*Q J Med* 2001; **94**:485–490

# Increased oxidative stress in Alzheimer's disease as assessed with 4-hydroxynonenal but not malondialdehyde

L.T. McGRATH, B.M. McGLEENON, S. BRENNAN, D. McCOLL, S. McILROY and A.P. PASSMORE

*From the Department of Geriatric Medicine, Queens University of Belfast, Belfast, UK*

*Received 21 January 2000 and in revised form 6 July 2001*

## Summary

**Oxidative stress is thought to play a major role in the pathogenesis of Alzheimer's disease (AD). Although there is strong post-mortem and experimental evidence of oxidative damage occurring in AD brains, the use of markers in the peripheral circulation to show oxidative stress is less convincing. We examined plasma from AD patients for markers of increased oxidative stress. We report elevated levels of 4-hydroxy-nonenal (4-HNE) in AD patients compared to controls (median 20.6, IQR 6.0–25.2 vs. 7.8, 3.3–14.5 μmol/l, respectively; $p = 0.001$) but not malondialdehyde (MDA), and lower levels of ascorbate in AD plasma when compared to age-matched controls (9.9, 6.0–33.7 vs. 24.2, 13.9–48.6 μmol/l; $p < 0.05$). Levels of 4-HNE in AD patients were inversely related to ascorbate ($r = -0.337$; $p = 0.07$) and Folstein Mini-Mental State Examination (MMSE) ($r = -0.474$; $p = 0.015$). The concentration of protein sulphydryls, free-radical scavengers, was directly related to the MMSE result ($r = 0.427$; $p = 0.03$). Increased production of 4-HNE indicates increased oxidative stress (lipid peroxidation), which is not evident using the more common marker MDA. This elevation of 4-HNE was related to the degree of cognitive impairment (MMSE).**

## Introduction

The clinical features of Alzheimer's disease (AD) are coupled with a progressive loss of neurones in several different regions of the brain. One theory on the pathogenesis of AD postulates that neurodegeneration is the result of oxidative stress and damage to vulnerable cerebral tissues. The brain has a high lipid content and poor antioxidant defences. In addition, a high metabolic rate and an abundant supply of the necessary transition metals, make the brain an ideal target for free radical attack.

Free radicals are compounds possessing an unpaired electron, which renders them highly reactive and capable of causing oxidative damage to all the major macro-molecules in cells, including lipids, proteins and nucleic acids. A major family of free radicals is the reactive oxygen species, derived metabolically from molecular oxygen via the superoxide anion. Oxidative attack on proteins results in the formation of protein carbonyls, often with loss of functionality of the parent protein. Free radicals can attack polyunsaturated fatty acids in cell-membrane phospholipids, resulting in the formation of lipid peroxides which can then fragment to numerous small compounds which may be measured to assess lipid peroxidation. Two of these compounds, MDA and 4-HNE, are accepted routine methods of assessing lipid peroxidation.

Under normal circumstances, the brain is protected from such damage by a careful balance between pro-oxidant and antioxidant mechanisms which include antioxidant enzymes and free-radical-scavenging chemicals such as ascorbate, vitamin E and protein sulphydryls. In AD, this balance appears to be disturbed, with pathological studies of biopsy and post-mortem cerebral tissue reporting excess DNA oxidation,[1] protein

*Address correspondence to Dr A.P. Passmore, Department of Geriatric Medicine, Whitla Medical Building, 97 Lisburn Road, Belfast BT9 7BL. e-mail: p.passmore@qub.ac.uk*

oxidation[2,3] and lipid peroxidation,[4,5] and increased activity of the antioxidant enzyme superoxide dismutase (SOD).[1]

Oxidative stress tends to be highly compartmentalized, and it is usually impossible to access affected tissue directly in living subjects. Evidence of oxidative stress from examining effects in the systemic circulation of AD patients has been less conclusive. There are reports of increased DNA oxidation in the leucocytes of AD patients,[6] alongside reports of altered activity of free radicals and SOD activity in plasma,[7,8] and lowered antioxidant activity.[9,10]

A major limitation of assessing oxidative stress by examining markers in the peripheral circulation is that it gives a global assessment, with no indication as to the actual site of the oxidative stress. There is strong evidence to support the hypothesis that, within the CNS, beta amyloid protein (Aβ) is the source of this oxidative stress, with evidence of cytotoxic activity in neural and endothelial cell cultures.[11] These findings have given weight to the proposal that oxidative stress plays a key role in disease development, and may involve Aβ protein and the brain microvasculature. We postulated that if oxidative stress were a core mechanism of AD, with involvement of the microvasculature, then effects of this excess oxidation would be measurable in the circulation. We examined the serum from AD patients for evidence of increased oxidative damage to lipids and proteins, and assessed antioxidant status.

