clin. Chem. 4, 601–623.

Davis, R.E., Miller, S., Herrnstadt, C., Ghosh, S.S., Fahy, E., Shinobu, L.A., Galasko, D., Thal, L.J., Beal, M.F., Howell, N., Parker Jr., W.D., 1997. Mutations in mitochondrial cytochrome c oxidase genes segregate with late-onset Alzheimer disease. Proc. Natl. Acad. Sci. U.S.A. 9, 4526–4531.

de Leon, M.J., DeSanti, S., Zinkowski, R., Mehta, P.D., Pratico, D., Segal, S., Clark, C., Kerkman, D., DeBernardis, J., Li, J., Lair, L., Reisberg, B., Tsui, W., Rusinek, H., 2004. MRI and CSF studies in the early diagnosis of Alzheimer's disease. J. Intern. Med. 3, 205–223.

de Leon, M.J., DeSanti, S., Zinkowski, R., Mehta, P.D., Pratico, D., Segal, S., Rusinek, H., Li, J., Tsui, W., Saint Louis, L.A., Clark, C.M., Tarshish, C., Li, Y., Lair, L., Javier, E., Rich, K., Lesbre, P., Mosconi, L., Reisberg, B., Sadowski, M., DeBernadis, J.F., Kerkman, D.J., Hampel, H., Wahlund, L.O., Davies, P., 2006. Longitudinal CSF and MRI biomarkers improve the diagnosis of mild cognitive impairment. Neurobiol. Aging 3, 394–401.

de Leon, M.J., Mosconi, L., Li, J., De Santi, S., Yao, Y., Tsui, W.H., Pirraglia, E., Rich, K., Javier, E., Brys, M., Glodzik, L., Switalski, R., Saint Louis, L.A., Pratico, D., 2007. Longitudinal CSF isoprostane and MRI atrophy in the progression to AD. J. Neurol. 12, 1666–1675.

Deshpande, A., Mina, E., Glabe, C., Busciglio, J., 2006. Different conformations of amyloid beta induce neurotoxicity by distinct mechanisms in human cortical neurons. J. Neurosci. 22, 6011–6018.

Di Domenico, F., Cenini, G., Sultana, R., Perluigi, M., Uberti, D., Memo, M., Butterfield, A.D., 2009. Glutathionylation of the Pro-apoptotic Protein p53 in Alzheimer's Disease Brain: Implications for AD Pathogenesis. Neurochem. Res. 4, 727–733.

Ding, Q., Keller, J.N., 2001. Proteasomes and proteasome inhibition in the central nervous system. Free Radic. Biol. Med. 5, 574–584.

Ding, Q., Markesbery, W.R., Chen, Q., Li, F., Keller, J.N., 2005. Ribosome dysfunction is an early event in Alzheimer's disease. J. Neurosci. 40, 9171–9175.

Ding, Q., Markesbery, W.R., Cecarini, V., Keller, J.N., 2006. Decreased RNA, and increased RNA oxidation, in ribosomes from early Alzheimer's disease. Neurochem. Res. 5, 705–710.

Ding, Q., Dimayuga, E., Keller, J.N., 2007. Oxidative damage, protein synthesis, and protein degradation in Alzheimer's disease. Curr. Alzheimer Res. 1, 73–79.

Dirican, M., Sarandol, E., Serdar, Z., Ocak, N., Dilek, K., 2007. Oxidative status and prevalent cardiovascular disease in patients with chronic renal failure treated by hemodialysis. Clin. Nephrol. 3, 144–150.

Dizdaroglu, M., Jaruga, P., Birincioglu, M., Rodriguez, H., 2002. Free radical-induced damage to DNA: mechanisms and measurement. Free Radic. Biol. Med. 11, 1102–1115.

Droge, W., 2002. Free radicals in the physiological control of cell function. Physiol. Rev. 1, 47–95.

Edwards, J.A., Wang, L.G., Setlow, R.B., Kaminskas, E., 1989. O6-methylguanine-DNA methyltransferase in lymphocytes of the elderly with and without Alzheimer's disease. Mutat. Res. 5–6, 267–272.

El Khoury, J., Hickman, S.E., Thomas, C.A., Cao, L., Silverstein, S.C., Loike, J.D., 1996. Scavenger receptor-mediated adhesion of microglia to beta-amyloid fibrils. Nature 6593, 716–719.

Engelhart, M.J., Geerlings, M.I., Ruitenberg, A., van Swieten, J.C., Hofman, A., Witteman, J.C., Breteler, M.M., 2002. Dietary intake of antioxidants and risk of Alzheimer disease. JAMA 24, 3223–3229.

Esterbauer, H., Schaur, R.J., Zollner, H., 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic. Biol. Med. 1, 81–128.

Feillet-Coudray, C., Tourtauchaux, R., Niculescu, M., Rock, E., Tauveron, I., Alexandre-Gouabau, M.C., Rayssiguier, Y., Jalenques, I., Mazur, A., 1999. Plasma levels of 8-epiPGF2alpha, an in vivo marker of oxidative stress, are not affected by aging or Alzheimer's disease. Free Radic. Biol. Med. 3–4, 463–469.

Fernandes, M.A., Proenca, M.T., Nogueira, A.J., Grazina, M.M., Oliveira, L.M., Fernandes, A.I., Santiago, B., Santana, I., Oliveira, C.R., 1999. Influence of apolipoprotein E genotype on blood redox status of Alzheimer's disease patients. Int. J. Mol. Med. 2, 179–186.

Fiala, E.S., Conaway, C.C., Mathis, J.E., 1989. Oxidative DNA and RNA damage in the livers of Sprague-Dawley rats treated with the hepatocarcinogen 2-nitropropane. Cancer Res 20, 5518–5522.

Fillenbaum, G.G., Kuchibhatla, M.N., Hanlon, J.T., Artz, M.B., Pieper, C.F., Schmader, K.E., Dysken, M.W., Gray, S.L., 2005. Dementia and Alzheimer's disease in community-dwelling elders taking vitamin C and/or vitamin E. Ann. Pharmacother. 12, 2009–2014.

Fishel, M.L., Vasko, M.R., Kelley, M.R., 2007. DNA repair in neurons: so if they don't divide what's to repair? Mutat. Res. 1–2, 24–36.

Forman, M.S., Mufson, E.J., Leurgans, S., Pratico, D., Joyce, S., Leight, S., Lee, V.M., Trojanowski, J.Q., 2007. Cortical biochemistry in MCI and Alzheimer disease: lack of correlation with clinical diagnosis. Neurology 10, 757–763.

Frank, R.A., Galasko, D., Hampel, H., Hardy, J., de Leon, M.J., Mehta, P.D., Rogers, J., Siemers, E., Trojanowski, J.Q., 2003. Biological markers for therapeutic trials in Alzheimer's disease. Proceedings of the biological markers working group; NIA initiative on neuroimaging in Alzheimer's disease. Neurobiol. Aging 4, 521–536.

Friguet, B., Szweda, L.I., 1997. Inhibition of the multicatalytic proteinase (proteasome) by 4-hydroxy-2-nonenal cross-linked protein. FEBS Lett. 1, 21–25.

Gabbita, S.P., Lovell, M.A., Markesbery, W.R., 1998. Increased nuclear DNA oxidation in the brain in Alzheimer's disease. J. Neurochem. 5, 2034–2040.

Gabbita, S.P., Aksenov, M.Y., Lovell, M.A., Markesbery, W.R., 1999. Decrease in peptide methionine sulfoxide reductase in Alzheimer's disease brain. J. Neurochem. 4, 1660–1666.

Gale, C.R., Martyn, C.N., Cooper, C., 1996. Cognitive impairment and mortality in a cohort of elderly people. BMJ 7031, 608–611.

Glabe, C.G., 2006. Common mechanisms of amyloid oligomer pathogenesis in degenerative disease. Neurobiol. Aging 4, 570–575.

Glaso, M., Nordbo, G., Diep, L., Bohmer, T., 2004. Reduced concentrations of several vitamins in normal weight patients with late-onset dementia of the Alzheimer type without vascular disease. J. Nutr. Health Aging 5, 407–413.

Gomez-Ramos, A., Diaz-Nido, J., Smith, M.A., Perry, G., Avila, J., 2003. Effect of the lipid peroxidation product acrolein on tau phosphorylation in neural cells. J. Neurosci Res. 6, 863–870.

Gotz, M.E., Dirr, A., Freyberger, A., Burger, R., Riederer, P., 1993. The thiobarbituric acid assay reflects susceptibility to oxygen induced lipid peroxidation in vitro rather than levels of lipid hydroperoxides in vivo: a methodological approach. Neurochem. Int. 3, 255–262.

