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Forum Review

F_2-Isoprostanes in Alzheimer and Other Neurodegenerative Diseases

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ABSTRACT

Increased free radical-mediated injury to brain is proposed to be an integral component of several neurodegenerative diseases, including Alzheimer's disease (AD). Lipid peroxidation is a major outcome of free radical-mediated injury to brain, where it directly damages membranes and generates a number of oxidized products. F_2-Isoprostanes (F_2-IsoPs), one group of lipid peroxidation products derived from arachidonic acid, are especially useful as *in vivo* biomarkers of lipid peroxidation. F_2-IsoP concentration is selectively increased in diseased regions of brain from patients who died from advanced AD, where pathologic changes include amyloid β (Aβ) amyloidogenesis, neurofibrillary tangle formation, and extensive neuron death. Interestingly, cerebral F_2-IsoPs are not reproducibly elevated in aged mouse models of cerebral Aβ amyloidogenesis only. There is broad agreement that increased cerebrospinal fluid (CSF) levels of F_2-IsoPs also are present in patients with early AD. Demonstrated applications of quantifying CSF F_2-IsoPs have improved laboratory diagnostic accuracy of AD and objective assessment of antioxidant therapeutics. In contrast, quantification of F_2-IsoPs in plasma and urine of AD patients has produced conflicting data. These results indicate that brain lipid peroxidation is a potential therapeutic target early in the course of AD, and that CSF F_2-IsoPs may aid in the assessment of antioxidant experimental therapeutics and laboratory diagnosis of AD. *Antioxid. Redox Signal.* 7, 269–275.

INTRODUCTION

ABUNDANT *IN VITRO* AND *IN VIVO* DATA have strongly implicated free radical-mediated injury to diseased regions of brain as a shared mechanism among several neurodegenerative diseases. Although free radical damage may be common among neurodegenerative diseases, the sources of free radicals likely are specific to different types of neurodegeneration. For example, *in vivo* and *in vitro* data indicate that oligomers or higher order aggregates of amyloid β (Aβ) peptides both directly and indirectly stimulate free radical production in diseased regions of brain of patients with Alzheimer's disease (AD) (2).

Due to the high concentration of polyunsaturated fatty acids in brain relative to other organs, lipid peroxidation is one of the major outcomes of free radical-mediated injury to brain (22). A critically important aspect of lipid peroxidation is that it is self-propagating and will proceed until substrate is consumed or termination occurs. In this way, lipid peroxidation is fundamentally different from other forms of free radical injury in that it is a self-sustaining process capable of extensive tissue damage (28). There are two broad outcomes to lipid peroxidation, *viz*., structural damage to membranes and generation of oxidized products, some of which are chemically reactive and covalently modify macromolecules (3, 13, 28). Although reactive products of lipid peroxidation are

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likely contributors to neurodegeneration, their use as *in vivo* biomarkers is severely limited because of their rapid and extensive metabolism and chemical instability (5, 22, 24). For these reasons, other products of oxidative damage that are chemically and metabolically stable *in situ* are superior *in vivo* biomarkers of oxidative damage. One such group of stable products of lipid peroxidation is the F_2-isoprostanes (F_2-IsoPs) (25).

ASSAYS FOR F_2-IsoPs

F_2-IsoPs are a complex mixture of 64 enantiomers contained within four regioisomeric families. Their chemistry has been discussed elsewhere in this volume. As they are formed by nonenzymatic processes, different subsets have been measured to reflect overall levels of lipid peroxidation.

In the study of neurodegeneration, F_2-IsoPs have been quantified by one of four different methods: commercially available enzyme-linked immunosorbent assays (4), two different gas chromatography (GC)-mass spectrometry (MS) stable isotope dilution methods that we refer to as the original method (25) or modified method (29), and most recently by liquid chromatography (LC)-MS (1). The two GC-MS methods are similar, and quantify subsets of F_2-IsoPs that coelute with the deuterated internal standard used; this will be key to comparing studies presented in detail below. The original GC-MS method uses a commercially available deuterated F_2-IsoP as internal standard, 8-*iso*-prostaglandin $F_{2\alpha}$ (8-*iso*-$PGF_{2\alpha}$; also known as $iPF_{2\alpha}$-III), and quantifies those F_2-IsoPs in the peak that comigrate with this standard (26). One of us (J.D.M.) and colleagues have shown by reverse-phase HPLC and electrospray ionization MS that this peak contains not only 8-*iso*-$PGF_{2\alpha}$, but also additional as yet uncharacterized F_2-IsoPs (26); it is for this reason that 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 additional internal standards, $iPF_{2\alpha}$-VI and 8-,12-*iso*-$iPF_{2\alpha}$-VI (29, 31). This assay quantifies the peak that comigrates 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.