# Methods

## Patients

We recruited 29 patients (18 female) from the memory clinic at the Belfast City Hospital. Patients were diagnosed using the NINCDS-ADRDA criteria[12] and were diagnosed as probable Alzheimer's disease, with Folstein Mini-Mental State Examination (MMSE)[13] scores ranging from 7 to 24. A full medical history was taken, and physical examination performed, and patients with evidence of other significant medical problems were excluded. All patients and controls had a nutritional assessment, consisting of both anthropometric measurements and a dietary history.[14] Subjects were excluded if they were considered to be malnourished on the dietary assessment (<19), or if the subject was taking antioxidant supplements.

The control population was recruited from local retirement clubs and from the Department of Podiatric Medicine, Queens University of Belfast. Healthy subjects ($n = 46$, 28 female) were age-matched, underwent a medical history and examination, scored >29/30 on the Folstein MMSE and were on no regular medication. Both patients and controls had laboratory analysis of urea and creatinine, liver function tests, fasting lipids and thyroid function tests. The nature and purpose of the study was explained to all participants, and to the relatives/carers of AD patients, prior to consent being given. The study was approved by the ethical committee of Queens University Belfast.

## Assays

Protein assay used a Sigma Diagnostics kit. Assay of malondialdehyde and 4-hydroxy nonenal used an LPO-586 kit from Calbiochem. Total cholesterol was analysed using enzymic kits from Boehringer Mannheim. Biuret kits for the assay of total protein were from Randox Laboratories.

## Sample collection and treatment

The sampling protocol was designed to minimize oxidation after collection. Venous blood (20 ml) was collected from subjects and divided between a heparinized tube and a tube containing EDTA. Samples were gently mixed, packed in ice and prepared immediately. All samples were maintained at 4 °C throughout preparation. The heparinized and EDTA-containing bloods were centrifuged at 3000 *g* for 10 min at 4 °C. Heparinized plasma was aliquoted and set aside for analysis of protein sulphydryls and protein carbonyls. For analysis of carbonyls, 500 μl plasma was mixed with 50 μl 0.2% (w/v) ethanolic butylated hydroxy toluene (BHT) as antioxidant. Plasma containing EDTA was aliquoted and set aside for analysis of ascorbate, MDA, 4-HNE and vitamin E. For analysis of ascorbate, 500 μl plasma was mixed with 500 μl 10% (w/v) aqueous metaposphoric acid and centrifuged at 3000 *g* for 5 min at 4 °C. The supernatant was removed and set aside for analysis. For analysis of MDA and 4-HNE, 500 μl plasma was mixed with 50 μl 0.2% (w/v) ethanolic BHT. All samples were stored at −80 °C and analysed within 4 weeks.

## Biochemical analyses in plasma

Total protein was determined using a commercially-available biuret method. MDA and 4-HNE were determined by a commercially-available assay based upon the method of Esterbauer and Cheeseman,[15] and the results were expressed as μmol/l. In brief, preformed malondialdehyde and 4-HNE were reacted with N-methyl-2-phenylindole and methanesulfonic acid. Substitution of

methanesulfonic acid with 12 M HCl allows measurement of MDA alone. Total cholesterol was measured enzymically using a commercially-available kit. Total plasma protein sulphydryls and protein carbonyls were assayed spectrophotometrically.[16,17] Protein carbonyl and sulphydryl results were expressed as μmol/g per g of protein. Ascorbate was measured by HPLC as described by Inyama *et al*.[18] Vitamin E was measured by HPLC according to the method of Hatam and Kayden,[19] standardized for the concentration of cholesterol and expressed as μmol per mmol cholesterol.

