NIH Public Access
Author Manuscript
Free Radic Biol Med. Author manuscript; available in PMC 2009 August 1.

Published in final edited form as:
Free Radic Biol Med. 2008 August 1; 45(3): 219–230.

Free radical-mediated damage to brain in Alzheimer's disease and its transgenic mouse models

Joshua A. Sonnen, **John C. Breitner**, **Mark A. Lovell**, **William R. Markesbery**, **Joseph F. Quinn**, and **Thomas J. Montine**
Departments of Pathology and of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA, Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY, and Department of Neurology, Oregon Health & Science University, Portland, OR

INTRODUCTION

Advances in our understanding of the etiologies and pathogenesis of Alzheimer's disease (AD) highlight a role for free radical-mediated injury to brain regions from early stages of this illness. Here we will review the evidence from transgenic mouse models of AD, autopsy samples, and human biofluids obtained during life paying particular attention to the stage of disease. In addition, we will review the epidemiologic literature that addresses the potential of anti-oxidants to prevent incident dementia from AD, and the clinical trial literature that addresses anti-oxidant preventative or therapeutic strategies for different stage of AD. Future efforts in preclinical models and ultimately clinical trials are needed to define optimally effective agents and combinations, doses, and timing to suppress safely this facet of AD.

I. Alzheimer's disease and its forms and stages

What is commonly referred to as Alzheimer's disease (AD) is really a syndrome, a common clinico-pathological entity with multiple causes. Rare early-onset forms of this syndrome are caused by highly penetrant autosomal dominant mutations in one of three different genes: amyloid precursor protein (APP) gene, presenilin (PS) 1 gene, or presenilin 2 gene [1]. In addition, apparently similar processes afflict adults with trisomy 21 or Down's syndrome. However, it is late-onset AD (LOAD) that represents a significant and growing public health burden, currently affecting between 2.5 and 4 million people in the U.S., and more than 10 million individuals worldwide [2]. The causes of LOAD are not yet clarified, but several environmental and genetic risk factors have been identified; the most potent of these, other than age, is the ε4 allele of the apolipoprotein (apo) E gene (*APOE*) [3]. LOAD is projected to grow to staggering prevalence in the next generation with an estimated 8 to 12 million patients by the year 2050 in the U.S. alone [4]. In addition to causing untold suffering by patients and their families, LOAD is the third most costly medical condition in the U.S. [5–7]. As the number of patients afflicted continues to mount, the need for safe and effective therapy to delay or avert LOAD will become imperative [8]. In the following we will use AD to refer to this disease in general terms, and use LOAD or other designations to refer to specific forms.

Pathologic processes of AD precede clinically diagnosed dementia by as much as 2 or 3 decades. Indeed, as early as 1976, Katzman proposed a chronic disease model for AD, including

Corresponding author: Thomas J. Montine, MD, PhD, Department of Pathology, University of Washington, Harborview Medical Center, 300 9th Ave, Seattle, WA 98104, 206-744-6776, tmontine@u.washington.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

a latent stage during which some structural damage accrues but there is no functional or behavioral change, followed by a prodromal stage during which more structural damage accrues and mild functional and behavioral changes first become evident, and ultimately by a clinical stage with substantial irreversible damage and behavioral abnormalities that manifest as the dementia syndrome [9]. Identification of individuals with latent and prodromal AD is an area of active investigation. Mild cognitive impairment (MCI) is defined by clinical criteria that identify a subset of individuals enriched for those with prodromal AD (reviewed in [10]). Data supporting the existence of latent AD are extensive and derive from pathologic, neuroimaging, and biomarker studies that all have shown AD-type neurodegenerative changes in a substantial subset of older individuals rigorously demonstrated to be cognitively normal [11–39]. Importantly, these concepts of latent and prodromal AD imply an opportunity to intervene early and prevent the clinical expression of dementia. Key to successful intervention will be knowledge of the critical pathogenic events at early stages of AD progression.

Abundant *in vitro* and *in vivo* data now support the proposal that accumulation of amyloid (A) β-containing senile plaques and paired helical filament tau-containing neurofibrillary tangles (NFTs) in brain regions in AD lie distal in a complex pathogenic cascade that includes earlier formation of abnormal Aβ aggregates. This somehow sets in motion a series of partially overlapping events including disturbed cell signaling, innate immune activation, mitochondrial dysfunction, excitotoxicity, abnormal glycation, and altered metabolism of metal ions, among others (reviewed in [40]). Several of these pathogenic processes can produce free radical stress [41] which in turn can promote further Aβ aggregation and thereby potentially propagate the pathogenic cascade [42] from reversible loss of synapses to death of neurons [43]. However, it is important to stress that the associations between these hallmark structures of AD and markers of free radical damage are complex [44].

Here we will review the evidence for increased free radical-mediated damage to brain in transgenic models of AD, autopsy samples, and human biofluids obtained during life paying particular attention to the stage of LOAD. In addition, we will review the epidemiologic literature that addresses the potential of anti-oxidants to prevent incident dementia from LOAD, and the clinical trial literature that addresses anti-oxidant preventative or therapeutic strategies for MCI or the dementia stage of LOAD. These epidemiologic studies and clinical trials typically rely on the concept of “probable” AD as a consensus designation for the highest clinical certainty that the dementia suffered by an individual derives from AD; “definite” AD requires autopsy confirmation [45]. The sensitivity and specificity of a clinical diagnosis of probable AD, compared against neuropathologic examination, has been determined in referral centers where clinical diagnoses generally achieve a sensitivity of approximately 90 to 95% but a specificity of only 50 to 60% because co-morbid conditions can contribute to cognitive impairment and dementia but are difficult to distinguish from AD by current clinical criteria.

II. Markers of free radical-mediated damage

Free radical-mediated stress is met by anti-oxidant defenses that, when overwhelmed or exhausted, result in damage to tissue. Free radical stress can be assessed by measuring the reserve in anti-oxidant defenses or increased expression of anti-oxidant enzymes. Free radical-mediated damage to brain typically is assayed by measuring oxidative modifications to lipids, proteins, or nucleic acids.

Peroxidation of polyunsaturated fatty acids, or lipid peroxidation, is especially important because it is a self-propagating reaction that will continue until terminated by defenses or until substrate is exhausted. Attack of polyunsaturated fatty acids by free radicals leads to structural damage to membranes and the generation of several aldehyde by-products including malondialdehyde and $C_3 - C_{10}$ straight chain aldehydes as well as α,β-unsaturated aldehydes including 4-hydroxy-2-nonenal (HNE) and acrolein. The α,β-unsaturated aldehydes in

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

particular may be primary effectors of tissue damage since they show high reactivity with nucleophiles including sulfhydryl groups of cysteine, histidine, and lysine [46], and impair key neuronal processes [47,48]. Lipid peroxidation also produces oxidized and endocyclized products of arachidonic acid (F_2-isoprostanes or F_2-IsoPs) or docosohexaenoic acid (F_4-neuroprostanes or F_4-NeuroPs) that are quantitative in vivo biomarkers of free radical damage [49,50].

Free radicals, particularly the hydroxyl radical, also attack nucleic acids leading to strand breaks, cross linking, and base modifications that may contribute to alterations in protein production and propagate neuron dysfunction and death. Mitochondrial DNA (mtDNA) is more susceptible to free radical-mediated damage than nuclear DNA (nDNA) because of its proximity to the site of production of reactive oxygen species (ROS), and because of its lack of protective histones, limited repair capacity, and lack of significant noncoding sequences. As reviewed by Dizdaroglu et al. [51], ROS attack of DNA can lead to the generation of more than 20 oxidized base adducts, the most prominent being 8-hydroxydeoxyguanine (8-OHdG) because of guanine's relatively low oxidation potential [51]. In addition to direct oxidation by ROS, DNA can also be modified by α,β-unsaturated aldehyde by-products of lipid peroxidation through an initial Michael addition of the exocyclic amino group followed by ring closure of N-1 onto the aldehyde group to generate a bulky exocyclic 1-N^2-propanodeoxyguanosine adduct [52]. These adducts are potentially biologically relevant because they may promote DNA-DNA and DNA-protein cross-linking that can limit transcription [53].

While protein oxidation is as biochemically complex as peroxidation of lipids and oxidation of nucleic acids, assays for protein oxidation are dominated by a relatively simple global assessment of protein carbonyls. The widely used spectrophotomertic assay for protein carbonyls detects primarily glutamic semialdehyde, an oxidation product of arginine and proline, or aminoadipic semialdehyde, an oxidation product of lysine. Like products of lipid peroxidation, there is overwhelming evidence from many experimental perspectives that protein oxidation is an effector of cellular dysfunction and not simply a reflection of damaged tissue [54].

III. Transgenic mice

Transgenic mice that reproduce selected facets of AD pathogenesis have been created, and many of these have been investigated for associations with free radical-mediated damage. All of the transgenic mouse models reviewed below are in fact models of dominantly inherited forms of AD or related neurodegenerative diseases, so that extrapolations to LOAD should be made with caution. Some strains of transgenic mice develop Aβ plaques, some accumulate NFTs, and some do both. The age of onset and the magnitude of pathologic change also vary across mouse strains. Mice expressing mutant human amyloid precursor protein (APP) develop Aβ plaques at about 10–12 months of life, but do not develop NFTs. Mice that express both mutant APP and mutant human presenilin-1 (APP-PS1) accumulate Aβ plaques at a younger age, but still do not develop NFTs. The onset of plaque pathology can also be accelerated by expressing two different mutant APP genes in a single mouse, again with no NFT formation. Accumulation of NFTs is achieved in mice carrying mutant human tau genes that are associated with frontotemporal dementia, a relatively uncommon neurodegenerative disease characterized by NFT formation but not senile plaques. Predictably, these mice do not develop Aβ plaques. The development of both Aβ plaques and NFTs has been achieved recently in a "triple transgenic" mouse expressing APP, PS1, and the mutant tau protein, and also with conditional expression neuronal expression of SV40 T antigen [55].

The role of oxidative damage in each of these transgenic mouse models has been examined to varying extents. These studies are summarized in Table 1. Since the transgenic mouse strains vary in both transgene and genetic background, we will consider the published findings for

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

each strain in turn. It is also important to realize that AD pathologic changes often occupy less than 5% of cortical or hippocampal volume in some strains, so that measurements using homogenates may "dilute" small amounts of damaged tissue with larger amounts of unaffected tissue. Histochemical techniques permit greater distinction of localized changes, but are not robustly quantitative. Consequently, measurements using homogenates and histochemical findings should be viewed as complementary.

Most of the animal studies of oxidative damage associated with AD pathology have used the Tg2576 strain, which expresses mutant human APP containing the "Swedish" mutation associated with autosomal dominant AD. These mice express the transgene from the time of birth, and begin to develop both Aβ plaques and spatial memory impairment at 10–12 months of age [56]. Several studies have examined the role of oxidative damage in this murine model of AD using F_2-IsoPs. A study examining serial brain, plasma, and urine F_2-IsoPs by the "modified method" (see Section V for description of F_2-IsoP methods) found elevation in all three at 8 months of age, before to the appearance of plaque pathology, with further marked increases at 12 and 18 months of age, correlating with the onset and progression of Aβ plaque accumulation [57]. However, other investigators comparing 14–20 month old Aβ plaque-bearing Tg2576 mice with age-matched wild type (wt) mice have repeatedly found no increase in cerebral cortical F_2-IsoPs ("original method") or F_4-Neuroprostanes (NeuroPs)[58,59]. Moreover, massive acute cerebral oxidative damage from status epilepticus induced in rats by kainate exposure fails to result in elevated plasma or urine F_2-IsoPs, seriously challenging the idea that these markers in peripheral biofluids reflect neurochemical changes in brain.[60] An immunohistochemical study of HNE-protein adducts in Tg2576 mice showed increased immunoreactive labeling in dystrophic neurites adjacent to plaques [61]; although we could not replicate this finding using polyclonal antiserum that identified HNE-protein adducts in sections from patients who died from LOAD [62,63]. Elevated brain levels of a cholesterol oxidation product have been reported in Tg2576 mice compared to wt mice [64]. Cole and colleagues have repeatedly reported increased hippocampal protein carbonyls in Tg2576 mice using a Western blot method [65,66], and have observed that this outcome can be profoundly influenced by concentration of anti-oxidant in diet. Another report found no increase in cerebral cortical protein carbonyls (measured by ELISA) in Tg2576 compared to wild type mice, even though the carbonyl measurement was sensitive to the effects of treatment [67]. Other evidence suggests free radical stress in brains of Tg2576 mice is expression of compensatory antioxidant enzymes. Immunohistochemical studies have localized superoxide dismutase and heme oxygenase in the immediate vicinity of Aβ plaques [61]. The deposition of Aβ plaques at 10–12 months of age also is temporally associated with an increase in cortical superoxide dismutase and glutathione peroxidase [68].