The recent commercial availability of deuterated $iPF_{2\alpha}$-VI has provided the opportunity to compare the original and modified GC-MS methods. As a first step, we determined the retention time of deuterated $iPF_{2\alpha}$-VI using the original GC method and compared it with the retention time of deuterated 8-*iso*-$PGF_{2\alpha}$ (23). Surprisingly, $iPF_{2\alpha}$-VI comigrated with 8-*iso*-$PGF_{2\alpha}$. The significance of this unexpected result is that in all data previously published by us using the original GC-MS method, $iPF_{2\alpha}$-VI is included in the peak we call F_2-IsoPs, along with 8-*iso*-$PGF_{2\alpha}$ ($iPF_{2\alpha}$-III) and other as yet uncharacterized F_2-IsoPs. In contrast, we found that another isomer, deuterated $iPF_{2\alpha}$-IV, does not comigrate with the F_2-IsoP peak quantified by the original GC-MS method.

F_2-IsoPs IN BRAIN

The first step in investigating lipid peroxidation in neurodegenerative disease was to establish relevance in human *postmortem* tissue. The advantages of this approach are that autopsy classification of neurodegenerative disease remains the gold standard and multiple regions of brain can be examined. A disadvantage is that almost all patients have advanced disease at the time of death. This point is particularly important because it means that finding changes *post mortem* does not inform about whether these processes occur early in the disease process, making them potential therapeutic targets, or are late stage consequences of disease.

Numerous studies have demonstrated significantly increased concentrations of reactive lipid peroxidation-derived aldehydes in diseased regions of brain obtained *post mortem* from patients with AD (8–10, 12, 38). However, activity of some of the major metabolizing enzymes for these reactive products of lipid peroxidation also is altered in diseased regions of brain in AD (27, 39, 40), raising serious issues when interpreting the significance of increased levels of these reactive products in neurodegenerative diseases. Are levels of these reactive products increased because of increased production or decreased metabolism? Has increased metabolism led to an underestimation of the extent of lipid peroxidation?

Due to these limitations in quantifying reactive products of lipid peroxidation, two groups have measured F_2-IsoPs in brain of patients who died with advanced AD. Pratico *et al*. demonstrated elevated $iPF_{2\alpha}$-III and $iPF_{2\alpha}$-VI levels in frontal and temporal lobes of AD patients compared with controls using the modified GC-MS method (30). We expanded these findings by measuring F_2-IsoPs in temporal and parietal cortex, hippocampus, and cerebellum of AD patients and age-matched controls, all with short *postmortem* intervals using the original GC-MS method (37). These data indicated greater lipid peroxidation in diseased regions of AD brain (Fig. 1).

A central feature of AD is an ~70% loss of neurons in affected regions of brain. Although the precise pathogenic mechanisms of AD remain to be determined, this disease is characterized by the confluence of two processes: those that culminate in accumulation of Aβ in plaques and the forma-

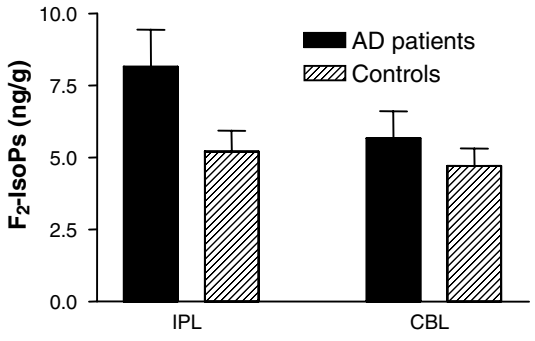

FIG. 1. F_2-IsoPs were determined in patients who died with AD and age-matched controls from two different regions of brain: the inferior parietal lobule (IPL) that is heavily involved by AD and the cerebellar cortex (CBL) that is not involved by AD. Deuterated 8-*iso*-$PGF_{2\alpha}$ ($iPF_{2\alpha}$-III) was used as internal standard and F_2-IsoPs measured using the original GC-MS method. F_2-IsoPs were significantly greater in IPL of AD patients ($p < 0.05$), but not in CBL ($p > 0.05$) with n = 8–10 samples in each group.