## Statistical analysis

Data were analysed using the SPSS package. Normality of distribution of the data was assessed using the Normal plot method. Values for ascorbate and HNE were not normally distributed. Comparisons for these two compounds were done using a Mann-Whitney test, and results were expressed as median (IQR) values. The remaining variables were normally distributed and comparisons were made using a non-paired *t* test with results expressed as mean ± SD. Pearson's correlation was applied to determine the correlation between plasma 4-HNE and other markers of oxidative stress and scavengers. Significance was determined by linear regression. A $p$ value of $< 0.05$ or less was considered significant.

# Results

## Patients

The clinical characteristics of the study group are shown in Table 1. A dietary assessment of both groups revealed a significantly lower score for AD patients compared to controls, $24.26 \pm 2.86$ vs. $27.72 \pm 1.71$, respectively ($p < 0.05$), indicating a higher risk of malnutrition. This was present despite similar BMI scores: $25.2 \pm 4.4$ and $26.6 \pm 3.9$ for AD patients and controls, respectively. There was no significant difference between the groups for any of the other parameters assessed, including sex.

## Serum analysis

Measures of oxidation in serum showed significantly elevated levels of 4-HNE in AD patients compared to controls: median 20.6 (6.0–25.2) vs. 7.8 (3.3–14.5) μmol/l, respectively, $p = 0.001$ (Figure 1, Table 2). The other measure of lipid peroxidation used in this study, MDA, showed no elevation in AD patients. There was no significant difference observed in carbonyls, the measure of protein oxidation (Table 2). Measurement of serum antioxidants demonstrated a significantly lower level of ascorbate in AD patients compared to controls, 9.9 (6.0–33.7) vs. 24.2 (13.9–48.6) μmol/l, respectively ($p < 0.05$) (Figure 2, Table 3). No difference was observed in levels of vitamin E or protein sulphydryls (Table 3). Plasma levels of 4-HNE in

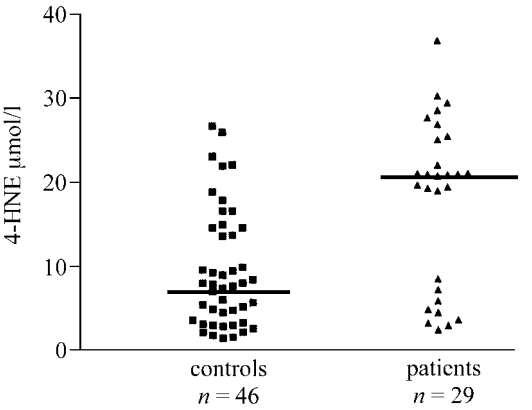


**Figure 1.** Plasma 4-hydroxynonenal (4-HNE) concentrations in Alzheimer's disease patients and controls. Scatter plot showing median value (horizontal bar). $p = 0.001$.

**Table 1** Patient and control characteristics

| | Alzheimer's disease | Controls | *p* |
|---|---|---|---|
| *n* | 29 | 46 | |
| Sex (M/F) | 11/18 | 18/28 | |
| Age (years) | 74 (56–87) | 73 (63–86) | 0.94 |
| Body mass index (kg/m$^2$) | $25.2 \pm 4.4$ | $26.6 \pm 3.9$ | 0.27 |
| Systolic blood pressure (mmHg) | $148 \pm 18$ | $153 \pm 12$ | 0.22 |
| Diastolic blood pressure (mmHg) | $82 \pm 8$ | $85 \pm 8$ | 0.22 |
| Fasting cholesterol (mmol/l) | $5.94 \pm 1.03$ | $6.00 \pm 1.13$ | 0.82 |
| Mini-Nutritional Assessment | $24.26 \pm 2.86$ | $27.71 \pm 1.71$ | $< 0.05$* |
| Folstein Mini-Mental Score | 7–24 | $> 29$ | $< 0.001$* |

Figures for age are means with range; remaining parameters are means ± SD. *Significant at $p < 0.05$.

**Table 2** Plasma markers of oxidative stress in Alzheimer's disease patients and controls

| | Alzheimer's disease | Controls | *p* |
|---|---|---|---|
| *n* | 29 | 46 | |
| Carbonyls (μmol/g protein) | 0.58 ± 0.25 | 0.55 ± 0.2 | 0.52 |
| MDA (μmol/l) | 5.97 ± 2.37 | 5.32 ± 1.50 | 0.16 |
| 4-HNE (μmol/l) | 20.65 (6.02–25.20) | 7.80 (3.35–14.50) | 0.001* |

MDA, malondialdehyde; 4-HNE, 4-hydroxynonenal. Figures for carbonyls and MDA are means ± SD; figures for 4-HNE are medians (IQR). *Significant at $p < 0.05$.