Fewer investigations of free radical-mediated injury have been conducted for the other transgenic mouse models of AD. In general, oxidative damage is greater in mice with single APP or PS1 mutations than in wild type animals, and higher still in doubly transgenic mice expressing the combination of mutant APP and mutant PS1 (APP-PS1) [69]. Cortical homogenates from these same mice have elevated protein carbonyls and HNE when compared to wild type. The role of oxidative damage in NFT accumulation has been examined to a limited extent. In mice that express a wild-type human tau isoform and develop insoluble tau aggregates, cerebral cortical protein carbonyl levels are increased when measured by Western blot [70]. Oxidative damage to nucleic acids is also increased in these mice, as measured by immunohistochemistry for 8-OHdG. Levels of oxidative damage in CNS tissue have not yet been reported for a P301 tau transgenic mouse that develops NFTs primarily in spinal cord [71], nor for a model of P301 tau conditional expression that develops NFTs in forebrain [72]. A transgenic mouse expressing mutant APP, PS1, and tau recently has been developed as a more "complete" model of AD pathologic changes, developing both Aβ plaques and NFTs

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

[73,74]. However, we are unaware of any study of this strain that has examined the role of oxidative damage.

As shown in Table 1, the evidence for increased free radical-mediated damage in cerebral cortex and hippocampus (HP) of the most widely used transgenic model of AD, the Tg2576 mouse, is variable, and suggests that local factors such as inbred substrains or diet may be as important as transgene expression in producing oxidative damage in these mice. Studies of oxidative damage in other transgenic mouse models are fewer but appear to show more consistently increased free radical damage to cerebral cortex, especially APP-PS1 models.

Several of the laboratories that regularly observe free radical-mediated damage in the cerebral cortex or HP of aged Tg2576 mice have explored cause-and-effect relationships by treating these mice with a variety of antioxidants (Table 2) Although some investigators have expressed disappointment with α-tocopherol in these experiments, [75], others have shown that vitamin E (isoforms not specified) can attenuate both oxidative damage and Aβ plaque accumulation if it is initiated early in life [76]. The hypothesis that oxidative stress promotes Aβ deposition is strengthened further by a study that crossed Tg2576 mice with mice deficient in α-tocopherol transfer protein, finding that these double mutants have increased cerebral oxidative damage and accelerated Aβ deposition [77]. Mice expressing a human tau gene have also been treated with α-tocopherol, resulting in decreased brain protein carbonyls, 8OHdG, and tau pathology [70]. While α-tocopherol is the dominant isoform in synthetic vitamin E supplements, γ-tocopherol is the dominant isoform from food. Most transgenic mouse studies and, to date, all clinical trials, have used α-tocopherol, even though peripherally administered γ-tocopherol is at least as effective as α-tocopherol in suppressing cerebral F_2-IsoP and F_4-NeuroP formation after intra-cerebroventricular kainate injection, a model of excitotoxic neurodegeneration [78], as well as suppressing striatal dopaminergic toxicity from methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [79].

A readily available antioxidant with great promise based on transgenic mouse models of AD is curcumin, a component of the curry spice turmeric. Orally administered curcumin reduces brain protein carbonyls and Aβ burden in Tg2576 mice [80], even when initiated after the age of plaque formation [81]. Another readily available and non-toxic antioxidant is melatonin. As with vitamin E, melatonin treatment of Tg2576 mice early in life attenuates Aβ deposition [82], but initiation after the age of plaque formation does not alter Aβ burden[83]. Yet another well tolerated antioxidant, α-lipoic acid, improves hippocampal-dependent memory in Tg2576 mice but has no effect on cerebral lipid peroxidation or Aβ plaque burden [59]. Similarly, pyrrolidine dithiocarbamate, a clinically tolerated metal chelator, potent antioxidant, and inhibitor of nuclear factor κB, improves spatial learning in APP-PS1 mice but has no effect on Aβ burden [84]. Finally, coenzyme Q supplementation protects mutant APP transgenic mice from vulnerability to ischemia [85], but has not been shown to affect Aβ deposition.

IV. Autopsy

Numerous autopsy studies have evaluated free radical-mediated damage in tissue obtained from individuals who were diagnosed at death with MCI or dementia from LOAD (Table 3). Excellent reviews are available on regionally increased oxidative damage to lipids in diseased regions of brain from patients with MCI or LOAD [86,87]. Studies of lipid peroxidation in LOAD are more numerous than studies of other markers, and have been reviewed thoroughly elsewhere [88,89]. Importantly, the levels of markers of lipid peroxidation in brain regions from patients with MCI are comparable to those observed in patients with LOAD.

Multiple studies report increased protein carbonyls in diseased regions of brain from patients with LOAD (reviewed by [87,90]), and again patients with MCI have been observed to have comparable levels [91]. Using redox proteomics, one group has identified a small number of

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

specific proteins targeted by carbonyl formation as well as other oxidative modifications. Their results have focused on about 15 proteins that have broad cellular functions and are distributed across several different organelles or cellular compartments. This work has been reviewed recently in this journal [92]. Notably, there is at least partial overlap in the oxidized proteins identified by this approach in tissue from patients with MCI or LOAD.

Several studies now have also demonstrated significantly elevated 8-OHdG as well as other oxidatively modified bases in nDNA and mtDNA from diseased regions of brain from patients with MCI or LOAD (reviewed in [86,93]). As with lipid peroxidation and protein carbonyl markers, levels of 8-OHdG and other oxidized DNA bases in MCI are comparable to those observed in LOAD brain. Again, consistent with mtDNA's proximity to ROS generation in the mitochondria and the relatively limited DNA repair capacity in mitochondria the extent of oxidation in mtDNA is approximately 10-fold greater than in nDNA [94,95]. There have been a few studies of lipid peroxidation-derived aldehyde-DNA adducts. One of these showed an approximately 2-fold increased in acrolein-guanosine adducts in nDNA isolated from HP of LOAD patients compared to controls [96]; however, HNE-guanosine adduct concentrations in nDNA from parietal lobe (PL) and HP of LOAD subjects were found not to be significantly different from controls [52]. These findings are consistent with earlier studies of Gotz et al. [97], who showed no significant differences in HNE-guanosine adducts using ^{32}P post-labeling of deoxyguanosine adducts from DNA isolated from HP, PL, and cerebellum (CBLM) of LOAD subjects compared to controls. One group studied the cellular distribution of DNA adducts by immunohistochemistry and observed more pronounced immunoreactivity in LOAD subjects compared to age-matched control subjects. This immunoreactivity was not restricted to NFT-bearing neurons or to cells closely localized to senile plaques [98].

Increased oxidative RNA damage has been reported in a variety of neurological disorders including LOAD (reviewed in [99]). Increased RNA oxidation has also been observed in a presymptomatic subject who inherited a familial AD mutation [100] and in Down syndrome subjects with AD pathologic changes [99]. More recently, Ding et al. [101] showed significantly elevated 8-OHG in PL but not CBLM of MCI patients that correlated with decreased ribosomal and transfer RNA and decreased protein synthesis capacity. Using immunohistochemistry and an 8-OHG antibody, Lovell and Markesbery [102] showed significantly increased RNA oxidation in degenerating hippocampal neurons in MCI and LOAD patients compared to age-matched controls.

Overall, the studies described above underscore that free radical-mediated damage to human brain from the processes of AD appears to be focused largely in neurons but is otherwise a largely indiscriminant process that damages many different cellular components. Data from lipid peroxidation, protein oxidation, nDNA oxidation, mtDNA oxidation, and RNA oxidation all show comparable increases in brain regions from patients with MCI and LOAD, indicating advanced free radical-mediated damage at this pre-dementia stage.

V. Biofluid studies

Much of the work on biomarkers of oxidative damage in AD has focused on F_2-IsoPs. For that reason, a brief overview of the different approaches to quantifying unesterified F_2-IsoPs in biofluids is in order. Since F_2-IsoPs are a mixture are comprised of 4 regioisomers each of which are theoretically comprised of 8 racemic diastereomers, different isomers have been measured to reflect overall levels of lipid peroxidation. In studies of neurodegeneration, F_2-IsoPs have been quantified by one of four different methods: commercially available enzyme-linked immunosorbent assays (ELISAs) [103], two different gas chromatography- (GC-) mass spectrometry (MS) stable isotope dilution methods that we refer to as the "original method" [104] or "modified method" [105], and most recently by liquid chromatography- (LC-) MS [106]. The two GC-MS methods are similar, and quantify subsets of F_2-IsoPs that

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

co-elute with the deuterated internal standards used; this will be key to comparing studies presented below. The original GC-MS method used a deuterated 8-iso-$PGF_{2\alpha}$ internal standard (also known as $iPF_{2\alpha}$ -III) and quantified those F_2-IsoPs in the peak that co-migrated with this standard [107]; this peak not only contains 8-iso-$PGF_{2\alpha}$ but also other F_2-IsoPs [107]. For this reason the subset quantified by the original GC-MS method is conservatively referred to as "F_2-IsoPs". The modified GC-MS method uses a different GC protocol and different deuterated internal standards, $iPF_{2\alpha}$-VI and 8-,12-iso-$iPF_{2\alpha}$-VI [105,108]. This assay quantifies the peak that co-migrates with each deuterated standard and refers to what is quantified as $iPF_{2\alpha}$-III, $iPF_{2\alpha}$-VI, or 8-,12-iso-$iPF_{2\alpha}$-VI.

Cerebrospinal fluid (CSF) derives in part from a transudate of plasma at the choroids plexus and in part from the extracellular fluid of brain; it is this latter feature that motivates many neurochemical studies of brain metabolism by sampling and analyzing CSF. CSF obtained from the lateral ventricles at autopsy has been assayed for F_2-IsoPs. These studies represent a bridge between *post mortem* tissue studies (*vide supra*) and the analysis of CSF from the lumbar cistern of living patients described below. Using either the original or modified methods, the concentrations of F_2-IsoPs in ventricular CSF are elevated on average between 56 and 168% in patients with mild to moderate dementia from LOAD compared with age-matched controls by both the original and modified methods [109,110]. In addition, ventricular CSF F_2-IsoP concentrations in patients with LOAD are significantly correlated with histologic indices of neurodegeneration [111].

The first study of probable AD patients showed that F_2-IsoPs are significantly elevated by approximately 60% in lumbar CSF of patients with early dementia as contrasted with fluid from age-matched hospitalized patients without neurologic disease [112]. This same result was replicated in additional groups of probable AD patients and controls using both the original method [113–115] and the modified method [108], although the latter showed an average increase in LOAD exceeding 200%. In distinction to autopsy studies where tissue oxidative damage markers were equivalent in brains of patients with MCI and LOAD, lumbar CSF F_2-IsoPs concentrations from MCI patients were intermediate between those from controls and patients with probable AD [116].