Growdon, J., 1998. Consensus report of the working group on: "Molecular and biochemical markers of alzheimer's disease". The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group. Neurobiol Aging 2, 109–116.

Gutteridge, J.M., 1982. Free-radical damage to lipids, amino acids, carbohydrates and nucleic acids determined by thiobarbituric acid reactivity. Int. J. Biochem. 7, 649–653.

Gutteridge, J.M., Halliwell, B., 1990. The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem. Sci. 4, 129–135.

Hajimohammadreza, I., Brammer, M., 1990. Brain membrane fluidity and lipid peroxidation in Alzheimer's disease. Neurosci. Lett. 2–3, 333–337.

Halliwell, B., Gutteridge, J.M, 1999. Free Radicals in Biology and Medicine, 3rd ed. Oxford University Press, Oxford.

Halliwell, B., Whiteman, M., 2004. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br. J. Pharmacol. 2, 231–255.

Hayn, M., Kremser, K., Singewald, N., Cairns, N., Nemethova, M., Lubec, B., Lubec, G., 1996. Evidence against the involvement of reactive oxygen species in the pathogenesis of neuronal death in Down's syndrome and Alzheimer's disease. Life Sci. 7, 537–544.

Hebert, L.E., Scherr, P.A., Bienias, J.L., Bennett, D.A., Evans, D.A., 2003. Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch. Neurol. 8, 1119–1122.

Helmer, C., Peuchant, E., Letenneur, L., Bourdel-Marchasson, I., Larrieu, S., Dartigues, J.F., Dubourg, L., Thomas, M.J., Barberger-Gateau, P., 2003. Association between antioxidant nutritional indicators and the incidence of dementia: results from the PAQUID prospective cohort study. Eur. J. Clin. Nutr. 12, 1555–1561.

Hensley, K., Carney, J.M., Mattson, M.P., Aksenova, M., Harris, M., Wu, J.F., Floyd, R.A., Butterfield, D.A., 1994. A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer disease. Proc. Natl. Acad. Sci. U.S.A. 8, 3270–3274.

Hensley, K., Hall, N., Subramaniam, R., Cole, P., Harris, M., Aksenov, M., Aksenova, M., Gabbita, S.P., Wu, J.F., Carney, J.M., et al., 1995. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. J. Neurochem. 5, 2146–2156.

Hensley, K., Maidt, M.L., Yu, Z., Sang, H., Markesbery, W.R., Floyd, R.A., 1998. Electrochemical analysis of protein nitrotyrosine and dityrosine in the Alzheimer brain indicates region-specific accumulation. J. Neurosci. 20, 8126–8132.

Honda, K., Smith, M.A., Zhu, X., Baus, D., Merrick, W.C., Tartakoff, A.M., Hattier, T., Harris, P.L., Siedlak, S.L., Fujioka, H., Liu, Q., Moreira, P.I., Miller, F.P., Nunomura, A., Shimohama, S., Perry, G., 2005. Ribosomal RNA in Alzheimer disease is oxidized by bound redox-active iron. J. Biol. Chem. 22, 20978–20986.

Hou, L., Kang, I., Marchant, R.E., Zagorski, M.G., 2002. Methionine 35 oxidation reduces fibril assembly of the amyloid abeta-(1–42) peptide of Alzheimer's disease. J. Biol. Chem. 43, 40173–40176.

Ichihashi, K., Osawa, T., Toyokuni, S., Uchida, K., 2001. Endogenous formation of protein adducts with carcinogenic aldehydes: implications for oxidative stress. J. Biol. Chem. 26, 23903–23913.

Ihara, Y., Hayabara, T., Sasaki, K., Kawada, R., Nakashima, Y., Kuroda, S., 2000. Relationship between oxidative stress and apoE phenotype in Alzheimer's disease. Acta Neurol. Scand. 6, 346–349.

Iida, T., Furuta, A., Nishioka, K., Nakabeppu, Y., Iwaki, T., 2002. Expression of 8-oxoguanine DNA glycosylase is reduced and associated with neurofibrillary tangles in Alzheimer's disease brain. Acta Neuropathol. 1, 20–25.

Irie, Y., Saeki, M., Kamisaki, Y., Martin, E., Murad, F., 2003. Histone H1.2 is a substrate for denitrase, an activity that reduces nitrotyrosine immunoreactivity in proteins. Proc. Natl. Acad. Sci. U.S.A. 10, 5634–5639.

Irizarry, M.C., Yao, Y., Hyman, B.T., Growdon, J.H., Pratico, D., 2007. Plasma F2A isoprostane levels in Alzheimer's and Parkinson's disease. Neurodegener. Dis. 6, 403–405.

Jacobsen, E., Beach, T., Shen, Y., Li, R., Chang, Y., 2004. Deficiency of the Mre11 DNA repair complex in Alzheimer's disease brains. Brain Res. Mol. Brain Res. 1, 1–7.

Jones, D.P., 2006. Redefining oxidative stress. Antioxid. Redox Signal. 9–10, 1865–1879.

Joseph, J., Shukitt-Hale, B., Denisova, N.A., Martin, A., Perry, G., Smith, M.A., 2001. Copernicus revisited: amyloid beta in Alzheimer's disease. Neurobiol. Aging 1, 131–146.

Kadiiska, M.B., Gladen, B.C., Baird, D.D., Graham, L.B., Parker, C.E., Ames, B.N., Basu, S., Fitzgerald, G.A., Lawson, J.A., Marnett, L.J., Morrow, J.D., Murray, D.M., Plastaras, J., Roberts 2nd, L.J., Rokach, J., Shigenaga, M.K., Sun, J., Walter, P.B., Tomer, K.B., Barrett, J.C., Mason, R.P., 2005. Biomarkers of Oxidative Stress Study III. Effects of the nonsteroidal anti-inflammatory agents indomethacin and meclofenamic acid on measurements of oxidative products of lipids in CCl4 poisoning. Free Radic. Biol. Med. 6, 711–718.

Kadioglu, E., Sardas, S., Aslan, S., Isik, E., Esat Karakaya, A., 2004. Detection of oxidative DNA damage in lymphocytes of patients with Alzheimer's disease. Biomarkers 2, 203–209.

Kawaguchi-Niida, M., Shibata, N., Morikawa, S., Uchida, K., Yamamoto, T., Sawada, T., Kobayashi, M., 2006. Crotonaldehyde accumulates in glial cells of Alzheimer's disease brain. Acta Neuropathol. 5, 422–429.

Kawamoto, E.M., Munhoz, C.D., Glezer, I., Bahia, V.S., Caramelli, P., Nitrini, R., Gorjao, R., Curi, R., Scavone, C., Marcourakis, T., 2005. Oxidative state in platelets and erythrocytes in aging and Alzheimer's disease. Neurobiol. Aging 6, 857–864.

Kedar, N.P., 2003. Can we prevent Parkinson's and Alzheimer's disease? J. Postgrad. Med. 3, 236–245.

Keller, J.N., Mattson, M.P., 1998. Roles of lipid peroxidation in modulation of cellular signaling pathways, cell dysfunction, and death in the nervous system. Rev. Neurosci. 2, 105–116.

Keller, J.N., Mark, R.J., Bruce, A.J., Blanc, E., Rothstein, J.D., Uchida, K., Waeg, G., Mattson, M.P., 1997. 4-Hydroxynonenal, an aldehydic product of membrane lipid peroxidation, impairs glutamate transport and mitochondrial function in synaptosomes. Neuroscience 3, 685–696.

Keller, J.N., Schmitt, F.A., Scheff, S.W., Ding, Q., Chen, Q., Butterfield, D.A., Markesbery, W.R., 2005. Evidence of increased oxidative damage in subjects with mild cognitive impairment. Neurology 7, 1152–1156.

Kim, K.M., Jung, B.H., Paeng, K.J., Kim, I., Chung, B.C., 2004. Increased urinary F(2)-isoprostanes levels in the patients with Alzheimer's disease. Brain Res. Bull. 1, 47–51.

Kinsella, T.J., Dobson, P.P., Fornace Jr., A.J., Barrett, S.F., Ganges, M.B., Robbins, J.H., 1987. Alzheimer's disease fibroblasts have normal repair of *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine-induced DNA damage determined by the alkaline elution technique. Biochem. Biophys. Res. Commun. 2, 355–361.

Klein, W.L., Krafft, G.A., Finch, C.E., 2001. Targeting small Abeta oligomers: the solution to an Alzheimer's disease conundrum? Trends Neurosci. 4, 219–224.

Korolainen, M.A., Goldsteins, G., Nyman, T.A., Alafuzoff, I., Koistinaho, J., Pirttila, T., 2006. Oxidative modification of proteins in the frontal cortex of Alzheimer's disease brain. Neurobiol. Aging 1, 42–53.