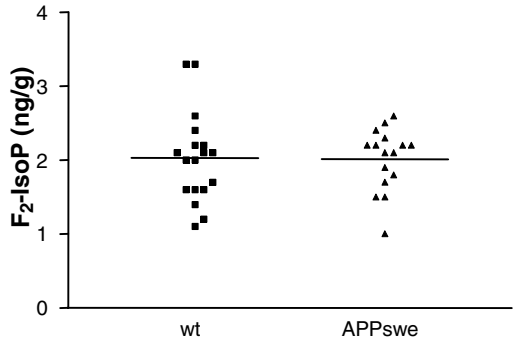

FIG. 2. Scatter plot (line is mean) of frontal cortex F_2-IsoPs in APP_{swe} transgenic mice aged 16–20 months and age-matched littermate controls ($p = 0.9$). Deuterated 8-*iso*-$PGF_{2\alpha}$ ($iPF_{2\alpha}$-III) was used as internal standard and F_2-IsoPs measured using the original GC-MS method. There was no correlation with age or significant stratification for gender within genotypes.

tion of neurofibrillary tangles (NFTs) in characteristic regions of brain. Efforts to develop transgenic mouse models of AD pathogenesis have focused on Aβ accumulation and deposition. The most widely used is a transgenic mouse that overexpresses a mutant form of the gene encoding the precursor of Aβ peptides that was discovered in a Swedish kindred; these APP_{swe} mice develop age-dependent cerebral Aβ amyloidogenesis (6). For reasons not fully understood, however, APP_{swe} mice do not demonstrate significant NFT formation or neuron loss (7). In addition, there are conflicting reports on whether F_2-IsoPs are elevated in these mice. One group has reported that cerebral 8-,12-*iso*-$iPF_{2\alpha}$-VI, as measured by the modified method, is significantly increased in APP_{swe} mice at ~6 months of age, a time that precedes Aβ amyloidogenesis, and then continues to rise as mice age further (32). We have been unable to reproduce this finding when measuring F_2-IsoPs by the original GC-MS method, both in a published report (35) and in a subsequent repeated attempt; combined data from our studies are presented in Fig. 2. Although this may be related to differences in the genetic background, diets, or environmental exposures of these mice, it does raise an important point about the proposed role of increased oxidative damage in AD. If indeed cerebral lipid peroxidation is increased in APP_{swe} mice, then cerebral lipid peroxidation is associated with Aβ amyloidogenesis, but apparently is insufficient to cause neurodegeneration. In contrast, if cerebral F_2-IsoPs are *not* increased in APP_{swe} mice, then these results are consistent with the hypothesis that lipid peroxidation, as well as other forms of oxidative damage, is a key link between Aβ peptide accumulation and neurodegeneration (11).

F_2-IsoPs IN VENTRICULAR CEREBROSPINAL FLUID (CSF)

Ventricular CSF obtained from the lateral ventricles at autopsy also has been assayed for F_2-IsoPs. These studies represent a bridge between *postmortem* tissue analysis and the analysis of CSF from the lumbar cistern of living patients described below. We and others have determined the concentration of F_2-IsoPs in ventricular CSF obtained *post mortem* and have shown significant elevations in AD patients compared with age-matched controls (14, 30) (Table 1). Although different deuterated standards were used and slightly different results achieved, both methods had similar control values, and both showed a significant increase in ventricular CSF F_2-IsoPs in AD patients compared with controls. In addition, we demonstrated that ventricular CSF F_2-IsoP concentrations in AD patients are significantly correlated with indices of neurodegeneration (17).

F_2-IsoPs IN LUMBAR CSF

Patients with AD undergoing *postmortem* examination typically have advanced disease with illness progressing over a decade or more. Therefore, a serious limitation to interpretation of results described above is that the increased brain and ventricular CSF F_2-IsoPs might be a late-stage consequence of disease. Obviously, a late-stage consequence would be a less attractive therapeutic target than a process contributing to disease progression at an earlier stage. Therefore, two groups have quantified F_2-IsoPs in lumbar CSF obtained *intra vitam* from the lumbar cistern in patients with AD and other neurodegenerative diseases.