We stress that increased lumbar CSF F_2-IsoPs are not specific to probable AD but have been observed in patients with Huntington's disease (HD), as well as acute brain injury from either stroke or closed head trauma [117]. Nevertheless, lumbar CSF F_2-IsoPs can provide useful diagnostic insight in the appropriate context. We quantified lumbar CSF F_2-IsoPs, CSF $A\beta_{42}$, and CSF total tau levels in patients with probable AD, dementias other than AD, and age-matched controls [115]. Individuals were classified as AD or non-AD by a commercially available test using CSF $A\beta_{42}$ and tau levels (95% sensitivity, 50% specificity), by CSF F_2-IsoP and $A\beta_{42}$ levels (90% sensitivity, 83% specificity), and by combined analysis using CSF F_2-IsoP, $A\beta_{42}$, and tau levels (84% sensitivity, 89% specificity). In a longitudinal study of 17 individuals followed over 4 years, others reported that elevated CSF F_2-IsoPs have 100% accuracy in identifying those who later progressed to either MCI or dementia [118].

Lumbar CSF F_2-IsoPs can also provide an objective measure of AD progression and response to antioxidant therapeutics [119,120]. We pursued a longitudinal assessment of lumbar CSF F_2-IsoPs in a group of patients with mild probable AD followed for 1 year [119]. The percent change in CSF F_2-IsoPs observed in these 40 AD patients was stratified for dietary antioxidant supplementation with α-tocopherol, either alone or in combination with ascorbate (no one took ascorbate alone). Patients without supplementation showed an approximately 50% increase in CSF F_2-IsoPs over the 1 year period. Consonant with recently reported epidemiologic observations on the apparent mitigation in risk of incident AD with combined use of

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

supplemental tocopherols and ascorbate [121], we observed a significant pharmacologic effect only in the group that supplemented their diets with both α-tocopherol and ascorbate [119].

Although obtaining CSF from the lumbar cistern has no significant risks when performed by experienced physicians, even in the elderly, [122], spinal taps can be stressful and are not easily obtained in most clinics. For these reasons, several investigators have pursued quantification of F_2-IsoPs in plasma or urine – even though rat models of massive acute oxidative damage to brain do not lead to elevations in plasma or urine F_2-IsoPs [123]. Until recently, the literature on plasma and urine F_2-IsoPs has been confusing, with different assays giving different outcomes in samples from patients with LOAD or MCI. This has led to speculation of major differences between the original and modified GC-MS assays despite their very close quantitative agreement in CSF. We have reviewed this topic in detail previously [124] and find that two studies are important in untangling these conflicting studies. An investigation of peripheral F_2-IsoPs in LOAD with a new LC-MS-MS method sensitively quantified the four classes of F_2-IsoPs in urine: $iPF_{2\alpha}$-III, $iPF_{2\alpha}$-IV, $iPF_{2\alpha}$-V, and $iPF_{2\alpha}$-VI [106]; yet, these investigators were unable to detect a significant difference in urine $iPF_{2\alpha}$ concentrations between AD patients and controls. Most importantly, the same investigator who repeatedly reported increased plasma F_2-IsoPs in MCI and LOAD by the modified GC-MS method recently reported that no increase was seen in a separate set of samples [125].

We conclude that multiple analytical methods show that CSF F_2-IsoPs are increased in CSF of patients with early dementia from LOAD and, although less robustly demonstrated, from individuals with MCI. Despite a conflicting past literature, all methodologies including the modified GC-MS method using 8-,12-*iso*-$iPF_{2\alpha}$-VI, now agree that peripheral F_2-IsoPs are not reproducibly increased in patients with MCI or LOAD.

VI. Epidemiologic studies

As is true in transgenic animal models, the only definitive evidence of a therapeutic or preventive effect of an intervention strategy in humans comes from experimentation, i.e., from randomized controlled trials. A number of trials are described in the following section. Trials are costly and time-consuming, however, and there are many questions that they cannot answer practically (e.g., long-term effects of interventions). For these reasons, trials are often preceded by observational studies of associations between antecedent “exposures” to a putative risk factor or intervention strategy and an alteration in the subsequent expression of the disease in question. Observational studies can never provide proof of causality, but increasing sophistication in their theory and conduct over the past two decades has resulted in more reliable results. It is now unusual that a finding reported in multiple well-designed epidemiological studies is later refuted by trial data. [126] When this does happen, the explanation is often that, inadvertently, the observational and the trial data really addressed different questions, or at least different facets of the same question. [127] For example, at least two prevention strategies – use of non-steroidal anti-inflammatory drugs (NSAIDs) and post-menopausal hormone replacement therapy – appear to have quite different effects depending on the stage of AD pathogenesis at which individuals are exposed, with the suggestion that both may be effective during latency but not later stages [127–129].

Human dietary patterns have been associated with a varying degree of risk for cognitive decline and dementia. One of the first such studies showed that people who ate fish at least once a week had a 60% lower risk of developing AD [130] and a slower rate of cognitive decline [131]. The relationship between fat intake (both saturated and trans-unsaturated fat) and cognitive decline also appears to be linear [132], and a high intake of copper with a diet abundant in saturated and trans fats may predict an increased rate of cognitive decline [133]. By contrast, total intake of n-3 polyunsaturated fatty acids may be associated with a reduced risk of AD [130]. These findings are congruent with data from animal models showing that fat

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

intake may be related not only to vascular risks but also to a direct effect of polyunsaturated fatty acids on Aβ accumulation [134]. Other evidence suggests that the "Mediterranean diet" is associated with a lower risk for AD [135] and a lower mortality once AD is established [136]. One hypothesis to explain this relationship is that the Mediterranean diet reduces vascular risks; however, a recent analysis suggests this is not the case [137]. The inclusion of vascular risks in multiple logistic regression models suggests independent effects of vascular risk factors (*viz*., presence of hypertension, diabetes, stroke, heart disease, elevated lipid levels) and other relevant covariates (caloric intake, smoking, and body mass index) on the magnitude of association between the Mediterranean diet and risk for AD. The authors hypothesized that other biological factors (e.g., oxidative stress, immune activation) are likely responsible, but acknowledged that there could be error in measuring vascular risk in such a study. Further exploration of this issue is needed.

Of particular interest are dietary content or nutritional "supplements" containing antioxidant vitamins C and E. There are a few points to keep in mind when considering these associative studies. Diets high in antioxidants will also be high in other micronutrients that may work in concert or independently of the subset of antioxidant micronutrients that are focus of dietary questionnaires. Supplements provide a more controlled exposure but lack other possibly beneficial micronutrients that accompany a diet high in antioxidants. As noted above with the transgenic mouse studies, the form of vitamin E contained in most supplements (α-tocopherol) is different from the major form consumed in Western diets (γ-tocopherol), and while these different isomers of vitamin E have complementary biological activities [138], both can act as effective antioxidants in the CNS following peripheral injection [78]. Finally, none of the studies described in the remainder of this section included autopsy validation of the diagnostic accuracy of the possible or probable AD endpoints.

Two prospective observational studies have reported lower risks of dementia or AD in participants consuming increased amounts of antioxidants in food [139,140] while other research groups have failed to observe such an association [141,142]. One of these groups has extended their analysis and observed that α– and γ-tocopherol taken in food had independent but equivalent reduction in risk for AD [143].

Observational studies of supplement use are also noteworthy. An early study of 633 participants found no incident AD cases over four years among individuals who reported use of vitamin E or C supplements at baseline [19], while an investigation of 3,385 men found reduced prevalence of vascular and mixed dementias, but not AD, among users of both vitamin E and C supplements [144]. These results contrast with one of the above-cited studies that showed no association between AD and antioxidant vitamin consumption in either dietary or supplement form [141]. A possible explanation for some of the discord in the above observations may be found in a study of 3,227 seniors of both sexes who showed significantly reduced prevalence and incidence of AD in participants who used both vitamin E and C supplements, but not either one alone [121] –a finding that echoes the study relating CSF F_2-IsoP levels to antioxidant supplement use in patients with mild AD described above [119]. The benefits of combined water-soluble (e.g., ascorbate) and lipid-soluble (e.g., tocopherol isoforms) antioxidant interventions warrants further investigation.

A few epidemiologic studies have also observed an association between antioxidant vitamin consumption and better cognitive test performance in individuals who are not demented. One observed that supplemental vitamin E or vitamin C was associated with better cognitive test performance [145]. Other investigators recently have made a similar observation in participants in the Cache County study [146], and in the Chicago Health and Aging Project where again both α- and γ-tocopherol from food had protective associations [143].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

While these epidemiologic studies of antioxidant consumption and the risk of incident dementia are difficult to summarize neatly, it seems likely that a diet rich in tocopherols and ascorbate can lower the risk for incident AD. Moreover, it seems likely that these vitamins, especially in combination, may be effective at partially blocking or slowing age-related cognitive decline, a presumed harbinger of incident AD and perhaps vascular dementia.

VII. Clinical trials of antioxidants

There have been few major clinical trials using antioxidants alone to treat AD. As far as we are aware, none has incorporated a measure of oxidative injury and so it is unclear whether the regimen used in the trial actually had the presumed pharmacologic effect. This is a very important point to consider in light of recent analysis suggesting that previous null trials of anti-oxidant supplements to prevent cardiovascular disease might have used ineffective doses; indeed, there is no reason to expect a therapeutic effect without a pharmacologic effect [147].

Sano et al [148] conducted a double-blind, placebo-controlled, randomized multicenter trial that used a factorial design to test effects of α-tocopherol (2000 IU daily), the monoamine oxidase-(MAO) B inhibitor selegiline (10 mg daily), or both in combination vs. placebo in 341 moderately severe AD patients followed for two years. The primary outcome was the time to occurrence of any of the following: death, institutionalization, loss of basic activities of daily living (ADLs), or severe dementia. The study revealed mild slowing of disease progression in the α-tocopherol, selegiline, and combination groups compared to the placebo group. Unexpectedly, α-tocopherol appeared to be slightly superior to selegiline or combination therapy in delaying the time to primary outcome. There was a significant difference in falls, syncopal episodes, and dental treatments in the treated groups, but no significant differences between groups in adverse effects after adjustment for multiple comparisons.

Petersen et al [149] conducted a randomized, double-blind, placebo-controlled three-arm study of the effect of 2,000 IU of “synthetic” α-tocopherol or 10 mg of the cholinesterase inhibitor donepezil daily vs. placebo for three years in patients with amnestic MCI. The primary outcome was development of clinically possible or probable AD by standard diagnostic criteria. A total of 769 patients were enrolled but 239 dropped out during the follow-up phase. By the end of the observation period, α-tocopherol showed no benefit, although donepezil therapy was associated with a lower rate of progression to AD during the first 12 months of therapy. Major adverse events were judged to be inconsequential. The implications of this study are not clear in relation to the study by Sano et al [150] that showed slight slowing of progress of AD by α-tocopherol in more advanced patients.

The Women’s Health Study (WHS) [151] added a placebo-controlled, double-blind end-of-study trial using 600 IU of α-tocopherol every other day starting 5.6 years after initiation of the WHS and running for four years’ duration. The initial trial enrolled 6,377 women, of whom 5,073 had mental status follow-up. No difference in decline in global cognition was observed between the α-tocopherol and placebo groups. However, there was an encouraging trend for partial protection of a composite verbal memory measure in the α-tocopherol group. Because memory decline is a hallmark of early AD, this trend might have proven more promising in a longer trial.