Kosova, F., Cetin, B., Akinci, M., Aslan, S., Ari, Z., Sepici, A., Altan, N., Cetin, A., 2007. Advanced oxidation protein products, ferrous oxidation in xylenol orange, and malondialdehyde levels in thyroid cancer. Ann. Surg. Oncol. 9, 2616–2620.

Kozekov, I.D., Nechev, L.V., Moseley, M.S., Harris, C.M., Rizzo, C.J., Stone, M.P., Harris, T.M., 2003. DNA interchain cross-links formed by acrolein and crotonaldehyde. J. Am. Chem. Soc. 1, 50–61.

Kruman, I.I., 2004. Why do neurons enter the cell cycle? Cell Cycle 6, 769–773.

Kuhla, B., Haase, C., Flach, K., Luth, H.J., Arendt, T., Munch, G., 2007. Effect of pseudophosphorylation and cross-linking by lipid peroxidation and advanced glycation end product precursors on tau aggregation and filament formation. J. Biol. Chem. 10, 6984–6991.

La Rue, A., Koehler, K.M., Wayne, S.J., Chiulli, S.J., Haaland, K.Y., Garry, P.J., 1997. Nutritional status and cognitive functioning in a normally aging sample: a 6-y reassessment. Am. J. Clin. Nutr. 1, 20–29.

Laboissiere, M.C., Sturley, S.L., Raines, R.T., 1995. The essential function of protein-disulfide isomerase is to unscramble non-native disulfide bonds. J. Biol. Chem. 47, 28006–28009.

Lambert, M.P., Barlow, A.K., Chromy, B.A., Edwards, C., Freed, R., Liosatos, M., Morgan, T.E., Rozovsky, I., Trommer, B., Viola, K.L., Wals, P., Zhang, C., Finch, C.E., Krafft, G.A., Klein, W.L., 1998. Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proc. Natl. Acad. Sci. U.S.A. 11, 6448–6453.

Lauderback, C.M., Hackett, J.M., Huang, F.F., Keller, J.N., Szweda, L.I., Markesbery, W.R., Butterfield, D.A., 2001. The glial glutamate transporter, GLT-1, is oxidatively modified by 4-hydroxy-2-nonenal in the Alzheimer's disease brain: the role of Abeta1-42. J. Neurochem. 2, 413–416.

Laurin, D., Masaki, K.H., Foley, D.J., White, L.R., Launer, L.J., 2004. Midlife dietary intake of antioxidants and risk of late-life incident dementia: the Honolulu-Asia Aging Study. Am. J. Epidemiol. 10, 959–967.

LeDoux, S.P., Druzhyna, N.M., Hollensworth, S.B., Harrison, J.F., Wilson, G.L., 2007. Mitochondrial DNA repair: a critical player in the response of cells of the CNS to genotoxic insults. Neuroscience 4, 1249–1259.

Lee, H.G., Casadesus, G., Zhu, X., Takeda, A., Perry, G., Smith, M.A., 2004. Challenging the amyloid cascade hypothesis: senile plaques and amyloid-beta as protective adaptations to Alzheimer disease. Ann. N. Y. Acad. Sci. 1–4.

Lee, S.H., Kim, I., Chung, B.C., 2007. Increased urinary level of oxidized nucleosides in patients with mild-to-moderate Alzheimer's disease. Clin. Biochem. 13–14, 936–938.

Lepage, G., Munoz, G., Champagne, J., Roy, C.C., 1991. Preparative steps necessary for the accurate measurement of malondialdehyde by high-performance liquid chromatography. Anal. Biochem. 2, 277–283.

Li, W.P., Chan, W.Y., Lai, H.W., Yew, D.T., 1997. Terminal dUTP nick end labeling (TUNEL) positive cells in the different regions of the brain in normal aging and Alzheimer patients. J. Mol. Neurosci. 2, 75–82.

Li, F., Calingasan, N.Y., Yu, F., Mauck, W.M., Toidze, M., Almeida, C.G., Takahashi, R.H., Carlson, G.A., Flint Beal, M., Lin, M.T., Gouras, G.K., 2004. Increased plaque burden in brains of APP mutant MnSOD heterozygous knockout mice. J. Neurochem. 5, 1308–1312.

Linnane, A.W., Marzuki, S., Ozawa, T., Tanaka, M., 1989. Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. Lancet 8639, 642–645.

Liu, X., Lovell, M.A., Lynn, B.C., 2005. Development of a method for quantification of acrolein-deoxyguanosine adducts in DNA using isotope dilution-capillary LC/MS/MS and its application to human brain tissue. Anal. Chem. 18, 5982–5989.

Liu, X., Lovell, M.A., Lynn, B.C., 2006. Detection and quantification of endogenous cyclic DNA adducts derived from trans-4-hydroxy-2-nonenal in human brain tissue by isotope dilution capillary liquid chromatography nanoelectrospray tandem mass spectrometry. Chem. Res. Toxicol. 5, 710–718.

Loidl-Stahlhofen, A., Hannemann, K., Spiteller, G., 1994. Generation of alpha-hydroxyaldehydic compounds in the course of lipid peroxidation. Biochim. Biophys. Acta 2, 140–148.

Long, J., Liu, C., Sun, L., Gao, H., Liu, J., 2008. Neuronal mitochondrial toxicity of malondialdehyde: inhibitory effects on respiratory function and enzyme activities in rat brain mitochondria. Neurochem. Res. 4, 786–794.

LoPachin, R.M., Barber, D.S., Gavin, T., 2008. Molecular mechanisms of the conjugated alpha,beta-unsaturated carbonyl derivatives: relevance to neurotoxicity and neurodegenerative diseases. Toxicol. Sci. 2, 235–249.

Lovell, M.A., Markesbery, W.R., 2001. Ratio of 8-hydroxyguanine in intact DNA to free 8-hydroxyguanine is increased in Alzheimer disease ventricular cerebrospinal fluid. Arch. Neurol. 3, 392–396.

Lovell, M.A., Markesbery, W.R., 2007. Oxidative DNA damage in mild cognitive impairment and late-stage Alzheimer's disease. Nucleic Acids Res. 22, 7497–7504.

Lovell, M.A., Markesbery, W.R., 2008. Oxidatively modified RNA in mild cognitive impairment. Neurobiol. Dis. 2, 169–175.

Lovell, M.A., Ehmann, W.D., Butler, S.M., Markesbery, W.R., 1995. Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. Neurology 8, 1594–1601.

Lovell, M.A., Ehmann, W.D., Mattson, M.P., Markesbery, W.R., 1997. Elevated 4-hydroxynonenal in ventricular fluid in Alzheimer's disease. Neurobiol. Aging 5, 457–461.

Lovell, M.A., Robertson, J.D., Teesdale, W.J., Campbell, J.L., Markesbery, W.R., 1998. Copper, iron and zinc in Alzheimer's disease senile plaques. J. Neurol. Sci. 1, 47–52.

Lovell, M.A., Gabbita, S.P., Markesbery, W.R., 1999. Increased DNA oxidation and decreased levels of repair products in Alzheimer's disease ventricular CSF. J. Neurochem. 2, 771–776.

Lovell, M.A., Xie, C., Markesbery, W.R., 2000a. Acrolein, a product of lipid peroxidation, inhibits glucose and glutamate uptake in primary neuronal cultures. Free Radic. Biol. Med. 8, 714–720.

Lovell, M.A., Xie, C., Markesbery, W.R., 2000b. Decreased base excision repair and increased helicase activity in Alzheimer's disease brain. Brain Res. 1, 116–123.

Lovell, M.A., Xie, C., Markesbery, W.R., 2001. Acrolein is increased in Alzheimer's disease brain and is toxic to primary hippocampal cultures. Neurobiol. Aging 2, 187–194.

Lu, T., Pan, Y., Kao, S.Y., Li, C., Kohane, I., Chan, J., Yankner, B.A., 2004. Gene regulation and DNA damage in the ageing human brain. Nature 6994, 883–891.

Lucassen, P.J., Chung, W.C., Kamphorst, W., Swaab, D.F., 1997. DNA damage distribution in the human brain as shown by in situ end labeling; area-specific differences in aging and Alzheimer disease in the absence of apoptotic morphology. J. Neuropathol. Exp. Neurol. 8, 887–900.

Luchsinger, J.A., Tang, M.X., Shea, S., Mayeux, R., 2003. Antioxidant vitamin intake and risk of Alzheimer disease. Arch. Neurol. 2, 203–208.

Luczaj, W., Skrzydlewska, E., 2003. DNA damage caused by lipid peroxidation products. Cell Mol. Biol. Lett. 2, 391–413.

Luth, H.J., Munch, G., Arendt, T., 2002. Aberrant expression of NOS isoforms in Alzheimer's disease is structurally related to nitrotyrosine formation. Brain Res. 1–2, 135–143.