**Table 3** Plasma levels of antioxidants in Alzheimer's disease patients and controls

| | Alzheimer's disease | Controls | *p* |
|---|---|---|---|
| *n* | 29 | 46 | |
| Ascorbate (μmol/l) | 9.9 (6.0–33.7) | 24.2 (13.9–48.6) | 0.04* |
| Vitamin E (μmol/mmol cholesterol) | 4.08 ± 0.90 | 4.47 ± 1.39 | 0.21 |
| Sulphydryls (μmol/g protein) | 3.74 ± 0.96 | 3.87 ± 1.05 | 0.60 |

Figures for ascorbate are medians (IQR); figures for vitamin E and sulphydryls are means ± SD. *Significant at $p < 0.05$.

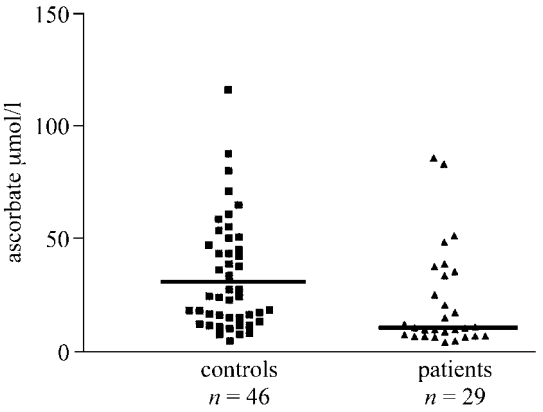


**Figure 2.** Plasma ascorbate concentrations in Alzheimer's disease patients and controls. Scatter plot showing median value (horizontal bar). $p = 0.04$.

AD patients demonstrated a degree of inverse correlation with plasma ascorbate levels ($r = -0.337$; $p = 0.07$) and with MMSE ($r = -0.474$; $p = 0.015$). Also in AD patients, total plasma sulphydryls demonstrated a positive correlation with MMSE ($r = 0.427$; $p = 0.03$). These correlations were not seen in healthy controls.

## Discussion

There are numerous reports of excess oxidation within the brain of AD patients, but outside the CNS, the evidence is less definite. Blood components have been widely examined for evidence of a systemic oxidative effect, with changes in membrane fluidity,[20] platelet activation[21] and more specifically, reports of leukocyte oxidation.[6]

We have now found evidence of lipid peroxidation in the serum of patients with AD, with significantly higher levels of 4-HNE, an aldehydic product of lipid peroxidation. An important observation was that the concentration of 4-HNE in patients was related to the degree of cognitive impairment. Although 4-HNE levels were higher, other measures of lipid peroxidation (MDA) and protein oxidation (carbonyls) were not elevated in AD patients. Other studies have failed to show any difference in serum MDA in AD.[10,22,23] The type of assay commonly used may account for this discrepancy, as often no specific breakdown product is measured, and results are given as total thiobarbituric-acid-reacting substances (TBARS). We have found a specific elevation of 4-HNE, which might not have been detected if this general method was used. Elevated levels of 4-HNE have been reported in the brain tissue[24] and ventricular fluid of AD patients.[25] In addition, HNE has been identified in the amyloid component of senile plaques.[26] The effects of this compound have been the focus of much recent research. 4-HNE has been shown to accumulate in areas of oxidative stress, with evidence that it inhibits DNA and RNA, and inactivates and modifies enzyme systems.[27] We know from functional studies that the blood-brain barrier (BBB) is grossly intact in patients with AD,[28,29] but there are several reports of abnormal small-vessel structure, particularly affecting the endothelium, which could affect BBB function.[30,31] This might contribute to the presence of oxidative

products in the systemic circulation, and changes in blood components that are in contact with the altered microvasculature.