None of these studies used vitamin C in combination with α-tocopherol. It has long been known that vitamin C recycles α-tocopherol and makes it more effective [152] and the Cache County study (observational) reported an apparent benefit of vitamin C in combination with α-tocopherol, but not with either alone (*vide supra*). However, two trials have used combinations of antioxidants including vitamins E and C, although their focus has not been on AD. In The UK Heart Protection Study, 20,536 adults (ages 40–80) with coronary disease, other occlusive arterial disease, or diabetes received 600 mg “synthetic” vitamin E, 250 mg vitamin C, and 20

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

mg β-carotene daily, or placebo [153]. Assuming that the large randomized groups were cognitively equal at baseline, an ancillary study interviewed participants at the final follow-up using the modified Telephone Interview for Cognitive Status [154,155]. No significant differences were found in cognitive test scores after 5 years between the treatment and placebo groups. The Age-Related Eye Disease Study used 400 IU vitamin E, 500 mg vitamin C, 15 mg β-carotene, 80 mg zinc 80, and 2 mg cupric oxide daily vs. placebo in 3,640 elderly subjects [156]. Again assuming treatment group equivalence in cognition at baseline, after a median of 6.9 years of treatment an end-of-trial ancillary study administered a battery of six cognitive tests to 2,166 subjects. Mean age at this point was 75 years. No significant difference in any of the six cognitive tests between treatment and placebo groups was observed.

Grodstein et al [157] reported on the effect of β-carotene on cognition in the Physician's Health Study II (PHS-II), a long-term randomized trial with the primary outcomes of cardiovascular disease and cancer prevention. Subjects received 50 mg β-carotene on alternate days or placebo. Some subjects had been randomized to β-carotene in the original Physician's Health Study ("old recruits") and had mean treatment exposure of 18 years. The β-carotene arm also included "new recruits" who had received the treatment for a mean of one year. During the final year of PHS-II almost 6,000 men over 65 years old were given a telephone cognitive test battery. The "old recruits" who had received β-carotene had significantly higher mean global cognitive scores and verbal memory scores than those on placebo. The trial also provided some evidence that this apparent effect of β-carotene was not explained by any short-term cognitive effect of the treatment, since there was no difference in scores between the treated and placebo groups of "new recruits." This rather complex end-of-trial study raises the question of whether long-term exposure to antioxidants may be needed if there is to be an effect on the pathophysiologic process involved in cognitive decline.

N-acetylcysteine (NAC) has some antioxidant properties, and a brief double-blind, placebo-controlled, randomized trial of NAC was undertaken in 43 probable AD patients [158]. Participants received placebo or 50 mg/kg/day of NAC in three diluted doses. Primary outcome measures were 6-month changes in Mini Mental State Examination Scores and ADLs. Assignment to NAC failed to produce significant alteration in primary outcome measures but did result in significant improvement in letter fluency and Wechsler Memory Scale figure reproduction. No significant adverse effects were reported. Although brief and inadequately powered, this trial raises the possibility that NAC should be tried in lengthier and more comprehensive trials.

A 1997 report described a double-blind, placebo-controlled, randomized trial of antioxidant extracts of *Ginkgo biloba* leaf in patients with mild to severe AD or multi-infarct dementia [159]. The study enrolled 309 participants who received either *Ginkgo* extract (120 mg/day) or placebo. The active treatment group showed significant improvement on the Alzheimer's Disease Assessment Scale, cognitive component (ADAS-cog) and on the Geriatric Evaluation by a Relative's Rating Instrument. Twenty-seven percent of treated patients achieved at least a 4 point improvement on the ADAS-cog. No significant adverse events were reported. A recent double-blind, placebo controlled pilot study of 118 cognitively intact individuals 85 years of age or older demonstrated a small protective effect from *Ginko* extract on progression to MCI, and a smaller decline in memory scores when controlled the medication adherence level [160]. However this same study also had more ischemic strokes and transient ischemic attacks in the treatment group.

There are currently two ongoing large antioxidant studies aimed at the prevention of AD, but no data are yet available. The Ginkgo Evaluation of Memory study is an ongoing, double-blind, placebo controlled, randomized trial designed to determine the efficacy of *Ginkgo biloba* extract for prevention of dementia and AD [161]. Subjects receive 240 mg *Ginkgo biloba* or

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

placebo daily. The primary outcome is incidence of all-cause dementia, with emphasis on AD. The trial has completed recruitment of over 3,000 elderly volunteers. Initially a healthy volunteer effect produced lower-than-expected incidence rates of dementia [162]. However, several years into the study, the incidence rate has now increased steadily and approximates that in many comparable population studies.

The Prevention of Alzheimer's Disease by Vitamin E and Selenium (PREADVISE) study is an ancillary study of over 6,600 males, (ages 60 to 89.5 years) enrolled in the Selenium and Vitamin E Cancer Prevention Trial (SELECT). SELECT is a double-blinded phase III 2 × 2 factorial double-placebo randomized controlled trial in which participants receive daily doses of either α-tocopherol (racemic) (400 IU), selenium (200 μg), the two in combination, or placebo. Individuals entering PREADVISE are cognitively intact. The treatment period will range from seven to twelve years depending on when the participant entered SELECT. The outcomes are incidence of MCI, AD, or other dementias. Results are expected in 2012.

In summary, despite promising basic science studies and some epidemiologic findings to suggest that antioxidants are potentially beneficial in AD, clinical trials to date offer variable support for such benefit. The lack of efficacy of α – tocopherol in the MCI trial [149] is not surprising, not only because use of other vitamins such as C was proscribed, but also because current data indicate that oxidative damage and neuropathological findings of MCI are equivalent to those in the early dementia stage of the disease.

VIII. Conclusion

Transgenic mouse models show that increased oxidative damage is a relatively early event in the pathogenesis of AD that can be suppressed by antioxidants. Data from autopsies of individuals who died with MCI and CSF analysis confirm that advanced oxidative damage to diseased regions of brain occurs early in the pathogenesis of AD, even before the onset of dementia. Some epidemiologic studies suggest that increased antioxidant consumption from food or supplements can suppress processes that underlie age-related cognitive decline and may (perhaps relatedly) reduce the risk of incident AD; however, while these data are hopeful that partial prevention of AD may be achieved with antioxidants, several epidemiologic studies do not support this idea. The results of clinical trials so far have been modest at best, but likely have been undermined by focusing on what are now known to be advanced stages of oxidative damage. Clinical trials for AD prevention by antioxidants are still in their infancy. The results of the two largest ongoing trials cited above are eagerly awaited. As suggested by the PHS-II [157], treatment with antioxidants may require long-term studies, a consideration for planning future trials. Meanwhile, major efforts should be undertaken to define more effective and safe antioxidant agents for clinical trials.

Acknowledgements

This work was supported by grants from the NIH (AG05136, AG024010, AG24011, AG005119, AG028383, AG08017, AG000258), Department of Veteran's Affairs Merit Review, and the Nancy and Buster Alvord Endowment.

List of abbreviations

HNE
: 4-hydroxy-2-nonenal

FapyAdenine
: 4,6-diamino-5-formamidopyrimidine

5-OHC
: 5-hydroxycytosine

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

8-OHA
8-hydroxyadenine

5-OHU
5-hydroxyuracil

ADLs
activities of daily living

AD
Alzheimer's disease

A β
amyloid

APP
amyloid precursor protein

CBLM
cerebellum

CSF
cerebrospinal fluid

ELISAs
enzyme-linked immunosorbent assays

IsoPs
F_2-isoprostanes

NeuroPs
F_4-neuroprostanes

FL
frontal lobe

GC
gas chromatography

HP
hippocampus

LOAD
late-onset AD

MS
mass spectrometry

MCI
Mild Cognitive Impairment

NAC
N-acetylcysteine

NFTs
neurofibrillary tangles

NSAIDs
non-steroidal anti-inflammatory drugs

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

OL
occipital lobe

PL
parietal lobe

PHS-II
Physician's Health Study II

PS
presenilin

PREADVISE
Prevention of Alzheimer's Disease by Vitamin E and Selenium

ROS
reactive oxygen species

SELECT
Selenium and Vitamin E Cancer Prevention Trial

TL
temporal lobe

WHS
Women's Health Study

References

1. Tsuang DW, Bird TD. Genetics of dementia. Med Clin North Am 2002;86:591–614. [PubMed: 12171060]
2. Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch Neurol 2003;60:1119–1122. [PubMed: 12925369]
3. Strittmatter WJ, Roses AD. Apolipoprotein E and Alzheimer's disease. Proc Natl Acad Sci 1995;92:4725–4727. [PubMed: 7761390]
4. Hebert LE, Beckett LA, Scherr PA, Evans DA. Annual incidence of Alzheimer disease in the United States projected to the years 2000 through 2050. Alzheimer Dis Assoc Disord 2001;15:169–173. [PubMed: 11723367]
5. Ernst RL, Hay JW. The US economic and social costs of Alzheimer's disease revisited. Am J Public Health 1994;84:1261–1264. [PubMed: 8059882]
6. McCormick WC, Hardy J, Kukull WA, Bowen JD, Teri L, Zitzer S, Larson EB. Healthcare utilization and costs in managed care patients with Alzheimer's disease during the last few years of life. J Am Geriatr Soc 2001;49:1156–1160. [PubMed: 11559373]
7. Welch HG, Walsh JS, Larson EB. The cost of institutional care in Alzheimer's disease: nursing home and hospital use in a prospective cohort. J Am Geriatr Soc 1992;40:221–224. [PubMed: 1538039]
8. Brookmeyer R, Gray S, Kawas C. Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. Am J Public Health 1998;88:1337–1342. [PubMed: 9736873]
9. Katzman R. Editorial: The prevalence and malignancy of Alzheimer disease. A major killer. Arch Neurol 1976;33:217–218. [PubMed: 1259639]
10. Petersen R, Doody R, Kurz A, Mohs R, Morris J, Rabins P, Ritchie K, Rossor M, Thal L, Winblad B. Current concepts in mild cognitive impairment. Arch Neurol 2001;58:1985–1982. [PubMed: 11735772]
11. Arriagada PV, Marzloff K, Hyma BT. Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. Neurology 1992;42:1681–1688. [PubMed: 1307688]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