Lyras, L., Cairns, N.J., Jenner, A., Jenner, P., Halliwell, B., 1997. An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease. J. Neurochem. 5, 2061–2069.

Malinski, T., 2007. Nitric oxide and nitroxidative stress in Alzheimer's disease. J. Alzheimers Dis. 2, 207–218.

Marcus, D.L., Thomas, C., Rodriguez, C., Simberkoff, K., Tsai, J.S., Strafaci, J.A., Freedman, M.L., 1998. Increased peroxidation and reduced antioxidant enzyme activity in Alzheimer's disease. Exp. Neurol. 1, 40–44.

Mariani, E., Polidori, M.C., Cherubini, A., Mecocci, P., 2005. Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview. J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 1, 65–75.

Mariani, E., Monastero, R., Mecocci, P., 2007. Mild cognitive impairment: a systematic review. J. Alzheimers Dis. 1, 23–35.

Mark, R.J., Hensley, K., Butterfield, D.A., Mattson, M.P., 1995. Amyloid beta-peptide impairs ion-motive ATPase activities: evidence for a role in loss of neuronal Ca^{2+} homeostasis and cell death. J. Neurosci. 9, 6239–6249.

Markesbery, W.R., Carney, J.M., 1999. Oxidative alterations in Alzheimer's disease. Brain Pathol. 1, 133–146.

Markesbery, W.R., Lovell, M.A., 1998. Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. Neurobiol. Aging 1, 33–36.

Markesbery, W.R., Lovell, M.A., 2007. Damage to lipids, proteins, DNA, and RNA in mild cognitive impairment. Arch. Neurol. 7, 954–956.

Markesbery, W.R., Kryscio, R.J., Lovell, M.A., Morrow, J.D., 2005. Lipid peroxidation is an early event in the brain in amnestic mild cognitive impairment. Ann. Neurol. 5, 730–735.

Marques, C.A., Keil, U., Bonert, A., Steiner, B., Haass, C., Muller, W.E., Eckert, A., 2003. Neurotoxic mechanisms caused by the Alzheimer's disease-linked Swedish amyloid precursor protein mutation: oxidative stress, caspases, and the JNK pathway. J. Biol. Chem. 30, 28294–28302.

Martin, B.L., Wu, D., Jakes, S., Graves, D.J., 1990. Chemical influences on the specificity of tyrosine phosphorylation. J. Biol. Chem. 13, 7108–7111.

Martin-Aragon, S., Bermejo-Bescos, P., Benedi, J., Felici, E., Gil, P., Ribera, J.M., Villar, A.M., 2009. Metalloproteinase's activity and oxidative stress in mild cognitive impairment and Alzheimer's disease. Neurochem. Res. 2, 373–378.

Martin-Gallan, P., Carrascosa, A., Gussinye, M., Dominguez, C., 2003. Biomarkers of diabetes-associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications. Free Radic. Biol. Med. 12, 1563–1574.

Mattson, M.P., 1998. Modification of ion homeostasis by lipid peroxidation: roles in neuronal degeneration and adaptive plasticity. Trends Neurosci. 2, 53–57.

Mattson, M.P., 2004. Pathways towards and away from Alzheimer's disease. Nature 7000, 631–639.

Maxwell, C.J., Hicks, M.S., Hogan, D.B., Basran, J., Ebly, E.M., 2005. Supplemental use of antioxidant vitamins and subsequent risk of cognitive decline and dementia. Dement. Geriatr. Cogn. Disord. 1, 45–51.

Mazur-Kolecka, B., Frackowiak, J., Kowal, D., Krzeslowska, J., Dickson, D., 2002. Oxidative protein damage in cells engaged in beta-amyloidosis is related to apoE genotype. Neuroreport 4, 465–468.

McGeer, P.L., McGeer, E.G., Yasojima, K., 2000. Alzheimer disease and neuroinflammation. J. Neural. Transm. Suppl. 53–57.

McGrath, L.T., McGleenon, B.M., Brennan, S., McColl, D., Mc, I.S., Passmore, A.P., 2001. Increased oxidative stress in Alzheimer's disease as assessed with 4-hydroxynonenal but not malondialdehyde. QJM 9, 485–490.

McIntosh, L.J., Trush, M.A., Troncoso, J.C., 1997. Increased susceptibility of Alzheimer's disease temporal cortex to oxygen free radical-mediated processes. Free Radic. Biol. Med. 2, 183–190.

Mecocci, P., MacGarvey, U., Kaufman, A.E., Koontz, D., Shoffner, J.M., Wallace, D.C., Beal, M.F., 1993. Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. Ann. Neurol. 4, 609–616.

Mecocci, P., MacGarvey, U., Beal, M.F., 1994. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. Ann. Neurol. 5, 747–751.

Mecocci, P., Polidori, M.C., Ingegni, T., Cherubini, A., Chionne, F., Cecchetti, R., Senin, U., 1998. Oxidative damage to DNA in lymphocytes from AD patients. Neurology 4, 1014–1017.

Mecocci, P., Polidori, M.C., Troiano, L., Cherubini, A., Cecchetti, R., Pini, G., Straatman, M., Monti, D., Stahl, W., Sies, H., Franceschi, C., Senin, U., 2000. Plasma antioxidants and longevity: a study on healthy centenarians. Free Radic. Biol. Med. 8, 1243–1248.

Mecocci, P., Polidori, M.C., Cherubini, A., Ingegni, T., Mattioli, P., Catani, M., Rinaldi, P., Cecchetti, R., Stahl, W., Senin, U., Beal, M.F., 2002. Lymphocyte oxidative DNA damage and plasma antioxidants in Alzheimer disease. Arch. Neurol. 5, 794–798.

Melov, S., 2004. Modeling mitochondrial function in aging neurons. Trends Neurosci. 10, 601–606.

Mieyal, J.J., Gallogly, M.M., Qanungo, S., Sabens, E.A., Shelton, M.D., 2008. Molecular mechanisms and clinical implications of reversible protein S-glutathionylation. Antioxid. Redox Signal. 11, 1941–1988.

Migliore, L., Fontana, I., Colognato, R., Coppede, F., Siciliano, G., Murri, L., 2005a. Searching for the role and the most suitable biomarkers of oxidative stress in Alzheimer's disease and in other neurodegenerative diseases. Neurobiol. Aging 5, 587–595.

Migliore, L., Fontana, I., Trippi, F., Colognato, R., Coppede, F., Tognoni, G., Nucciarone, B., Siciliano, G., 2005b. Oxidative DNA damage in peripheral leukocytes of mild cognitive impairment and AD patients. Neurobiol. Aging 5, 567–573.

Miyata, M., Smith, J.D., 1996. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. Nat. Genet. 1, 55–61.

Montine, K.S., Olson, S.J., Amarnath, V., Whetsell Jr., W.O., Graham, D.G., Montine, T.J., 1997. Immunohistochemical detection of 4-hydroxy-2-nonenal adducts in Alzheimer's disease is associated with inheritance of APOE4. Am. J. Pathol. 2, 437–443.

Montine, T.J., Markesbery, W.R., Morrow, J.D., Roberts 2nd, L.J., 1998. Cerebrospinal fluid F2-isoprostane levels are increased in Alzheimer's disease. Ann. Neurol. 3, 410–413.

Montine, T.J., Beal, M.F., Cudkowicz, M.E., O'Donnell, H., Margolin, R.A., McFarland, L., Bachrach, A.F., Zackert, W.E., Roberts, L.J., Morrow, J.D., 1999a. Increased CSF F2-isoprostane concentration in probable AD. Neurology 3, 562–565.

Montine, T.J., Markesbery, W.R., Zackert, W., Sanchez, S.C., Roberts 2nd, L.J., Morrow, J.D., 1999b. The magnitude of brain lipid peroxidation correlates with the extent of degeneration but not with density of neuritic plaques or neurofibrillary tangles or with APOE genotype in Alzheimer's disease patients. Am. J. Pathol. 3, 863–868.

Montine, T.J., Sidell, K.R., Crews, B.C., Markesbery, W.R., Marnett, L.J., Roberts 2nd, L.J., Morrow, J.D., 1999c. Elevated CSF prostaglandin E2 levels in patients with probable AD. Neurology 7, 1495–1498.

Montine, T.J., Shinobu, L., Montine, K.S., Roberts 2nd, L.J., Kowall, N.W., Beal, M.F., Morrow, J.D., 2000. No difference in plasma or urinary F2-isoprostanes among patients with Huntington's disease or Alzheimer's disease and controls. Ann. Neurol. 6, 950.