TABLE 1. COMPARISON OF ORIGINAL AND MODIFIED GC-MS METHODS IN AD PATIENTS AND AGE-MATCHED CONTROLS

	Original GC-MS Method (14–16, 18–21, 34)				*Modified GC-MS Method (30, 31, 33)*			
Fluid	*St.*	*Control*	*AD*	Δ	*St.*	*Control*	*AD*	Δ
V-CSF (pg/ml)	III	46	72	+	III	41	49	ND
					VI	38	102	+
L-CSF (pg/ml)	III	23–26	31–50	+	*iso*-VI	15 or 25	66 or 68	+
Plasma (pg/ml)	III	41	43	ND	*iso*-VI	180 or 190	610 or 680	+
Urine (pg/mg Cr)	III	1.5 or 2.4	1.3 or 2.4	ND	*iso*-VI	1.5 or 1.8	4.6 or 4.9	+

Data are the mean values from published references (noted in parentheses). St. is the deuterated internal standard used, either III ($iPF_{2\alpha}$-III, also known as 8-*iso*-$PGF_{2\alpha}$), VI ($iPF_{2\alpha}$-VI), or *iso*-VI (8-,12-*iso*-$iPF_{2\alpha}$-VI), V-CSF and L-CSF indicate ventricular and lumbar CSF. Δ stands for change in AD patients compared with age-matched controls; + indicates a statistically significant increase, whereas ND indicates no significant difference between AD and control values.

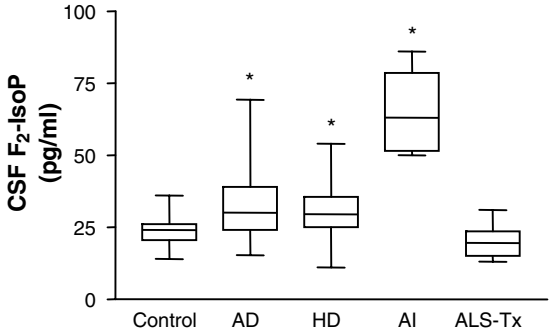

FIG. 3. Box and whiskers plot of lumbar CSF F_2-IsoPs determined in control individuals and patients with early AD, HD, acute injury (AI) to brain, or treated ALS (ALS-Tx). Deuterated 8-*iso*-$PGF_{2\alpha}$ ($iPF_{2\alpha}$-III) was used as internal standard and F_2-IsoPs measured using the original GC-MS method. Nonparametric ANOVA showed $p < 0.0001$ with corrected repeat comparisons with controls significantly different for AD, HD, and AI (*$p < 0.05$).

The first study of probable AD patients early in the course of dementia showed that F_2-IsoPs are significantly elevated in lumbar CSF compared with age-matched hospitalized patients without neurologic disease (15). This study used the original GC-MS method and quantified those F_2-IsoPs that comigrate with deuterated 8-*iso*-$PGF_{2\alpha}$ ($iPF_{2\alpha}$-III), thus including $iPF_{2\alpha}$-VI as mentioned above. The average duration of dementia in these probable AD patients at the time of CSF examination was <2 years, whereas the average duration of AD is between 9 and 12 years. This same result has been observed by us in additional groups of probable AD patients and controls (16, 18, 20). The combined results from these studies are presented in Fig. 3. As expected, the concentration of F_2-IsoPs was lower in lumbar CSF than ventricular CSF, due to both a rostrocaudal gradient (36) and likely lower levels earlier in the disease. Importantly, a different laboratory examining probable AD patients and controls using the modified GC-MS method and deuterated 8-,12-*iso*-$iPF_{2\alpha}$-VI as standard obtained similar results (31). Although different internal standards were used in these studies, there again was good agreement for values obtained in control individuals and AD patients (Table 1). Finally, these investigators extended their studies to patients with amnestic mild cognitive impairment (MCI), a condition that appears to represent, at least in some patients, a transition between normal aging and early AD; individuals with MCI were reported to have 8-,12-*iso*-$iPF_{2\alpha}$-VI lumbar CSF concentrations that were intermediate between controls and patients with AD (33).