12. Berg L, McKeel DW, Miller JP, Baty J, Morris JC. Neuropathological indexes of Alzheimer's disease in demented and nondemented persons aged 80 years and older. Arch Neurol 1993;50:349–358. [PubMed: 8460956]
13. Crystal HA, Dickson DW, Sliwinski MJ, Lipton RB, Grober E, Marks-Nelson H, Antis P. Pathological markers associated with normal aging and dementia in the elderly. Ann Neurol 1993;34:566–573. [PubMed: 8215244]
14. Davis DG, Schmitt FA, Wekstein DR, Markesbery W. Alzheimer neuropathological alterations in aged cognitively normal subjects. J Neuropathol Exp Neurol 1999;58:376–388. [PubMed: 10218633]
15. Green MS, Kaye JA, Ball MJ. The Oregon brain aging study: neuropathology accompanying health aging in the oldest old. Neurology 2000;54:105–113. [PubMed: 10636134]
16. Haroutunian V, Purohit DP, Perl DP, Marin D, Khan K, Lantz M, Davis KL, Mohs RC. Neurofibrillary tangles in nondemented elderly subjects and mild Alzheimer disease. Arch Neurol 1999;56:713–718. [PubMed: 10369312]
17. Hulette CM, Welsh-Bohmer KA, Murray MG, Saunders AM, Mash DC, McIntyre LM. Neuropathological and neuropsychological changes in "normal" aging: evidence for preclinical Alzheimer disease in cognitively normal individuals. J Neuropathol Exp Neurol 1998;57:1168–1174. [PubMed: 9862640]
18. Morris JC, Price AL. Pathologic correlates of nondemented aging, mild cognitive impairment, and early-stage Alzheimer's disease. J Mol Neurosci 2001;17:101–118. [PubMed: 11816784]
19. Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Ann Neurol 1999;45:358–368. [PubMed: 10072051]
20. Price JL, Davis PB, Morris JC, White DL. The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. Neurobiol Aging 1991;12:295–312. [PubMed: 1961359]
21. Riley KP, Snowdon DA, Markesbery WR. Alzheimer's neurofibrillary pathology and the spectrum of cognitive function: findings from the nun study. Ann Neurol 2002;51:567–577. [PubMed: 12112102]
22. Schmitt FA, Davis DG, Wekstein DR, Smith CD, Ashford JW, Markesbery WR. "Preclinical" AD revisited. Neuropathology of cognitively normal older adults. Neurology 2000;55:370–376. [PubMed: 10932270]
23. Xuereb JH, Brayne C, Dufouil C, Gertz H, Wischik C, Harrington C, Mukaetova-Ladinska E, McGee MA, O'Sullivan A, O'Connor D, Paykel ES, Huppert FA. Neuropathological findings in the very old. Results from the first 101 brains of a population-based longitudinal study of dementing disorders. Ann N Y Acad Sci 2000;903:490–496. [PubMed: 10818543]
24. Sonnen JA, Larson EB, Crane PK, Haneuse S, Li G, Schellenberg GD, Craft S, Leverenz JB, Montine TJ. Pathological correlates of dementia in a longitudinal, population-based sample of aging. Ann Neurol. 2007
25. Mintun MA, Larossa GN, Sheline YI, Dence CS, Lee SY, Mach RH, Klunk WE, Mathis CA, DeKosky ST, Morris JC. [11C]PIB in a nondemented population: potential antecedent marker of Alzheimer disease. Neurology 2006;67:446–452. [PubMed: 16894106]
26. Kauwe JS, Jacquart S, Chakraverty S, Wang J, Mayo K, Fagan AM, Holtzman DM, Morris JC, Goate AM. Extreme cerebrospinal fluid amyloid beta levels identify family with late-onset Alzheimer's disease presenilin 1 mutation. Ann Neurol 2007;61:446–453. [PubMed: 17366635]
27. Glodzik-Sobanska L, Pirraglia E, Brys M, de Santi S, Mosconi L, Rich KE, Switalski R, Louis LS, Sadowski MJ, Martiniuk F, Mehta P, Pratico D, Zinkowski RP, Blennow K, de Leon MJ. The effects of normal aging and ApoE genotype on the levels of CSF biomarkers for Alzheimer's disease. Neurobiol Aging. 2007
28. Sunderland T, Mirza N, Putnam KT, Linker G, Bhupali D, Durham R, Soares H, Kimmel L, Friedman D, Bergeson J, Csako G, Levy JA, Bartko JJ, Cohen RM. Cerebrospinal fluid beta-amyloid1-42 and tau in control subjects at risk for Alzheimer's disease: the effect of APOE epsilon4 allele. Biol Psychiatry 2004;56:670–676. [PubMed: 15522251]
29. Fagan AM, Mintun MA, Mach RH, Lee SY, Dence CS, Shah AR, LaRossa GN, Spinner ML, Klunk WE, Mathis CA, DeKosky ST, Morris JC, Holtzman DM. Inverse relation between in vivo amyloid

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

imaging load and cerebrospinal fluid Abeta42 in humans. Ann Neurol 2006;59:512–519. [PubMed: 16372280]

30. Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal Fluid tau/beta-Amyloid42 Ratio as a Prediction of Cognitive Decline in Nondemented Older Adults. Arch Neurol 2007;64:343–349. [PubMed: 17210801]
31. Ewers M, Buerger K, Teipel SJ, Scheltens P, Schroder J, Zinkowski RP, Bouwman FH, Schonknecht P, Schoonenboom NS, Andreasen N, Wallin A, DeBernardis JF, Kerkman DJ, Heindl B, Blennow K, Hampel H. Multicenter assessment of CSF-phosphorylated tau for the prediction of conversion of MCI. Neurology 2007;69:2205–2212. [PubMed: 18071141]
32. Buerger K, Teipel SJ, Zinkowski R, Sunderland T, Andreasen N, Blennow K, Ewers M, DeBernardis J, Shen Y, Kerkman D, Du Y, Hampel H. Increased levels of CSF phosphorylated tau in apolipoprotein E epsilon4 carriers with mild cognitive impairment. Neurosci Lett 2005;391:48–50. [PubMed: 16165272]
33. Buerger K, Zinkowski R, Teipel SJ, Tapiola T, Arai H, Blennow K, Andreasen N, Hofmann-Kiefer K, DeBernardis J, Kerkman D, McCulloch C, Kohnken R, Padberg F, Pirttila T, Schapiro MB, Rapoport SI, Moller HJ, Davies P, Hampel H. Differential diagnosis of Alzheimer disease with cerebrospinal fluid levels of tau protein phosphorylated at threonine 231. Arch Neurol 2002;59:1267–1272. [PubMed: 12164722]
34. Herukka SK, Helisalmi S, Hallikainen M, Tervo S, Soininen H, Pirttila T. CSF Abeta42, Tau and phosphorylated Tau, APOE epsilon4 allele and MCI type in progressive MCI. Neurobiol Aging 2007;28:507–514. [PubMed: 16546302]
35. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol 2006;5:228–234. [PubMed: 16488378]
36. Andreasen N, Vanmechelen E, Vanderstichele H, Davidsson P, Blennow K. Cerebrospinal fluid levels of total-tau, phospho-tau and A beta 42 predicts development of Alzheimer's disease in patients with mild cognitive impairment. Acta Neurol Scand Suppl 2003;179:47–51. [PubMed: 12603251]
37. Buerger K, Teipel SJ, Zinkowski R, Blennow K, Arai H, Engel R, Hofmann-Kiefer K, McCulloch C, Ptok U, Heun R, Andreasen N, DeBernardis J, Kerkman D, Moeller H, Davies P, Hampel H. CSF tau protein phosphorylated at threonine 231 correlates with cognitive decline in MCI subjects. Neurology 2002;59:627–629. [PubMed: 12196665]
38. Arai H, Ishiguro K, Ohno H, Moriyama M, Itoh N, Okamura N, Matsui T, Morikawa Y, Horikawa E, Kohno H, Sasaki H, Imahori K. CSF phosphorylated tau protein and mild cognitive impairment: a prospective study. Exp Neurol 2000;166:201–203. [PubMed: 11031097]
39. Andreasen N, Minthon L, Vanmechelen E, Vanderstichele H, Davidsson P, Winblad B, Blennow K. Cerebrospinal fluid tau and Abeta42 as predictors of development of Alzheimer's disease in patients with mild cognitive impairment. Neurosci Lett 1999;273:5–8. [PubMed: 10505638]
40. Selkoe DJ. Alzheimer disease: mechanistic understanding predicts novel therapies. Ann Intern Med 2004;140:627–638. [PubMed: 15096334]
41. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002;297:353–356. [PubMed: 12130773]
42. Woltjer RL, McMahan W, Milatovic D, Kjerulf JD, Shie FS, Rung LG, Montine KS, Montine TJ. Effects of chemical chaperones on oxidative stress and detergent-insoluble species formation following conditional expression of amyloid precursor protein carboxy-terminal fragment. Neurobiol Dis 2007;25:427–437. [PubMed: 17141508]
43. Selkoe D. Alzheimer's disease is a synaptic failure. Science 2002;298:789–791. [PubMed: 12399581]
44. Nunomura A, Castellani RJ, Zhu X, Moreira PI, Perry G, Smith MA. Involvement of oxidative stress in Alzheimer disease. J Neuropathol Exp Neurol 2006;65:631–641. [PubMed: 16825950]
45. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–344. [PubMed: 6610841]
46. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. Free Radic Biol Med 1991;11:81–128. [PubMed: 1937131]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

47. Picklo MJ, Montine TJ, Amarnath V, Neely MD. Carbonyl toxicology and Alzheimer's disease. Toxicology and Applied Pharmacology 2002;184:187–197. [PubMed: 12460747]
48. Keller JN, Mattson MP. Roles of lipid peroxidation in modulation of cellular signaling pathways, cell dysfunction, and death in the nervous system. Rev Neurosci 1998;9:105–116. [PubMed: 9711902]
49. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ 2nd. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci U S A 1990;87:9383–9387. [PubMed: 2123555]
50. Roberts LJ, Montine TJ, Markesbery WR, Tapper AR, Hardy P, Chemtob S, Dettbarn WD, Morrow JD. Formation of isoprostane-like compounds (neuroprostanes) in vivo from docosahexaenoic acid. Journal of Biological Chemistry 1998;273:13605–13612. [PubMed: 9593698]
51. Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H. Free radical-induced damage to DNA: mechanisms and measurement. Free Radic Biol Med 2002;32:1102–1115. [PubMed: 12031895]
52. Liu X, Lovell MA, Lynn BC. 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 2006;19:710–718. [PubMed: 16696574]
53. Kozekov ID, Nechev LV, Moseley MS, Harris CM, Rizzo CJ, Stone MP, Harris TM. DNA interchain cross-links formed by acrolein and crotonaldehyde. J Am Chem Soc 2003;125:50–61. [PubMed: 12515506]
54. Requena JR, Levine RL, Stadtman ER. Recent advances in the analysis of oxidized proteins. Amino Acids 2003;25:221–226. [PubMed: 14661085]
55. Park KH, Hallows JL, Chakrabarty P, Davies P, Vincent I. Conditional neuronal simian virus 40 T antigen expression induces Alzheimer-like tau and amyloid pathology in mice. J Neurosci 2007;27:2969–2978. [PubMed: 17360920]
56. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science 1996;274:99–102. [PubMed: 8810256]
57. Pratico D, Uryu K, Leight S, Trojanowski J, Lee V. Increased lipid peroxidation preceded amyloid plaque formation in an animal model of Alzheimer amyloidosis. J Neurosci 2001;21:4183–4187. [PubMed: 11404403]
58. Quinn J, Montine T, Morrow J, Woodward WR, Kulhanek D, Eckenstein F. Inflammation and cerebral amyloidosis are disconnected in an animal model of Alzheimer's disease. Journal of Neuroimmunology 2003;137:32–41. [PubMed: 12667645]
59. Quinn JF, Bussiere JR, Hammond RS, Montine TJ, Henson E, Jones RE, Stackman RW Jr. Chronic dietary alpha-lipoic acid reduces deficits in hippocampal memory of aged Tg2576 mice. Neurobiol Aging 2007;28:213–225. [PubMed: 16448723]
60. Montine TJ, Quinn JF, Milatovic D, Silbert LC, Dang T, Sanchez S, Terry E, Roberts LJ, Kaye JA, Morrow JD. Peripheral F-2-isoprostanes and F-4-neuroprostanes are not increased in Alzheimer's disease. Annals of Neurology 2002;52:175–179. [PubMed: 12210787]
61. Smith MA, Sayre LM, Anderson VE, Harris PL, Beal MF, Kowall N, Perry G. Cytochemical demonstration of oxidative damage in Alzheimer disease by immunochemical enhancement of the carbonyl reaction with 2,4-dinitrophenylhydrazine. J Histochem Cytochem 1998;46:731–735. [PubMed: 9603784]
62. Montine KS, Kim PJ, Olson SJ, Markesbery WR, Montine TJ. 4-hydroxy-2-nonenal pyrrole adducts in human neurodegenerative disease. Journal of Neuropathology and Experimental Neurology 1997;56:866–871. [PubMed: 9258256]
63. Montine KS, Olson SJ, Amarnath V, Whetsell WO, Graham DG, Montine TJ. Immunohistochemical detection of 4-hydroxy-2-nonenal adducts in Alzheimer's disease is associated with inheritance of APOE4. American Journal of Pathology 1997;150:437–443. [PubMed: 9033259]
64. Puglielli L, Friedlich AL, Setchell KD, Nagano S, Opazo C, Cherny RA, Barnham KJ, Wade JD, Melov S, Kovacs DM, Bush AI. Alzheimer disease beta-amyloid activity mimics cholesterol oxidase. J Clin Invest 2005;115:2556–2563. [PubMed: 16127459]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

65. Lim GP, Yang F, Chu T, Gahtan E, Ubeda O, Beech W, Overmier JB, Hsiao-Ashec K, Frautschy SA, Cole GM. Ibuprofen effects on Alzheimer pathology and open field activity in APPsw transgenic mice. Neurobiol Aging 2001;22:983–991. [PubMed: 11755007]