Montine, T.J., Kaye, J.A., Montine, K.S., McFarland, L., Morrow, J.D., Quinn, J.F., 2001. Cerebrospinal fluid abeta42, tau, and f2-isoprostane concentrations in patients with Alzheimer disease, other dementias, and in age-matched controls. Arch. Pathol. Lab. Med. 4, 510–512.

Montine, T.J., Quinn, J.F., Milatovic, D., Silbert, L.C., Dang, T., Sanchez, S., Terry, E., Roberts 2nd, L.J., Kaye, J.A., Morrow, J.D., 2002. Peripheral F2-isoprostanes and F4-neuroprostanes are not increased in Alzheimer's disease. Ann. Neurol. 2, 175–179.

Montine, T.J., Quinn, J., Kaye, J., Morrow, J.D., 2007. F(2)-isoprostanes as biomarkers of late-onset Alzheimer's disease. J. Mol. Neurosci. 1, 114–119.

Montuschi, P., Barnes, P.J., Roberts 2nd, L.J., 2004. Isoprostanes: markers and mediators of oxidative stress. FASEB J. 15, 1791–1800.

Moore, S.A., Yoder, E., Murphy, S., Dutton, G.R., Spector, A.A., 1991. Astrocytes, not neurons, produce docosahexaenoic acid (22:6 omega-3) and arachidonic acid (20:4 omega-6). J. Neurochem. 2, 518–524.

Moreira, P.I., Nunomura, A., Nakamura, M., Takeda, A., Shenk, J.C., Aliev, G., Smith, M.A., Perry, G., 2008. Nucleic acid oxidation in Alzheimer disease. Free Radic. Biol. Med. 8, 1493–1505.

Morocz, M., Kalman, J., Juhasz, A., Sinko, I., McGlynn, A.P., Downes, C.S., Janka, Z., Rasko, I., 2002. Elevated levels of oxidative DNA damage in lymphocytes from patients with Alzheimer's disease. Neurobiol. Aging 1, 47–53.

Morris, M.C., Beckett, L.A., Scherr, P.A., Hebert, L.E., Bennett, D.A., Field, T.S., Evans, D.A., 1998. Vitamin E and vitamin C supplement use and risk of incident Alzheimer disease. Alzheimer. Dis. Assoc. Disord. 3, 121–126.

Morris, M.C., Evans, D.A., Bienias, J.L., Tangney, C.C., Bennett, D.A., Aggarwal, N., Wilson, R.S., Scherr, P.A., 2002. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. JAMA 24, 3230–3237.

Morrow, J.D., 2005. Quantification of isoprostanes as indices of oxidant stress and the risk of atherosclerosis in humans. Arterioscler. Thromb. Vasc. Biol. 2, 279–286.

Morrow, J.D., Awad, J.A., Boss, H.J., Blair, I.A., Roberts 2nd, L.J., 1992. Non-cyclooxygenase-derived prostanoids (F2-isoprostanes) are formed in situ on phospholipids. Proc. Natl. Acad. Sci. U.S.A. 22, 10721–10725.

Morrow, J.D., Frei, B., Longmire, A.W., Gaziano, J.M., Lynch, S.M., Shyr, Y., Strauss, W.E., Oates, J.A., Roberts 2nd, L.J., 1995. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N. Engl. J. Med. 18, 1198–1203.

Mullaart, E., Boerrigter, M.E., Ravid, R., Swaab, D.F., Vijg, J., 1990. Increased levels of DNA breaks in cerebral cortex of Alzheimer's disease patients. Neurobiol. Aging 3, 169–173.

Nakamura, K., Kusano, K.F., Matsubara, H., Nakamura, Y., Miura, A., Nishii, N., Banba, K., Nagase, S., Miyaji, K., Morita, H., Saito, H., Emori, T., Ohe, T., 2005. Relationship between oxidative stress and systolic dysfunction in patients with hypertrophic cardiomyopathy. J. Card. Fail. 2, 117–123.

Nath, R.G., Ocando, J.E., Guttenplan, J.B., Chung, F.L., 1998. 1,N2-propanodeoxyguanosine adducts: potential new biomarkers of smoking-induced DNA damage in human oral tissue. Cancer Res. 4, 581–584.

Nayak, B.S., Pinto, S., 2007. Protein thiols and thiobarbituric acid reactive substance status in colon cancer patients. Scand. J. Gastroenterol. 7, 848–851.

Newman, S.F., Sultana, R., Perluigi, M., Coccia, R., Cai, J., Pierce, W.M., Klein, J.B., Turner, D.M., Butterfield, D.A., 2007. An increase in S-glutathionylated proteins in the Alzheimer's disease inferior parietal lobule, a proteomics approach. J. Neurosci. Res. 7, 1506–1514.

Niki, E., Noguchi, N., Gotoh, N., 1993. Dynamics of lipid peroxidation and its inhibition by antioxidants. Biochem. Soc. Trans. 2, 313–317.

Nourooz-Zadeh, J., Liu, E.H., Yhlen, B., Anggard, E.E., Halliwell, B., 1999. F4-isoprostanes as specific marker of docosahexaenoic acid peroxidation in Alzheimer's disease. J. Neurochem. 2, 734–740.

Nunomura, A., Perry, G., Pappolla, M.A., Wade, R., Hirai, K., Chiba, S., Smith, M.A., 1999. RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. J. Neurosci. 6, 1959–1964.

Nunomura, A., Perry, G., Aliev, G., Hirai, K., Takeda, A., Balraj, E.K., Jones, P.K., Ghanbari, H., Wataya, T., Shimohama, S., Chiba, S., Atwood, C.S., Petersen, R.B., Smith, M.A., 2001. Oxidative damage is the earliest event in Alzheimer disease. J. Neuropathol. Exp. Neurol. 8, 759–767.

Nunomura, A., Chiba, S., Lippa, C.F., Cras, P., Kalaria, R.N., Takeda, A., Honda, K., Smith, M.A., Perry, G., 2004. Neuronal RNA oxidation is a prominent feature of familial Alzheimer's disease. Neurobiol. Dis. 1, 108–113.

Nunomura, A., Honda, K., Takeda, A., Hirai, K., Zhu, X., Smith, M.A., Perry, G., 2006a. Oxidative damage to RNA in neurodegenerative diseases. J. Biomed. Biotechnol. 3, 82323.

Nunomura, A., Moreira, P.I., Zhu, X., Smith, M.A., Perry, G., 2006b. Involvement of oxidative stress in the early-stage of Alzheimer's disease: implications for therapeutics. Trends Alzheimer's Dis. Res..

Ozcankaya, R., Delibas, N., 2002. Malondialdehyde, superoxide dismutase, melatonin, iron, copper, and zinc blood concentrations in patients with Alzheimer disease: cross-sectional study. Croat. Med. J. 1, 28–32.

Palmer, A.M., Burns, M.A., 1994. Selective increase in lipid peroxidation in the inferior temporal cortex in Alzheimer's disease. Brain Res. 1–2, 338–342.

Pamplona, R., Dalfo, E., Ayala, V., Bellmunt, M.J., Prat, J., Ferrer, I., Portero-Otin, M., 2005. Proteins in human brain cortex are modified by oxidation, glycoxidation, and lipoxidation. Effects of Alzheimer disease and identification of lipoxidation targets. J. Biol. Chem. 22, 21522–21530.

Pappolla, M.A., Omar, R.A., Kim, K.S., Robakis, N.K., 1992. Immunohistochemical evidence of oxidative stress in Alzheimer's disease. Am. J. Pathol. 3, 621–628.

Parshad, R.P., Sanford, K.K., Price, F.M., Melnick, L.K., Nee, L.E., Schapiro, M.B., Tarone, R.E., Robbins, J.H., 1996. Fluorescent light-induced chromatid breaks distinguish Alzheimer disease cells from normal cells in tissue culture. Proc. Natl. Acad. Sci. U.S.A. 10, 5146–5150.

Pedersen, W.A., Cashman, N.R., Mattson, M.P., 1999. The lipid peroxidation product 4-hydroxynonenal impairs glutamate and glucose transport and choline acetyltransferase activity in NSC-19 motor neuron cells. Exp. Neurol. 1, 1–10.

Perrig, W.J., Perrig, P., Stahelin, H.B., 1997. The relation between antioxidants and memory performance in the old and very old. J. Am. Geriatr. Soc. 6, 718–724.

Petersen, R.C., Thomas, R.G., Grundman, M., Bennett, D., Doody, R., Ferris, S., Galasko, D., Jin, S., Kaye, J., Levey, A., Pfeiffer, E., Sano, M., van Dyck, C.H., Thal, L.J., 2005. Vitamin E and donepezil for the treatment of mild cognitive impairment. N. Engl. J. Med. 23, 2379–2388.