We also have determined lumbar CSF F_2-IsoPs by the original method in patients with amyotrophic lateral sclerosis (ALS), patients with Huntington's disease (HD), and patients who had acute injury, either stroke or closed head trauma (16) (Fig. 3). Acute injury patients were included as a reference for expected high levels of lumbar CSF F_2-IsoPs. Of these three additional groups, HD and acute injury patients showed significantly increased lumbar CSF F_2-IsoPs compared with controls; none were receiving antioxidant therapy. ALS patients had illnesses of differing duration and severity, varying from mild to marked. Almost all patients with ALS were taking riluzole, and most (75%) were taking α-tocopherol supplements ranging from 400 to 5,000 IU per day. CSF F_2-IsoP levels tended to be lower in ALS patients taking α-tocopherol supplements; however, this difference was not statistically significant in this relatively small series of patients. Linear regression analysis failed to demonstrate a significant relationship between age and lumbar CSF F_2-IsoP concentrations in any of these groups of patients or in controls, and there was no difference in CSF F_2-IsoP concentrations between men and women. These results show that elevation of lumbar CSF F_2-IsoPs is not specific to AD and suggest that some therapies may suppress lipid peroxidation in the central nervous system (CNS).

In addition to providing mechanistic information about neurodegenerative disease pathogenesis and a means to assess quantitatively the response to antioxidant therapeutics, lumbar CSF F_2-IsoP levels also may provide information that is useful in diagnosis of diseases where it is elevated early. We tested the hypothesis that quantification of lumbar CSF F_2-IsoPs, along with CSF $A\beta_{42}$ and total tau levels, improves laboratory diagnostic accuracy for AD in patients with probable AD, dementias other than AD, and age-matched controls (20). 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). These results indicate that lumbar CSF F_2-IsoP quantification can enhance the accuracy of the laboratory diagnosis of AD; however, this conclusion is based on a single study and these findings need to be replicated.

Another potential application of lumbar CSF F_2-IsoPs is objective assessment of response to therapeutics. Indeed, our study of treated patients with ALS suggested that some therapies may suppress oxidative damage in patients with neurodegenerative diseases. We pursued this question further in a longitudinal assessment of lumbar CSF F_2-IsoPs in a group of patients with mild probable AD followed for 1 year. Figure 4

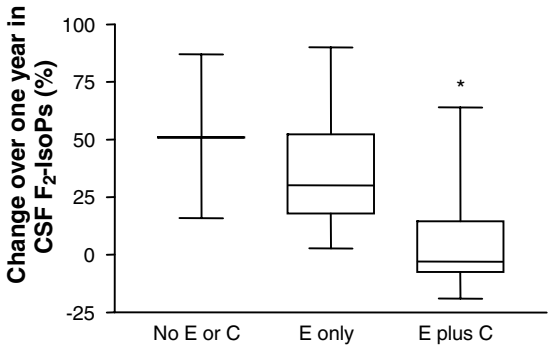

FIG. 4. Box and whiskers plot of the percent change in lumbar CSF F_2-IsoPs over 1 year in patients with mild AD who supplemented their diets with no α-tocopherol or ascorbate (no E or C, $n = 4$), α-tocopherol only (E only, $n = 16$), or both α-tocopherol and ascorbate (E plus C, $n = 9$). Deuterated 8-*iso*-$PGF_{2\alpha}$ ($iPF_{2\alpha}$-III) was used as internal standard and F_2-IsoPs measured using the original GC-MS method. ANOVA showed $p = 0.01$ with corrected repeat comparisons showing only the "E plus C" significantly different from "no E or C" (*$p < 0.05$).

shows the percent change in CSF F_2-IsoPs observed in these 40 AD patients stratified for dietary supplementation with no antioxidant vitamins, α-tocopherol, or α-tocopherol plus ascorbate (no one took ascorbate alone). Patients without dietary supplementation showed an ~50% increase in CSF F_2-IsoPs over the 1-year period. Consonant with recently reported epidemiologic observations on the stratification of risk for AD (43), we observed a significant pharmacologic effect only in that group that supplemented their diets with α-tocopherol and ascorbate, but not α-tocopherol alone (36).