66. Calon F, Lim GP, Yang F, Morihara T, Teter B, Ubeda O, Rostaing P, Triller A, Salem N Jr, Ashe KH, Frautschy SA, Cole GM. Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. Neuron 2004;43:633–645. [PubMed: 15339646]

67. Stackman RW, Eckenstein F, Frei B, Kulhanek D, Nowlin J, Quinn JF. Prevention of age-related spatial memory deficits in a transgenic mouse model of Alzheimer's disease by chronic Ginkgo biloba treatment. Exp Neurol 2003;184:510–520. [PubMed: 14637120]

68. Apelt J, Bigl M, Wunderlich P, Schliebs R. Aging-related increase in oxidative stress correlates with developmental pattern of beta-secretase activity and beta-amyloid plaque formation in transgenic Tg2576 mice with Alzheimer-like pathology. Int J Dev Neurosci 2004;22:475–484. [PubMed: 15465277]

69. Mohmmad Abdul H, Wenk GL, Gramling M, Hauss-Wegrzyniak B, Butterfield DA. APP and PS-1 mutations induce brain oxidative stress independent of dietary cholesterol: implications for Alzheimer's disease. Neurosci Lett 2004;368:148–150. [PubMed: 15351438]

70. Nakashima H, Ishihara T, Yokota O, Terada S, Trojanowski JQ, Lee VM, Kuroda S. Effects of alpha-tocopherol on an animal model of tauopathies. Free Radic Biol Med 2004;37:176–186. [PubMed: 15203189]

71. Lewis J, McGowan E, Rockwood J, Melrose H, Nacharaju P, Van Slegtenhorst M, Gwinn-Hardy K, Paul Murphy M, Baker M, Yu X, Duff K, Hardy J, Corral A, Lin WL, Yen SH, Dickson DW, Davies P, Hutton M. Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. Nat Genet 2000;25:402–405. [PubMed: 10932182]

72. Santacruz K, Lewis J, Spires T, Paulson J, Kotilinek L, Ingelsson M, Guimaraes A, DeTure M, Ramsden M, McGowan E, Forster C, Yue M, Orne J, Janus C, Mariash A, Kuskowski M, Hyman B, Hutton M, Ashe KH. Tau suppression in a neurodegenerative mouse model improves memory function. Science 2005;309:476–481. [PubMed: 16020737]

73. Oddo S, Caccamo A, Kitazawa M, Tseng BP, LaFerla FM. Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. Neurobiol Aging 2003;24:1063–1070. [PubMed: 14643377]

74. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 2003;39:409–421. [PubMed: 12895417]

75. Cole GM, Morihara T, Lim GP, Yang F, Begum A, Frautschy SA. NSAID and antioxidant prevention of Alzheimer's disease: lessons from in vitro and animal models. Ann N Y Acad Sci 2004;1035:68–84. [PubMed: 15681801]

76. Sung S, Yao Y, Uryu K, Yang H, Lee VM, Trojanowski JQ, Pratico D. Early vitamin E supplementation in young but not aged mice reduces Abeta levels and amyloid deposition in a transgenic model of Alzheimer's disease. Faseb J 2004;18:323–325. [PubMed: 14656990]

77. Nishida Y, Yokota T, Takahashi T, Uchihara T, Jishage K, Mizusawa H. Deletion of vitamin E enhances phenotype of Alzheimer disease model mouse. Biochem Biophys Res Commun 2006;350:530–536. [PubMed: 17026966]

78. Milatovic D, VanRollins M, Li K, Montine KS, Montine TJ. Suppression of murine cerebral F2-isoprostanes and F4-neuroprostanes from excitotoxicity and innate immune response in vivo by alpha- or gamma-tocopherol. J Chromatogr B Analyt Technol Biomed Life Sci 2005;827:88–93.

79. Itoh N, Masuo Y, Yoshida Y, Cynshi O, Jishage K, Niki E. gamma-Tocopherol attenuates MPTP-induced dopamine loss more efficiently than alpha-tocopherol in mouse brain. Neurosci Lett 2006;403:136–140. [PubMed: 16716512]

80. Lim GP, Chu T, Yang F, Beech W, Frautschy SA, Cole GM. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J Neurosci 2001;21:8370–8377. [PubMed: 11606625]

81. DeSouza L, Diehl G, Rodrigues MJ, Guo J, Romaschin AD, Colgan TJ, Siu KW. Search for cancer markers from endometrial tissues using differentially labeled tags iTRAQ and cICAT with

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

multidimensional liquid chromatography and tandem mass spectrometry. J Proteome Res 2005;4:377–386. [PubMed: 15822913]

82. Matsubara E, Bryant-Thomas T, Pacheco Quinto J, Henry TL, Poeggeler B, Herbert D, Cruz-Sanchez F, Chyan YJ, Smith MA, Perry G, Shoji M, Abe K, Leone A, Grundke-Ikbal I, Wilson GL, Ghiso J, Williams C, Refolo LM, Pappolla MA, Chain DG, Neria E. Melatonin increases survival and inhibits oxidative and amyloid pathology in a transgenic model of Alzheimer's disease. J Neurochem 2003;85:1101–1108. [PubMed: 12753069]
83. Quinn J, Kulhanek D, Nowlin J, Jones R, Pratico D, Rokach J, Stackman R. Chronic melatonin therapy fails to alter amyloid burden or oxidative damage in old Tg2576 mice: implications for clinical trials. Brain Res 2005;1037:209–213. [PubMed: 15777772]
84. Malm TM, Iivonen H, Goldsteins G, Keksa-Goldsteine V, Ahtoniemi T, Kanninen K, Salminen A, Auriola S, Van Groen T, Tanila H, Koistinaho J. Pyrrolidine dithiocarbamate activates Akt and improves spatial learning in APP/PS1 mice without affecting beta-amyloid burden. J Neurosci 2007;27:3712–3721. [PubMed: 17409235]
85. Li G, Zou L, Jack CR Jr, Yang Y, Yang ES. Neuroprotective effect of Coenzyme Q10 on ischemic hemisphere in aged mice with mutations in the amyloid precursor protein. Neurobiol Aging 2007;28:877–882. [PubMed: 16806588]
86. Lovell MA, Markesbery WR. Oxidative DNA damage in mild cognitive impairment and late-stage Alzheimer's disease. Nucleic Acids Res 2007;35:7497–7504. [PubMed: 17947327]
87. Markesbery WR, Lovell MA. Damage to lipids, proteins, DNA, and RNA in mild cognitive impairment. Arch Neurol 2007;64:954–956. [PubMed: 17620484]
88. Mielke MM, Lyketsos CG. Lipids and the pathogenesis of Alzheimer's disease: is there a link? Int Rev Psychiatry 2006;18:173–186. [PubMed: 16777671]
89. Montine TJ, Quinn JF, Montine KS, Kaye JA, Breitner JCS. Quantitative in vivo biomarkers of oxidative damage and their application to the diagnosis and management of Alzheimer's disease. Journal of Alzheimers Disease 2005;8:359–367.
90. Sultana R, Perluigi M, Butterfield DA. 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 2006;833:3–11.
91. Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA, Markesbery WR. Evidence of increased oxidative damage in subjects with mild cognitive impairment. Neurology 2005;64:1152–1156. [PubMed: 15824339]
92. Butterfield DA, Reed T, Newman SF, Sultana R. Roles of amyloid beta-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. Free Radic Biol Med 2007;43:658–677. [PubMed: 17664130]
93. Markesbery WR, Lovell MA. DNA oxidation in Alzheimer's disease. Antioxid Redox Signal 2006;8:2039–2045. [PubMed: 17034348]
94. Gabbita SP, Lovell MA, Markesbery WR. Increased nuclear DNA oxidation in the brain in Alzheimer's disease. J Neurochem 1998;71:2034–2040. [PubMed: 9798928]
95. Wang J, Xiong S, Xie C, Markesbery WR, Lovell MA. Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. J Neurochem 2005;93:953–962. [PubMed: 15857398]
96. Liu X, Lovell MA, Lynn BC. 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 2005;77:5982–5989. [PubMed: 16159131]
97. Gotz ME, Wacker M, Luckhaus C, Wanek P, Tatschner T, Jellinger K, Leblhuber F, Ransmayr G, Riederer P, Eder E. Unaltered brain levels of 1, N2-propanodeoxyguanosine adducts of trans-4-hydroxy-2-nonenal in Alzheimer's disease. Neurosci Lett 2002;324:49–52. [PubMed: 11983292]
98. Nunomura A, Perry G, Pappolla MA, Wade R, Hirai K, Chiba S, Smith MA. RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. J Neurosci 1999;19:1959–1964. [PubMed: 10066249]
99. Nunomura A, Honda K, Takeda A, Hirai K, Zhu X, Smith MA, Perry G. Oxidative damage to RNA in neurodegenerative diseases. J Biomed Biotechnol 2006:82323. [PubMed: 17047315]2006

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

100. Nunomura A, Chiba S, Lippa CF, Cras P, Kalaria RN, Takeda A, Honda K, Smith MA, Perry G. Neuronal RNA oxidation is a prominent feature of familial Alzheimer's disease. Neurobiol Dis 2004;17:108–113. [PubMed: 15350971]

101. Ding Q, Markesbery WR, Chen Q, Li F, Keller JN. Ribosome dysfunction is an early event in Alzheimer's disease. J Neurosci 2005;25:9171–9175. [PubMed: 16207876]

102. Lovell MA, Markesbery WR. Oxidatively modified RNA in mild cognitive impairment. Neurobiol Dis 2008;29:169–175. [PubMed: 17920285]

103. Feillet-Coudray C, Tourtauchaux R, Niculescu M, Rock E, Tauveron I, Alexandre-Gouabau MC, Rayssiguier Y, Jalenques I, Mazur A. Plasma levels of 8-epiPGF2alpha, an in vivo marker of oxidative stress, are not affected by aging or Alzheimer's disease. Free Rad Biol Med 1999;27:463–469. [PubMed: 10468223]

104. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ. A series of prostaglandin-like compounds produced in vivo in humans by a non-cyclooxygenase, free radical catalyzed mechanism. Proc Natl Sci USA 1990;87:9383–9387.

105. Pratico D, Barry OP, Lawson JA, Adiyaman M, Hwang SW, Khanapure SP, Iuliano L, Rokach J, Fitzgerald GA. IPF-alpha-I - an index of lipid peroxidation in humans. Proc Natl Acad Sci 1998;95:3449–3454. [PubMed: 9520386]

106. Bohnstedt KC, Karlberg B, Wahlund L, Jonhagen ME, Basun H, Schmidt S. 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 2003;796:11–19.