Picklo Sr., M.J., Montine, T.J., 2007. Mitochondrial effects of lipid-derived neurotoxins. J. Alzheimers Dis. 2, 185–193.

Pincemail, J., Defraigne, J.O., Limet, R., 1996. Oxidative stress in clinical situations—fact or fiction? Eur. J. Anaesthesiol. 3, 219–234.

Polidori, M.C., Mecocci, P., 2002. Plasma susceptibility to free radical-induced antioxidant consumption and lipid peroxidation is increased in very old subjects with Alzheimer disease. J. Alzheimers Dis. 6, 517–522.

Polidori, M.C., Cherubini, A., Senin, U., Mecocci, P., 2001a. Peripheral non-enzymatic antioxidant changes with human aging: a selective status report. Biogerontology 2, 99–104.

Polidori, M.C., Stahl, W., Eichler, O., Niestroj, I., Sies, H., 2001b. Profiles of antioxidants in human plasma. Free Radic. Biol. Med. 5, 456–462.

Polidori, M.C., Mattioli, P., Aldred, S., Cecchetti, R., Stahl, W., Griffiths, H., Senin, U., Sies, H., Mecocci, P., 2004. Plasma antioxidant status, immunoglobulin g oxidation and lipid peroxidation in demented patients: relevance to Alzheimer disease and vascular dementia. Dement. Geriatr. Cogn. Disord. 3–4, 265–270.

Polidori, M.C., Griffiths, H.R., Mariani, E., Mecocci, P., 2007. Hallmarks of protein oxidative damage in neurodegenerative diseases: focus on Alzheimer's disease. Amino Acids 4, 553–559.

Porter, N.A., Caldwell, S.E., Mills, K.A., 1995. Mechanisms of free radical oxidation of unsaturated lipids. Lipids 4, 277–290.

Praticò, D., 2008. Evidence of oxidative stress in Alzheimer's disease brain and antioxidant therapy: lights and shadows. Ann. N. Y. Acad. Sci. 70–78.

Praticò, D., Sung, S., 2004. Lipid peroxidation and oxidative imbalance: early functional events in Alzheimer's disease. J. Alzheimers Dis. 2, 171–175.

Praticò, D., Lee, V.M.Y., Trojanowski, J.Q., Rokach, J., Fitzgerald, G.A., 1998. Increased F2-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation in vivo. FASEB J. 15, 1777–1783.

Praticò, D., Clark, C.M., Lee, V.M., Trojanowski, J.Q., Rokach, J., FitzGerald, G.A., 2000. Increased 8,12-iso-iPF2alpha-VI in Alzheimer's disease: correlation of a noninvasive index of lipid peroxidation with disease severity. Ann. Neurol. 5, 809–812.

Praticò, D., Uryu, K., Leight, S., Trojanoswki, J.Q., Lee, V.M., 2001. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. J. Neurosci. 12, 4183–4187.

Praticò, D., Clark, C.M., Liun, F., Rokach, J., Lee, V.Y., Trojanowski, J.Q., 2002. Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. Arch. Neurol. 6, 972–976.

Pryor, W.A., Porter, N.A., 1990. Suggested mechanisms for the production of 4-hydroxy-2-nonenal from the autoxidation of polyunsaturated fatty acids. Free Radic. Biol. Med. 6, 541–543.

Quinn, J.F., Montine, K.S., Moore, M., Morrow, J.D., Kaye, J.A., Montine, T.J., 2004. Suppression of longitudinal increase in CSF F2-isoprostanes in Alzheimer's disease. J. Alzheimers Dis. 1, 93–97.

Ramassamy, C., Averill, D., Beffert, U., Theroux, L., Lussier-Cacan, S., Cohn, J.S., Christen, Y., Schoofs, A., Davignon, J., Poirier, J., 2000. Oxidative insults are associated with apolipoprotein E genotype in Alzheimer's disease brain. Neurobiol. Dis. 1, 23–37.

Reddy, P.H., Beal, M.F., 2008. Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. Trends Mol. Med. 2, 45–53.

Reddy, S., Finkelstein, E.I., Wong, P.S., Phung, A., Cross, C.E., van der Vliet, A., 2002. Identification of glutathione modifications by cigarette smoke. Free Radic. Biol. Med. 11, 1490–1498.

Reed, T., Perluigi, M., Sultana, R., Pierce, W.M., Klein, J.B., Turner, D.M., Coccia, R., Markesbery, W.R., Butterfield, D.A., 2008. Redox proteomic identification of 4-hydroxy-2-nonenal-modified brain proteins in amnestic mild cognitive impairment: insight into the role of lipid peroxidation in the progression and pathogenesis of Alzheimer's disease. Neurobiol. Dis. 1, 107–120.

Reich, E.E., Markesbery, W.R., Roberts 2nd, L.J., Swift, L.L., Morrow, J.D., Montine, T.J., 2001. Brain regional quantification of F-ring and D-/E-ring isoprostanes and neuroprostanes in Alzheimer's disease. Am. J. Pathol. 1, 293–297.

Repetto, M.G., Reides, C.G., Evelson, P., Kohan, S., de Lustig, E.S., Llesuy, S.F., 1999. Peripheral markers of oxidative stress in probable Alzheimer patients. Eur. J. Clin. Invest. 7, 643–649.

Rinaldi, P., Polidori, M.C., Metastasio, A., Mariani, E., Mattioli, P., Cherubini, A., Catani, M., Cecchetti, R., Senin, U., Mecocci, P., 2003. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. Neurobiol. Aging 7, 915–919.

Riviere, S., Birlouez-Aragon, I., Nourhashemi, F., Vellas, B., 1998. Low plasma vitamin C in Alzheimer patients despite an adequate diet. Int. J. Geriatr. Psychiatry 11, 749–754.

Roberts 2nd, L.J., Morrow, J.D., 1997. The generation and actions of isoprostanes. Biochim. Biophys. Acta 2, 121–135.

Roberts 2nd, L.J., Morrow, J.D., 2002. Products of the isoprostane pathway: unique bioactive compounds and markers of lipid peroxidation. Cell. Mol. Life Sci. 5, 808–820.

Roberts 2nd, L.J., Montine, T.J., Markesbery, W.R., Tapper, A.R., Hardy, P., Chemtob, S., Dettbarn, W.D., Morrow, J.D., 1998. Formation of isoprostane-like compounds (neuroprostanes) in vivo from docosahexaenoic acid. J. Biol. Chem. 22, 13605–13612.

Rottkamp, C.A., Atwood, C.S., Joseph, J.A., Nunomura, A., Perry, G., Smith, M.A., 2002. The state versus amyloid-beta: the trial of the most wanted criminal in Alzheimer disease. Peptides 7, 1333–1341.

Ryberg, H., Soderling, A.S., Davidsson, P., Blennow, K., Caidahl, K., Persson, L.I., 2004. Cerebrospinal fluid levels of free 3-nitrotyrosine are not elevated in the majority of patients with amyotrophic lateral sclerosis or Alzheimer's disease. Neurochem. Int. 1, 57–62.

Sano, M., Ernesto, C., Thomas, R.G., Klauber, M.R., Schafer, K., Grundman, M., Woodbury, P., Growdon, J., Cotman, C.W., Pfeiffer, E., Schneider, L.S., Thal, L.J., 1997. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. N. Engl. J. Med. 17, 1216–1222.

Sathiyapriya, V., Selvaraj, N., Nandeesha, H., Bobby, Z., Agrawal, A., Pavithran, P., 2007. Enhanced glycation of hemoglobin and plasma proteins is associated with increased lipid peroxide levels in non-diabetic hypertensive subjects. Arch. Med. Res. 8, 822–826.

Sayre, L.M., Zagorski, M.G., Surewicz, W.K., Krafft, G.A., Perry, G., 1997a. Mechanisms of neurotoxicity associated with amyloid beta deposition and the role of free radicals in the pathogenesis of Alzheimer's disease: a critical appraisal. Chem. Res. Toxicol. 5, 518–526.

Sayre, L.M., Zelasko, D.A., Harris, P.L., Perry, G., Salomon, R.G., Smith, M.A., 1997b. 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease. J. Neurochem. 5, 2092–2097.

Sayre, L.M., Perry, G., Smith, M.A., 2008. Oxidative stress and neurotoxicity. Chem. Res. Toxicol. 1, 172–188.

Schmidt, R., Hayn, M., Reinhart, B., Roob, G., Schmidt, H., Schumacher, M., Watzinger, N., Launer, L.J., 1998. Plasma antioxidants and cognitive performance in middle-aged and older adults: results of the Austrian Stroke Prevention Study. J. Am. Geriatr. Soc. 11, 1407–1410.

Schopfer, F.J., Baker, P.R., Freeman, B.A., 2003. NO-dependent protein nitration: a cell signaling event or an oxidative inflammatory response? Trends Biochem. Sci. 12, 646–654.