In tissue culture, 4-HNE has shown evidence of cytotoxicity and induction of chromosomal abnormalities at concentrations previously found in biological specimens.[32] Specifically in AD, 4-HNE has been implicated in mediating the neurotoxic effects of Aβ peptide.[33] This theory has been supported by the finding of 4-HNE in association with amyloid deposits in AD.[26] All of this points to 4-HNE having a significant role in mediating oxidative stress reactions. The elevated levels of 4-HNE in our study emphasize the importance of lipid peroxidation as an important reaction in Alzheimer's disease.

Serum levels of ascorbate were significantly lower in AD patients than in controls, but vitamin E was not different. A larger study of 79 AD patients showed both vitamin E and ascorbate (as well as other antioxidant measures) to be significantly lower in AD patients, although the vitamin E levels were not corrected for cholesterol.[9] In addition, this study failed to make any assessment of dietary intake in their subjects. Most studies exclude patients considered to be malnourished, and this is often decided on the basis of body mass index scores or plasma proteins and haemoglobin levels. We have used a Mini-Nutritional Score (MNA) on all subjects, which details both anthropometric measures, and a dietary history, to exclude patients who were considered to be malnourished.[14] The scale is simple to use in this population, although input from a carer is essential in cognitively impaired patients. Despite similar BMI scores, AD patients had significantly lower scores in their MNA, indicating that AD patients had specifically lower scores in the 'general assessment' part, which includes dependency levels, a medical history, and the dietary history. The majority of AD patients in this study were living at home with a carer who was responsible for the preparation of food. Thus no significant change in their dietary habits was evident as a result of the disease process. Like other similar studies into dietary antioxidants in AD, it remains difficult to ascertain whether any change is a consequence of the disease process with ascorbate being consumed during scavenging of free radicals, a change in dietary intake, or a predisposing factor in the development of AD. The finding of lower MNA scores in this group of AD patients suggests dietary deficiency, but we believe this only partly explains the deficiency. Another scavenger measured in this study was protein sulphydryls. While these were not elevated, their levels in AD patients were positively correlated with the degree of cognitive impairment, which would support the role of increased radical activity and scavenging/consumption.

The exact role of 4-HNE in the AD brain has yet to be established, but it is acknowledged that 4-HNE is a highly toxic compound capable of causing extensive lipid membrane damage. Kruman *et al.* have shown that 4-HNE mediates oxidative stress induced neuronal apoptosis.[34] This effect was not seen with other aldehydic products of lipid peroxidation. Antioxidants prevent apoptosis induced by oxidative stress, but they have no effect when preformed 4-HNE is added.[34] Glutathione, by binding 4-HNE, inhibits this apoptosis. Mark *et al.* have shown that 4-HNE is involved in the disruption of ion homeostasis and neuronal cell death induced by amyloid β peptide.[35] Page *et al.* have shown that 4-HNE specifically inhibits the NF-kB/Rel system. This may contribute to inflammatory/degenerative diseases.[36] There is evidence to suggest that ascorbate plays a role in maintaining levels of glutathione.[37] It is possible that lower levels of ascorbate in AD could contribute to lower glutathione levels and a resultant failure to detoxify accumulating 4-HNE. This would lead to a further cascade of oxidative damage. Thus the combination of low levels of ascorbate related to high levels of 4-HNE are important findings in light of the mounting evidence that oxidative stress plays a key role in the development of Alzheimer's disease. This is reinforced by the positive relationship between high levels of 4-HNE and impaired cognitive function.

## References


1. Mecocci P, MacGarvey U, Beal MF. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann Neurol* 1994; **36**:747–51.
2. Munch G, Thome J, Foley P, Schinzel R, Riederer P. Advanced glycation endproducts in ageing and Alzheimer's disease. *Brain Research Reviews* 1997; **23**:134–43.
3. Smith CD, Carney JM, Starke-Reed PE, *et al.* Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. *Proc Natl Acad Sci USA* 1991; **88**:10540–3.
4. Lovell MA, Ehmann WD, Butler SM, Markesbery WR. Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. *Neurology* 1995; **45**:1594–601.
5. Subbarao KV, Richardson JS, Ang LC. Autopsy samples of Alzheimer's cortex show increased peroxidation *in vitro*. *J Neurochem* 1990; **55**:342–5.
6. Mecocci P, Cherubini A, Senin U. Increased oxidative damage in lymphocytes of Alzheimer's disease patients. *JAGS* 1997; **45**:1536–7.
7. Ihara Y, Hayabara T, Sasaki K, *et al.* Free radicals and superoxide dismutase in blood of patients with Alzheimer's disease and vascular dementia. *J Neurol Sci* 1997; **153**:76–81.