107. Morrow JD, Roberts LJ II. Mass spectrometry of prostanoids: F2-isoprostanes produced by non-cyclooxygenase free radical catalyzed mechanism. Meth Enzymol 1994;233:163–174. [PubMed: 8015454]

108. Pratico D, Clack CM, Lee VMY, Trojanowski JQ, Rokach J, FitzGerald G. Increased 8,12-iso-iPF$_{2\alpha}$-IV in Alzheimer's disease: correlation of a noninvasive index of lipid peroxidation with disease severity. Ann Neurol 2000;48:809–812. [PubMed: 11079549]

109. Montine TJ, Markesbery WR, Morrow JD, Roberts LJ 2nd. Cerebrospinal fluid F2-isoprostane levels are increased in Alzheimer's disease. Ann Neurol 1998;44:410–413. [PubMed: 9749613]

110. Pratico D, Lee VM, Trojanowski JQ, Rokach J, Fitzgerald GA. Increased F$_2$-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation *in vivo*. FASEB J 1998;12:1777–1784. [PubMed: 9837868]

111. Montine TJ, Markesbery WR, Zackert W, Sanchez SC, Roberts LJ, Morrow JD. 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. American Journal of Pathology 1999;155:863–868. [PubMed: 10487843]

112. Montine TJ, Beal MF, Cudkowicz ME, Brown RH, O'Donnell H, Margolin RA, McFarland L, Bachrach AF, Zackert WE, Roberts LJ, Morrow JD. Increased cerebrospinal fluid F$_2$-isoprostane concentration in probable Alzheimer's disease. Neurology 1999;52:562–565. [PubMed: 10025788]

113. Montine TJ, Beal MF, Cudkowicz ME, O'Donnell H, Margolin RA, McFarland L, Bachrach AF, Zackert WE, Roberts LJ, Morrow JD. Increased CSF F2-isoprostane concentration in probable AD. Neurology 1999;52:562–565. [PubMed: 10025788]

114. Montine TJ, Sidell KR, Crews BC, Markesbery WR, Marnett LJ, Roberts LJ 2nd, Morrow JD. Elevated CSF prostaglandin E2 levels in patients with probable AD. Neurology 1999;53:1495–1498. [PubMed: 10534257]

115. Montine TJ, Kaye JA, Montine KS, McFarland L, Morrow JD, Quinn JF. Cerebrospinal fluid abeta42, tau, and f2-isoprostane concentrations in patients with Alzheimer disease, other dementias, and in age-matched controls. Arch Pathol Lab Med 2001;125:510–512. [PubMed: 11260625]

116. Pratico D, Clark CM, Liun F, Rokach J, Lee VY, Trojanowski JQ. Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. Arch Neurol 2002;59:972–976. [PubMed: 12056933]

117. Montine TJ, Beal MF, Robertson D, Cudkowicz ME, Biaggioni I, O'Donnell H, Zackert WE, Roberts LJ, Morrow JD. Cerebrospinal fluid F-2-isoprostanes are elevated in Huntington's disease. Neurology 1999;52:1104–1105. [PubMed: 10102447]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

118. de Leon MJ, Mosconi L, Li J, De Santi S, Yao Y, Tsui WH, Pirraglia E, Rich K, Javier E, Brys M, Glodzik L, Switalski R, Saint Louis LA, Pratico D. Longitudinal CSF isoprostane and MRI atrophy in the progression to AD. J Neurol 2007;254:1666–1675. [PubMed: 17994313]
119. Quinn JF, Montine KS, Moore M, Morrow JD, Kaye JA, Montine TJ. Suppression of longitudinal increase in CSFF2-isoprostanes in Alzheimer's disease. Journal of Alzheimers Disease 2004;6:93–97.
120. 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 LA, Wallin A, Blennow K, de Leon MJ. Prediction and longitudinal study of CSF biomarkers in mild cognitive impairment. Neurobiol Aging. 2007
121. Zandi PP, Anthony JC, Khachaturian AS, Stone SV, Gustafson D, Tschanz JT, Norton MC, Welsh-Bohmer KA, Breitner JC. Cache County Study Group. Reduced risk of Alzheimer disease in users of antioxidant vitamin supplements: the Cache County Study. Arch Neurol 2004;61:82–88. [PubMed: 14732624]
122. Peskind ER, Riekse R, Quinn JF, Kaye J, Clark CM, Farlow MR, Decarli C, Chabal C, Vavrek D, Raskind MA, Galasko D. Safety and acceptability of the research lumbar puncture. Alzheimer Dis Assoc Disord 2005;19:220–225. [PubMed: 16327349]
123. Montine TJ, Quinn JF, Milatovic D, Silbert LC, Dang T, Sanchez S, Terry E, Roberts LJ 2nd, Kaye JA, Morrow JD. Peripheral F2-isoprostanes and F4-neuroprostanes are not increased in Alzheimer's disease. Ann Neurol 2002;52:175–179. [PubMed: 12210787]
124. Montine TJ, Montine KS, McMahan W, Markesbery WR, Quinn JF, Morrow JD. F-2-isoprostanes in Alzheimer and other neurodegenerative diseases. Antioxidants & Redox Signaling 2005;7:269–275. [PubMed: 15650414]
125. Irizarry MC, Yao Y, Hyman BT, Growdon JH, Pratico D. Plasma F2A isoprostane levels in Alzheimer's and Parkinson's disease. Neurodegener Dis 2007;4:403–405. [PubMed: 17934322]
126. Concato J, Horwitz RI. Beyond randomised versus observational studies. Lancet 2004;363:1660–1661. [PubMed: 15158623]
127. Breitner JC. NSAIDs and Alzheimer's disease: how far to generalise from trials? Lancet Neurol 2003;2:527. [PubMed: 12941571]
128. Group AR, Lyketsos CG, Breitner JC, Green RC, Martin BK, Meinert C, Piantadosi S, Sabbagh M. Naproxen and celecoxib do not prevent AD in early results from a randomized controlled trial. Neurology 2007;68:1800–1808. [PubMed: 17460158]
129. Zandi PP, Carlson MC, Plassman BL, Welsh-Bohmer KA, Mayer LS, Steffens DC, Breitner JC. Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. Jama 2002;288:2123–2129. [PubMed: 12413371]
130. Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Wilson RS, Aggarwal N, Schneider J. Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 2003;60:940–946. [PubMed: 12873849]
131. Morris MC, Evans DA, Tangney CC, Bienias JL, Wilson RS. Fish consumption and cognitive decline with age in a large community study. Arch Neurol 2005;62:1849–1853. [PubMed: 16216930]
132. Morris MC, Evans DA, Bienias JL, Tangney CC, Wilson RS. Dietary fat intake and 6-year cognitive change in an older biracial community population. Neurology 2004;62:1573–1579. [PubMed: 15136684]
133. Morris MC, Evans DA, Tangney CC, Bienias JL, Schneider JA, Wilson RS, Scherr PA. Dietary copper and high saturated and trans fat intakes associated with cognitive decline. Arch Neurol 2006;63:1085–1088. [PubMed: 16908733]
134. Sahlin C, Pettersson FE, Nilsson LN, Lannfelt L, Johansson AS. Docosahexaenoic acid stimulates non-amyloidogenic APP processing resulting in reduced Abeta levels in cellular models of Alzheimer's disease. Eur J Neurosci 2007;26:882–889. [PubMed: 17714184]
135. Scarmeas N, Stern Y, Tang MX, Mayeux R, Luchsinger JA. Mediterranean diet and risk for Alzheimer's disease. Ann Neurol 2006;59:912–921. [PubMed: 16622828]
136. Scarmeas N, Luchsinger JA, Mayeux R, Stern Y. Mediterranean diet and Alzheimer disease mortality. Neurology 2007;69:1084–1093. [PubMed: 17846408]
137. Scarmeas N, Stern Y, Mayeux R, Luchsinger JA. Mediterranean diet, Alzheimer disease, and vascular mediation. Arch Neurol 2006;63:1709–1717. [PubMed: 17030648]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

138. Christen S, Woodall AA, Shigenaga MK, Southwell-Kelly PT, Duncan MW, Ames BN. gamma-Tocopherol traps mutagenic electrophiles such as NO(X) and complements alpha-tocopherol: Physiological implications. Proc Natl Acad Sci USA 1997;94:3217–3222. [PubMed: 9096373]
139. Engelhart MJ, Geerlings MI, Ruitenberg A, van Swieten JC, Hofman A, Witteman JC, Breteler MM. Dietary intake of antioxidants and risk of Alzheimer disease. JAMA 2002;287:3223–3229. [PubMed: 12076218]
140. Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Aggarwal N, Wilson RS, Scherr PA. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. JAMA 2002;287:3230–3237. [PubMed: 12076219]
141. Luchsinger J, Tang M, Shea S, Mayeux R. Antioxidant vitamin intake and risk of Alzheimer disease. Arch Neurol 2003;60:203–208. [PubMed: 12580704]
142. Fillenbaum GG, Kuchibhatla MN, Hanlon JT, Artz MB, Pieper CF, Schmader KE, Dysken MW, Gray SL. Dementia and Alzheimer's disease in community-dwelling elders taking vitamin C and/or vitamin E. Ann Pharmacother 2005;39:2009–2014. [PubMed: 16227448]
143. Morris MC, Evans DA, Tangney CC, Bienias JL, Wilson RS, Aggarwal NT, Scherr PA. Relation of the tocopherol forms to incident Alzheimer disease and to cognitive change. Am J Clin Nutr 2005;81:508–514. [PubMed: 15699242]
144. Goldman WP, Price JL, Storandt M, Grant EA, McKeel DW Jr, Rubin EH, Morris JC. Absence of cognitive impairment or decline in preclinical Alzheimer's disease. Neurology 2001;56:361–367. [PubMed: 11171902]
145. Masaki KH, Losonczy KG, Izmirlian G, Foley DJ, Ross GW, Petrovitch H, Havlik R, White LR. Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. Neurology 2000;54:1265–1272. [PubMed: 10746596]
146. Wengreen HJ, Munger RG, Corcoran CD, Zandi P, Hayden KM, Fotuhi M, Skoog I, Norton MC, Tschanz J, Breitner JC, Welsh-Bohmer KA. Antioxidant intake and cognitive function of elderly men and women: the Cache County Study. J Nutr Health Aging 2007;11:230–237. [PubMed: 17508099]
147. Roberts LJ 2nd, Oates JA, Linton MF, Fazio S, Meador BP, Gross MD, Shyr Y, Morrow JD. The relationship between dose of vitamin E and suppression of oxidative stress in humans. Free Radic Biol Med 2007;43:1388–1393. [PubMed: 17936185]
148. Sano M, Ernesto C, Thomas RG, Klauber MR, Schafer K, Grundman M, Woodbury P, Growdon J, Cotman CW, Pfeiffer E, Schneider LS, Thal LJ. 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 1997;336:1216–1222. [PubMed: 9110909]
149. Petersen RC, Thomas RG, Grundman M, Bennett D, Doody R, Ferris S, Galasko D, Jin S, Kaye J, Levey A, Pfeiffer E, Sano M, van Dyck CH, Thal LJ. Vitamin E and donepezil for the treatment of mild cognitive impairment. N Engl J Med 2005;352:2379–2388. [PubMed: 15829527]
150. Sano M, Ernesto C, Thomas RG, Klauber MR, Schafer K, Grundman M, Woodbury P, Growdon J, Cotman CW, Pfeiffer E, Schneider LS, Thal LJ. The Members of the Alzheimer's Disease Cooperative Study. A Controlled Trial of Selegiline, Alpha-Tocopherol, or Both as Treatment for Alzheimer's Disease. N Engl J Med 1997;336:1216–1222. [PubMed: 9110909]
151. Kang JH, Cook N, Manson J, Buring JE, Grodstein F. A randomized trial of vitamin E supplementation and cognitive function in women. Arch Intern Med 2006;166:2462–2468. [PubMed: 17159011]
152. Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev 1994;74:139–162. [PubMed: 8295932]
153. MRC/BHF. Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360:23–33. [PubMed: 12114037]
154. Brandt J, Spencer M, Folstein M. The Telephone Interview for Cognitive Status. Neuropsychiatry, Neuropsychology, and Behavioral Neurology 1988;1:111–117.
155. Welsh KA, Breitner J, Magruder-Habib K. Telephone screening for dementia in community dwelling elderly. Neurobiology of Aging 1990;11:260.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

156. Yaffe K, Clemons TE, McBee WL, Lindblad AS. Impact of antioxidants, zinc, and copper on cognition in the elderly: a randomized, controlled trial. Neurology 2004;63:1705–1707. [PubMed: 15534261]

157. Grodstein F, Kang JH, Glynn RJ, Cook NR, Gaziano JM. A randomized trial of beta carotene supplementation and cognitive function in men: the Physicians' Health Study II. Arch Intern Med 2007;167:2184–2190. [PubMed: 17998490]

158. Adair JC, Knoefel JE, Morgan N. Controlled trial of N-acetylcysteine for patients with probable Alzheimer's disease. Neurology 2001;57:1515–1517. [PubMed: 11673605]

159. Le Bars PL, Katz MM, Berman N, Itil TM, Freedman AM, Schatzberg AF. A placebo-controlled, double-blind, randomized trial of an extract of Ginkgo biloba for dementia. North American EGb Study Group. Jama 1997;278:1327–1332. [PubMed: 9343463]