Schuessel, K., Leutner, S., Cairns, N.J., Muller, W.E., Eckert, A., 2004. Impact of gender on upregulation of antioxidant defence mechanisms in Alzheimer's disease brain. J. Neural. Transm. 9, 1167–1182.

Seidl, R., Greber, S., Schuller, E., Bernert, G., Cairns, N., Lubec, G., 1997. Evidence against increased oxidative DNA-damage in Down syndrome. Neurosci. Lett. 3, 137–140.

Serra, J.A., Dominguez, R.O., de Lustig, E.S., Guareschi, E.M., Famulari, A.L., Bartolome, E.L., Marschoff, E.R., 2001. Parkinson's disease is associated with oxidative stress: comparison of peripheral antioxidant profiles in living Parkinson's, Alzheimer's and vascular dementia patients. J. Neural. Transm. 10, 1135–1148.

Shadel, G.S., 2005. Mitochondrial DNA, aconitase 'wraps' it up. Trends Biochem. Sci. 6, 294–296.

Shan, X., Lin, C.L., 2006. Quantification of oxidized RNAs in Alzheimer's disease. Neurobiol. Aging 5, 657–662.

Shan, X., Tashiro, H., Lin, C.L., 2003. The identification and characterization of oxidized RNAs in Alzheimer's disease. J. Neurosci. 12, 4913–4921.

Shan, X., Chang, Y., Lin, C.L., 2007. Messenger RNA oxidation is an early event preceding cell death and causes reduced protein expression. FASEB J. 11, 2753–2764.

Shao, C., Xiong, S., Li, G.M., Gu, L., Mao, G., Markesbery, W.R., Lovell, M.A., 2008. Altered 8-oxoguanine glycosylase in mild cognitive impairment and late-stage Alzheimer's disease brain. Free Radic. Biol. Med. 6, 813–819.

Shringarpure, R., Grune, T., Sitte, N., Davies, K.J., 2000. 4-Hydroxynonenal-modified amyloid-beta peptide inhibits the proteasome: possible importance in Alzheimer's disease. Cell. Mol. Life Sci. 12, 1802–1809.

Siegel, S.J., Bieschke, J., Powers, E.T., Kelly, J.W., 2007. The oxidative stress metabolite 4-hydroxynonenal promotes Alzheimer protofibril formation. Biochemistry 6, 1503–1510.

Siems, W., Quast, S., Carluccio, F., Wiswedel, I., Hirsch, D., Augustin, W., Hampi, H., Riehle, M., Sommerburg, O., 2002. Oxidative stress in chronic renal failure as a cardiovascular risk factor. Clin. Nephrol. S12–S19.

Sies, H., 1985. Oxidative Stress: Introductory Remarks. Academic Press, London.

Sinclair, A.J., Bayer, A.J., Johnston, J., Warner, C., Maxwell, S.R., 1998. Altered plasma antioxidant status in subjects with Alzheimer's disease and vascular dementia. Int. J. Geriatr. Psychiatry 12, 840–845.

Skinner, E.R., Watt, C., Besson, J.A., Best, P.V., 1993. Differences in the fatty acid composition of the grey and white matter of different regions of the brains of patients with Alzheimer's disease and control subjects. Brain 717–725.

Small, G.W., Rabins, P.V., Barry, P.P., Buckholtz, N.S., DeKosky, S.T., Ferris, S.H., Finkel, S.I., Gwyther, L.P., Khachaturian, Z.S., Lebowitz, B.D., McRae, T.D., Morris, J.C., Oakley, F., Schneider, L.S., Streim, J.E., Sunderland, T., Teri, L.A., Tune, L.E., 1997. Diagnosis and treatment of Alzheimer disease and related disorders. Consensus statement of the American Association for Geriatric Psychiatry, the Alzheimer's Association, and the American Geriatrics Society. JAMA 16, 1363–1371.

Smith, C.D., Carney, J.M., Starke-Reed, P.E., Oliver, C.N., Stadtman, E.R., Floyd, R.A., Markesbery, W.R., 1991. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc. Natl. Acad. Sci. U.S.A. 23, 10540–10543.

Smith, M.A., Perry, G., Richey, P.L., Sayre, L.M., Anderson, V.E., Beal, M.F., Kowall, N., 1996. Oxidative damage in Alzheimer's. Nature 6587, 120–121.

Smith, M.A., Harris, P.L., Sayre, L.M., Perry, G., 1997a. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. Proc. Natl. Acad. Sci. U.S.A. 18, 9866–9868.

Smith, M.A., Richey Harris, P.L., Sayre, L.M., Beckman, J.S., Perry, G., 1997b. Widespread peroxynitrite-mediated damage in Alzheimer's disease. J. Neurosci. 8, 2653–2657.

Sohal, R.S., Agarwal, S., Dubey, A., Orr, W.C., 1993. Protein oxidative damage is associated with life expectancy of houseflies. Proc. Natl. Acad. Sci. U.S.A. 15, 7255–7259.

Song, S., Jung, Y.K., 2004. Alzheimer's disease meets the ubiquitin-proteasome system. Trends Mol. Med. 11, 565–570.

Song, W.L., Lawson, J.A., Reilly, D., Rokach, J., Chang, C.T., Giasson, B., Fitzgerald, G.A., 2008. Neurofurans, novel indices of oxidant stress derived from docosahexaenoic acid. J. Biol. Chem. 1, 6–16.

Stadelmann, C., Bruck, W., Bancher, C., Jellinger, K., Lassmann, H., 1998. Alzheimer disease: DNA fragmentation indicates increased neuronal vulnerability, but not apoptosis. J. Neuropathol. Exp. Neurol. 5, 456–464.

Stadtman, E.R., Levine, R.L., 2003. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids 3–4, 207–218.

Stadtman, E.R., Moskovitz, J., Levine, R.L., 2003. Oxidation of methionine residues of proteins: biological consequences. Antioxid. Redox Signal. 5, 577–582.

Stocker, R., Keaney Jr., J.F., 2004. Role of oxidative modifications in atherosclerosis. Physiol. Rev. 4, 1381–1478.

Su, J.H., Anderson, A.J., Cummings, B.J., Cotman, C.W., 1994. Immunohistochemical evidence for apoptosis in Alzheimer's disease. Neuroreport 18, 2529–2533.

Su, J.H., Deng, G., Cotman, C.W., 1997. Neuronal DNA damage precedes tangle formation and is associated with up-regulation of nitrotyrosine in Alzheimer's disease brain. Brain Res. 1–2, 193–199.