F_2-IsoPs IN PLASMA AND URINE

Although obtaining CSF from the lumbar cistern is not associated with significant risks, even in the elderly, when performed by experienced physicians, 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. Like most data for peripheral biomarkers of neurodegenerative disease, the results have been conflicting. Some groups have reported increased F_2-IsoPs in plasma or urine of AD patients compared with age-matched controls (31, 33, 41, 42), whereas others, including us, have seen no difference (1, 4, 19, 21). It is important to remember two points from the CSF data: (a) very similar concentrations of F_2-IsoPs were measured in both ventricular and lumbar CSF from control individuals using either the original or modified GC-MS method, and (b) there was a statistically significant increase in AD for all studies, although the magnitude was larger with the modified method (Table 1). Despite this very close quantitative agreement for CSF, there are substantial quantitative differences between these two methods in plasma and urine (Table 1). The reasons why these values should differ so widely in the periphery, but not in CSF, are not clear. The most recent investigation of peripheral F_2-IsoPs in AD directly addressed this important issue by applying a new LC-MS-MS method that 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 (1); these investigators were unable to detect a significant difference in urine $iPF_{2\alpha}$ concentrations between AD patients and controls.

F_2-IsoPs are generated by every cell, and therefore, peripheral production unrelated to CNS disease could easily confound interpretation of blood or urine levels in AD patients. Thus, it is worth considering further those studies that measured both CSF and plasma F_2-IsoPs. Even if one imagined complete transfer of all increased CSF F_2-IsoPs from CSF to plasma, then based on the largest calculated total increase in F_2-IsoPs, ventricular CSF $iPF_{2\alpha}$-VI (from 38 pg/ml × 300 ml of CSF in controls to 102 pg/ml × 450 ml of CSF in AD), one would expect a maximum of an additional 34 ng of $iPF_{2\alpha}$-VI from CSF to plasma; this would yield an expected increase of only 12 pg/ml $iPF_{2\alpha}$-VI in AD plasma compared with controls (assuming 3,000 ml of plasma). However, an increase of ~460 pg/ml 8-,12-*iso*-$iPF_{2\alpha}$-VI was reported in plasma of AD patients. The basis for this large increase in plasma 8-,12-*iso*-$iPF_{2\alpha}$-VI compared with CSF in AD patients is not clear, but it seems highly improbable that the observed increase in plasma 8-,12-*iso*-$iPF_{2\alpha}$-VI can be accounted for solely by the increase in CSF. Indeed, we have tested this hypothesis directly by exposing rats to kainic acid that significantly increased cerebral F_2-IsoPs to a level comparable to AD brain; however, no significant increase in plasma or urine F_2-IsoPs was observed in the same rats (21).

We conclude by a variety of analytical methods that plasma and urine F_2-IsoPs, including $iPF_{2\alpha}$-VI, are not reproducibly increased in AD patients compared with controls. In addition, only a very small fraction of plasma F_2-IsoPs derive from CSF, and so interpretation of their changes in plasma or urine is limited with respect to CNS disease.

SUMMARY

Results from studies with F_2-IsoPs confirm earlier work showing that diseased regions of brain from patients with advanced AD have increased levels of lipid peroxidation products compared with controls. Importantly, patients with early AD or HD who were not receiving antioxidants had increased CSF F_2-IsoP levels compared with age-matched controls, thus establishing increased oxidative damage in the CNS as a potential therapeutic target in these diseases. Indeed, we have shown that dietary supplementation with α-tocopherol and ascorbate is associated with a significant suppression of longitudinal increase in lumbar CSF F_2-IsoPs in patients with early AD. Although highly desirable, peripheral F_2-IsoPs are not reproducibly increased in patients with AD or in an experimental model of cerebral oxidative damage. Moreover, even in those studies reporting increased 8-,12-*iso*-$iPF_{2\alpha}$-VI in plasma from AD patients, comparison with CSF concentrations raises serious concerns about the relevance of peripheral F_2-IsoPs to CNS disease. CSF F_2-IsoPs likely will be useful in the objective assessment of antioxidants targeted to the CNS and perhaps as adjuncts to the laboratory diagnosis of AD.

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ABBREVIATIONS

Aβ, amyloid β; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CNS, central nervous system; CSF, cerebrospinal fluid; F_2-IsoPs, F_2-isoprostanes; GC, gas chromatography; HD, Huntington's disease; 8-*iso*-$PGF_{2\alpha}$, 8-*iso*-prostaglandin $F_{2\alpha}$; LC, liquid chromatography; MCI, mild cognitive impairment; MS, mass spectrometry; NFT, neurofibrillary tangle.

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