160. Dodge HH, Zitzelberger T, Oken BS, Howieson D, Kaye J. A randomized placebo-controlled trial of ginkgo biloba for the prevention of cognitive decline. Neurology. 2008

161. DeKosky ST, Fitzpatrick A, Ives DG, Saxton J, Williamson J, Lopez OL, Burke G, Fried L, Kuller LH, Robbins J, Tracy R, Woolard N, Dunn L, Kronmal R, Nahin R, Furberg C. The Ginkgo Evaluation of Memory (GEM) study: design and baseline data of a randomized trial of Ginkgo biloba extract in prevention of dementia. Contemp Clin Trials 2006;27:238–253. [PubMed: 16627007]

162. Green RC, DeKosky ST. Primary prevention trials in Alzheimer disease. Neurology 2006;67:S2–5. [PubMed: 17101930]

163. Smith MA, Sayre LM, Anderson VE, Harris PL, Beal MF, Kowall N, Perry G. Cytochemical demonstration of oxidative damage in Alzheimer's disease by immunochemical enhancement of the carbonyl reaction with 2,4-dinitrophenylhydrazine. J Histochem Cytochem 1998;46:731–735. [PubMed: 9603784]

164. Schuessel K, Schafer S, Bayer TA, Czech C, Pradier L, Muller-Spahn F, Muller WE, Eckert A. Impaired Cu/Zn-SOD activity contributes to increased oxidative damage in APP transgenic mice. Neurobiol Dis 2005;18:89–99. [PubMed: 15649699]

165. Liang X, Wang Q, Hand T, Wu L, Breyer RM, Montine TJ, Andreasson K. Deletion of the prostaglandin E2 EP2 receptor reduces oxidative damage and amyloid burden in a model of Alzheimer's disease. J Neurosci 2005;25:10180–10187. [PubMed: 16267225]

166. Markesbery WR, Kryscio RJ, Lovell MA, Morrow JD. Lipid peroxidation is an early event in the brain in amnestic mild cognitive impairment. Ann Neurol 2005;58:730–735. [PubMed: 16240347]

167. Williams TI, Lynn BC, Markesbery WR, Lovell MA. 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 2006;27:1094–1099. [PubMed: 15993986]

168. Butterfield DA, Reed T, Perluigi M, De Marco C, Coccia R, Cini C, Sultana R. Elevated protein-bound levels of the lipid peroxidation product, 4-hydroxy-2-nonenal, in brain from persons with mild cognitive impairment. Neurosci Lett 2006;397:170–173. [PubMed: 16413966]

169. Yao Y, Clark CM, Trojanowski JQ, Lee VM, Pratico D. Elevation of 12/15 lipoxygenase products in AD and mild cognitive impairment. Ann Neurol 2005;58:623–626. [PubMed: 16037976]

170. Bader Lange ML, Cenini G, Piroddi M, Mohmmad Abdul H, Sultana R, Galli F, Memo M, Butterfield DA. Loss of phospholipid asymmetry and elevated brain apoptotic protein levels in subjects with amnestic mild cognitive impairment and Alzheimer disease. Neurobiol Dis. 2007

171. Montine TJ, Montine KS, McMahan W, Markesbery WR, Quinn JF, Morrow JD. F-isoprostanes in Alzheimer and other neurodegenerative diseases. Antioxid Redox Signal 2005;7:269–275. [PubMed: 15650414]

172. Butterfield DA, Poon HF, St Clair D, Keller JN, Pierce WM, Klein JB, Markesbery WR. Redox proteomics identification of oxidatively modified hippocampal proteins in mild cognitive impairment: insights into the development of Alzheimer's disease. Neurobiol Dis 2006;22:223–232. [PubMed: 16466929]

173. Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, Markesbery WR. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc Natl Acad Sci U S A 1991;88:10540–10543. [PubMed: 1683703]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

174. Choi J, Sullards MC, Olzmann JA, Rees HD, Weintraub ST, Bostwick DE, Gearing M, Levey AI, Chin LS, Li L. Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer diseases. J Biol Chem 2006;281:10816–10824. [PubMed: 16517609]

175. Choi J, Rees HD, Weintraub ST, Levey AI, Chin LS, Li L. Oxidative modifications and aggregation of Cu, Zn-superoxide dismutase associated with Alzheimer and Parkinson diseases. J Biol Chem 2005;280:11648–11655. [PubMed: 15659387]

176. Choi J, Levey AI, Weintraub ST, Rees HD, Gearing M, Chin LS, Li L. Oxidative modifications and down-regulation of ubiquitin carboxyl-terminal hydrolase L1 associated with idiopathic Parkinson's and Alzheimer's diseases. J Biol Chem 2004;279:13256–13264. [PubMed: 14722078]

177. Sultana R, Boyd-Kimball D, Poon HF, Cai J, Pierce WM, Klein JB, Merchant M, Markesbery WR, Butterfield DA. 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 2006;27:1564–1576. [PubMed: 16271804]

178. Marcum JL, Mathenia JK, Chan R, Guttmann RP. Oxidation of thiol-proteases in the hippocampus of Alzheimer's disease. Biochem Biophys Res Commun 2005;334:342–348. [PubMed: 16018967]

179. Korolainen MA, Goldsteins G, Nyman TA, Alafuzoff I, Koistinaho J, Pirttila T. Oxidative modification of proteins in the frontal cortex of Alzheimer's disease brain. Neurobiol Aging 2006;27:42–53. [PubMed: 16298240]

180. Wang J, Markesbery WR, Lovell MA. Increased oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment. J Neurochem 2006;96:825–832. [PubMed: 16405502]

181. Mecocci P, MacGarvey U, Beal MF. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. Ann Neurol 1994;36:747–751. [PubMed: 7979220]

182. Lyras L, Cairns NJ, Jenner A, Jenner P, Halliwell B. An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease. J Neurochem 1997;68:2061–2069. [PubMed: 9109533]

183. Lovell MA, Markesbery WR. Oxidatively modified RNA in mild cognitive impairment. Neurobiol Dis. 2007

184. Lovell MA, Markesbery WR. Oxidative damage in mild cognitive impairment and early Alzheimer's disease. J Neurosci Res. 2007

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1

Oxidative damage in murine models of AD

Strain	Marker of oxidative damage	Assay	Finding	Reference
APP (Tg2576)	Lipid peroxidation: F2-IsoPs	GC/MS (modified method)	Increased in hippo and in plasma	[57,83]
	Lipid peroxidation: F2-IsoPs	GC/MS (original method)	No increase in cortex of aged animals	[58]
	Lipid peroxidation: HNE	Immunohistochemistry	Increased in vicinity of Aβ plaques	[163]
	Lipid peroxidation: Oxidized cholesterol		Increased in cortex	[64]
	Oxidized protein: carbonyls	"oxy-blot" westerns	Increased in cerebral cortex	[65]
	carbonyls	ELISA	No increase in Cerebral cortex of aged animals	[67]
	Antioxidant enzymes SOD and HO-1	Immunohistochemistry	Increased in vicinity of Aβ plaques	[163]
Other APP (give name)	Antioxidant enzymes and HNE	Immunohistochemistry	Increased HNE; decreased SOD in Thy1-APP751 but not PDGF-APP695	[164]
APP-PS1	F2-IsoPs	GC/MS (original method)	Increased in APP-PS1	[165]
	Protein carbonyls, HNE	Slot-blot assay	Both increased	[69]
Tau (Human tau isoform)	Protein carbonyls, 8OHdG	Carbonyls by western, 8OhdG by immunohistochemistry	Both increased	[70]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 2

Antioxidant interventions in murine models of AD

Strain	Antioxidant intervention	Outcome	Reference
APP (Tg2576)	Vitamin E, Isoform not specified	Decreased F_2-IsoPs in both old and young mice; decreased Aβ only in young mice	[76]
Human tau	α-tocopherol	Decreased carbonyls, decreased 8-OHdG, decreased tau pathologic changes	[70]
APP (Tg2576)	Melatonin	Decreased Aβ burden in young mice	[82]
APP (Tg2576)	Melatonin	No effect on F_2-IsoPs or Aβ burden in older plaque-bearing mice	[83]
APP (Tg2576)	Lipoic acid	Improved spatial memory but no change in Aβ	[59]
APP-PS1	pyrrolidine dithiocarbamate	Improved spatial memory but no change in Aβ	[84]
APP (Tg2576)	Co-enzyme Q	Reduced vulnerability to ischemia, effect on Aβ not assessed	[85]
APP (Tg2576)	Curcumin	Reduced carbonyls and reduced Aβ burden	[80]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 3

Macromolecular modifications of reactive oxygen species measure in human autopsy tissue from MCI and AD.

	MCI	LOAD
Lipid	↑ thiobarbituric acid reactive substances and malondialdehyde in TL [91]; ↑ F_2-IsoP in FL, PL and OL, F_4-NeuroP in PL and OL, [166]; ↑ HNE in HP, SMTG and CBLM and acrolein in SMTG [167]; ↑ protein bound HNE in HP and PL [168]; ↑ 12 (s)-and 15 (s) hydroxyeicosatetraenoic acid [a][169]; loss of phosphatidyl asymmetry in PL [b] [170]	↑ HNE, acrolein, F_2-IsoPs, and F_4-NeuroPs[c] (reviewed in [88, 171]); ↑ 12 (s)-and 15 (s) hydroxyeicosatetraenoic acid [a] [169]
Protein	↑ Protein carbonyls in SMTG [91]; ↑ PIN-1, αp-enolase, glutamine synthetase, and pyruvate kinase [d] in the HP [172];	↑ Protein carbonyls in FL and OL [173]; ↑DJ-1 [174], Cu-Zn superoxide dismutase [175] and ubiquitin carboxyl terminal hydrolase [d] [176]; ↑peptidyl prolyl cis trans isomerase, phosphoglycerate mutase 1, ubiquitin carboxyl terminal hydrolase 1, dihydropyrimidinase related protein-2, carbonic anhydrase II, triose phosphate isomerase, alpha-enolase and gamma-SNAP [d] in HPG and NS difference in oxidatively modified protein from CBLM [177]; ↑ oxidation of cysteine proteases in LOAD HP [178]; ↓ oxidative modification of mitochondrial glutamate dehydrogenase and cytosolic malate dehydrogenase FL [179]
DNA	↑ 8-OHG in nDNA from FL and TL; ↑ 5-OHC in nDNA from FL, PL, and TL; and in mtDNA[f] of FL; 8-OHA were significantly elevated in nDNA of FL, PL, and TL; FapyAdenine[f] in nDNA and mt DNA in FL, PL, and TL [180].	3 × ↑ 8-OHG in PL in mtDNA but NS in nDNA [181]; ↑ 8-OHG, 8-OHA, and 5- OHC in total DNA in PL; ↑ thymine glycol, FapyAdenine, FapyGuanine and 5-OHU in multiple brain regions [182]; reviewed in [86].
RNA	↑8-OHG in PL, HP and CBLM [101,183] ↓ rRNA and tRNA and protein synthesis capacity in PL but NS in CBLM[101]	↑8-OHG in HP[184]

Abbreviations: **5-OHC** - 5-hydroxycytosine; **8-OHA** - 8-hydroxyadenine; **FapyAdenine** -4,6-diamino-5-formamidopyrimidine; **5-OHU** - 5-hydroxyuracil (degradation product of cytosine in various brain regions in AD)

[a] by-products of 12/15 lipoxygenase peroxidation of arachidonic acid.

[b] Thought to be mediated by HNE.

[c] Values in MCI and LOAD brain are comparable.

[d] Specifically oxidatively modified proteins identified by redox proteomics.

[e] suggesting attempted correction of oxidative modification of proteins.

[f] Two-way ANOVA showed that 8-OHG ($p < 0.04$), fapyadenine ($p < 0.001$) and 5-OHC ($p < 0.004$) in mtDNA were significantly increased in neocortical regions compared to CBLM