Subbarao, K.V., Richardson, J.S., Ang, L.C., 1990. Autopsy samples of Alzheimer's cortex show increased peroxidation in vitro. J. Neurochem. 1, 342–345.
Sugaya, K., Reeves, M., McKinney, M., 1997. Topographic associations between DNA fragmentation and Alzheimer's disease neuropathology in the hippocampus. Neurochem. Int. 2, 275–281.
Sultana, R., Boyd-Kimball, D., Poon, H.F., Cai, J., Pierce, W.M., Klein, J.B., Markesbery, W.R., Zhou, X.Z., Lu, K.P., Butterfield, D.A., 2006a. Oxidative modification and down-regulation of Pin1 in Alzheimer's disease hippocampus: a redox proteomics analysis. Neurobiol. Aging 7, 918–925.
Sultana, R., Boyd-Kimball, D., Poon, H.F., Cai, J., Pierce, W.M., Klein, J.B., Merchant, M., Markesbery, W.R., Butterfield, D.A., 2006b. Redox proteomics identification of oxidized proteins in Alzheimer's disease hippocampus and cerebellum: an approach to understand pathological and biochemical alterations in AD. Neurobiol. Aging 11, 1564–1576.
Sultana, R., Perluigi, M., Butterfield, D.A., 2006c. Redox proteomics identification of oxidatively modified proteins in Alzheimer's disease brain and in vivo and in vitro models of AD centered around Abeta(1–42). J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 1, 3–11.
Sultana, R., Perluigi, M., Butterfield, D.A., 2006d. Protein oxidation and lipid peroxidation in brain of subjects with Alzheimer's disease: insights into mechanism of neurodegeneration from redox proteomics. Antioxid. Redox Signal. 11–12, 2021–2037.
Sultana, R., Poon, H.F., Cai, J., Pierce, W.M., Merchant, M., Klein, J.B., Markesbery, W.R., Butterfield, D.A., 2006e. Identification of nitrated proteins in Alzheimer's disease brain using a redox proteomics approach. Neurobiol. Dis. 1, 76–87.
Sultana, R., Piroddi, M., Galli, F., Butterfield, D.A., 2008. Protein levels and activity of some antioxidant enzymes in hippocampus of subjects with amnestic mild cognitive impairment. Neurochem. Res. 12, 2540–2546.
Szymanski, M., Barciszewska, M.Z., Erdmann, V.A., Barciszewski, J., 2005. A new frontier for molecular medicine: noncoding RNAs. Biochim. Biophys. Acta 1, 65–75.
Tamagno, E., Bardini, P., Guglielmotto, M., Danni, O., Tabaton, M., 2006. The various aggregation states of beta-amyloid 1–42 mediate different effects on oxidative stress, neurodegeneration, and BACE-1 expression. Free Radic. Biol. Med. 2, 202–212.
Te Koppele, J.M., Lucassen, P.J., Sakkee, A.N., Van Asten, J.G., Ravid, R., Swaab, D.F., Van Bezooijen, C.F., 1996. 8OHdG levels in brain do not indicate oxidative DNA damage in Alzheimer's disease. Neurobiol. Aging 6, 819–826.
Tohgi, H., Abe, T., Yamazaki, K., Murata, T., Ishizaki, E., Isobe, C., 1999. Alterations of 3-nitrotyrosine concentration in the cerebrospinal fluid during aging and in patients with Alzheimer's disease. Neurosci. Lett. 1, 52–54.
Tuppo, E.E., Forman, L.J., Spur, B.W., Chan-Ting, R.E., Chopra, A., Cavalieri, T.A., 2001. Sign of lipid peroxidation as measured in the urine of patients with probable Alzheimer's disease. Brain Res. Bull. 5, 565–568.
Uchida, K., 2000. Role of reactive aldehyde in cardiovascular diseases. Free Radic. Biol. Med. 12, 1685–1696.
Uchida, K., 2003a. Histidine and lysine as targets of oxidative modification. Amino Acids 3–4, 249–257.
Uchida, K., 2003b. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. Prog. Lipid Res. 4, 318–343.
Uchida, K., Kanematsu, M., Morimitsu, Y., Osawa, T., Noguchi, N., Niki, E., 1998. 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. 26, 16058–16066.
Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 1, 44–84.
Vatassery, G.T., 2004. Impairment of brain mitochondrial oxidative phosphorylation accompanying vitamin E oxidation induced by iron or reactive nitrogen species: a selective review. Neurochem. Res. 11, 1951–1959.
Volkel, W., Alvarez-Sanchez, R., Weick, I., Mally, A., Dekant, W., Pahler, A., 2005. Glutathione conjugates of 4-hydroxy-2(E)-nonenal as biomarkers of hepatic oxidative stress-induced lipid peroxidation in rats. Free Radic. Biol. Med. 11, 1526–1536.
Volkel, W., Sicilia, T., Pahler, A., Gsell, W., Tatschner, T., Jellinger, K., Leblhuber, F., Riederer, P., Lutz, W.K., Gotz, M.E., 2006. Increased brain levels of 4-hydroxy-2-nonenal glutathione conjugates in severe Alzheimer's disease. Neurochem. Int. 8, 679–686.
Voss, P., Grune, T., 2007. The nuclear proteasome and the degradation of oxidatively damaged proteins. Amino Acids 4, 527–534.
Waddington, E., Croft, K., Clarnette, R., Mori, T., Martins, R., 1999. Plasma F2-isoprostane levels are increased in Alzheimer's disease: evidence of increased oxidative stress in vivo. Alzheimer's Rep. 2, 277–282.
Wallace, D.C., 2005. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu. Rev. Genet. 359–407.
Walsh, D.M., Klyubin, I., Fadeeva, J.V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J., Selkoe, D.J., 2002. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 6880, 535–539.
Wamer, W.G., Wei, R.R., 1997. In vitro photooxidation of nucleic acids by ultraviolet A radiation. Photochem. Photobiol. 3, 560–563.
Wang, J., Xiong, S., Xie, C., Markesbery, W.R., Lovell, M.A., 2005. Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. J. Neurochem. 4, 953–962.
Wang, J., Markesbery, W.R., Lovell, M.A., 2006. Increased oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment. J. Neurochem. 3, 825–832.
Weissman, L., Jo, D.G., Sorensen, M.M., de Souza-Pinto, N.C., Markesbery, W.R., Mattson, M.P., Bohr, V.A., 2007. Defective DNA base excision repair in brain from individuals with Alzheimer's disease and amnestic mild cognitive impairment. Nucleic Acids Res. 16, 5545–5555.
Williams, T.I., Lovell, M.A., Lynn, B.C., 2005. Analysis of derivatized biogenic aldehydes by LC tandem mass spectrometry. Anal. Chem. 10, 3383–3389.
Williams, T.I., Lynn, B.C., Markesbery, W.R., Lovell, M.A., 2006. Increased levels of 4-hydroxynonenal and acrolein, neurotoxic markers of lipid peroxidation, in the brain in Mild Cognitive Impairment and early Alzheimer's disease. Neurobiol. Aging 8, 1094–1099.
Wilson, R., Lyall, K., Smyth, L., Fernie, C.E., Riemersma, R.A., 2002. Dietary hydroxy fatty acids are absorbed in humans: implications for the measurement of 'oxidative stress' in vivo. Free Radic. Biol. Med. 2, 162–168.
Winblad, B., Palmer, K., Kivipelto, M., Jelic, V., Fratiglioni, L., Wahlund, L.O., Nordberg, A., Backman, L., Albert, M., Almkvist, O., Arai, H., Basun, H., Blennow, K., de Leon, M., DeCarli, C., Erkinjuntti, T., Giacobini, E., Graff, C., Hardy, J., Jack, C., Jorm, A., Ritchie, K., van Duijn, C., Visser, P., Petersen, R.C., 2004. Mild cognitive impairment—beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J. Intern. Med. 3, 240–246.
Yan, S.D., Chen, X., Schmidt, A.M., Brett, J., Godman, G., Zou, Y.S., Scott, C.W., Caputo, C., Frappier, T., Smith, M.A., et al., 1994. Glycated tau protein in Alzheimer disease: a mechanism for induction of oxidant stress. Proc. Natl. Acad. Sci. U.S.A. 16, 7787–7791.
Yan, S.D., Yan, S.F., Chen, X., Fu, J., Chen, M., Kuppusamy, P., Smith, M.A., Perry, G., Godman, G.C., Nawroth, P., et al., 1995. Non-enzymatically glycated tau in Alzheimer's disease induces neuronal oxidant stress resulting in cytokine gene expression and release of amyloid beta-peptide. Nat. Med. 7, 693–699.
Yan, S.D., Chen, X., Fu, J., Chen, M., Zhu, H., Roher, A., Slattery, T., Zhao, L., Nagashima, M., Morser, J., Migheli, A., Nawroth, P., Stern, D., Schmidt, A.M., 1996. RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. Nature 6593, 685–691.
Yan, L.J., Levine, R.L., Sohal, R.S., 1997. Oxidative damage during aging targets mitochondrial aconitase. Proc. Natl. Acad. Sci. U.S.A. 21, 11168–11172.
Yao, Y., Zhukareva, V., Sung, S., Clark, C.M., Rokach, J., Lee, V.M., Trojanowski, J.Q., Pratico, D., 2003. Enhanced brain levels of 8,12-iso-iPF2alpha-VI differentiate AD from frontotemporal dementia. Neurology 4, 475–478.
Yehuda, S., Rabinovitz, S., Carasso, R.L., Mostofsky, D.I., 2002. The role of polyunsaturated fatty acids in restoring the aging neuronal membrane. Neurobiol. Aging 5, 843–853.
Zandi, P.P., Anthony, J.C., Khachaturian, A.S., Stone, S.V., Gustafson, D., Tschanz, J.T., Norton, M.C., Welsh-Bohmer, K.A., Breitner, J.C., 2004. Reduced risk of Alzheimer disease in users of antioxidant vitamin supplements: the Cache County Study. Arch. Neurol. 1, 82–88.
Zhu, X., Rottkamp, C.A., Boux, H., Takeda, A., Perry, G., Smith, M.A., 2000. Activation of p38 kinase links tau phosphorylation, oxidative stress, and cell cycle-related events in Alzheimer disease. J. Neuropathol. Exp. Neurol. 10, 880–888.
Zhu, X., Raina, A.K., Perry, G., Smith, M.A., 2004. Alzheimer's disease: the two-hit hypothesis. Lancet Neurol. 4, 219–226.
Zhu, X., Lee, H.G., Perry, G., Smith, M.A., 2007a. Alzheimer disease, the two-hit hypothesis: an update. Biochim. Biophys. Acta 4, 494–502.
Zhu, X., Su, B., Wang, X., Smith, M.A., Perry, G., 2007b. Causes of oxidative stress in Alzheimer disease. Cell Mol. Life Sci. 17, 2202–2210.