8. Marcus DL, Thomas C, Rodriguez C, *et al*. Increased peroxidation and reduced antioxidant enzyme activity in Alzheimer's disease. *Exp Neurol* 1998; **150**:40–4.
9. Foy CJ, Passmore AP, Vahidassr MD, Young IS, Lawson JT. Plasma chain-breaking antioxidants in Alzheimer's disease, vascular dementia and Parkinson's disease. *Q J Med* 1999; **92**:39–45.
10. Sinclair AJ, Bayer AJ, Johnston J, Warner C, Maxwell SR. Altered plasma antioxidant status in subjects with Alzheimer's disease and vascular dementia. *Int J Geriatr Psychiatry* 1998; **13**:840–5.
11. Behl C. Amyloid beta-protein toxicity and oxidative stress in Alzheimer's disease. *Cell Tissue Res* 1997; **290**:471–80.
12. 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 Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984; **34**:939–44.
13. Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state': a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; **12**:189–98.
14. Guigoz Y, Vellas B, Garry PJ. Assessing the nutritional status of the elderly: The Mini Nutritional Assessment as part of the geriatric evaluation. *Nutr Rev* 1996; **54**:S59–65.
15. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods in Enzymology* 1990; **186**:407–21.
16. Brown RK, Kelly FJ. Evidence for increased oxidative damage in patients with cystic fibrosis. *Paediatr Res* 1994; **36**:487–93.
17. Levine RL, Garland D, Oliver CN. The determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990; **186**:464–77.
18. Inyami K, Yoshiura M, Iwamoto T. Simultaneous determination of uric acid and ascorbic acid in human serum by reversed-phase high-performance liquid chromatography with electrochemical detection. *Anal Biochem* 1984; **141**:238–43.
19. Hatam LJ, Kayden HJ. A high performance liquid chromatographic method for the determination of tocopherol in plasma and cellular elements of the blood. *J Lipid Res* 1979; **20**:639–45.
20. Zubenko GS, Cohen BM, Boller F, Malinakova I, Keefe N. Platelet membrane abnormality in Alzheimer's Disease. *Ann Neurol* 1987; **22**:237–44.
21. Sevush S, Jy W, Horstman LL, Mao WW, Kolodny L, Ahn YS. Platelet activation in Alzheimer disease. *Arch Neurol* 1998; **55**:530–6.
22. Ahlskog JE, Uitti RJ, Low PA. No evidence for systemic oxidant stress in Parkinson's or Alzheimer's disease. *Movement Disord* 1995; **10**:566–73.
23. Jeandel C, Nicolas MB, Dubois F, Nabet-Belleville F, Penin F, Cuny G. Lipid peroxidation and free radical scavengers in Alzheimer's disease. *Gerontology* 1989; **35**:275–82.
24. Markesbery WR, Lovell MA. Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. *Neurobiol Aging* 1998; **19**:33–6.
25. Lovell MA, Ehmann WD, Mattson MP, Markesbery WR. Elevated 4-hydroxynonenal in ventricular fluid in Alzheimer's disease. *Neurobiol Aging* 1997; **18**:457–61.
26. Ando Y, Brannstrom T, Uchida K, *et al*. Histochemical detection of 4-hydroxynonenal protein in Alzheimer amyloid. *J Neurol Sci* 1998; **156**:172–6.
27. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. *Free Radic Biol Med* 1991; **11**:81–128.
28. Caserta MT, Caccioppo D, Lapin GD, Ragin A, Groothuis DR. Blood-brain barrier integrity in Alzheimer's disease patients and elderly control subjects. *J Neuropsychiatry Clin Neurosci* 1998; **10**:78–84.
29. Friedland RP, Yano Y, Budinger TF. Quantitative evaluation of blood-brain-barrier integrity in Alzheimer type dementia: positron emission tomographic studies with rubidium-82. *Eur Neurol* 1983; **22**(suppl. 2):15–20.
30. Buee L, Hof PR, Delacourte A. Brain microvascular changes in Alzheimer's disease and other dementias. *Ann N Y Acad Sci* 1997; **826**:7–24.
31. Hashimura T, Kimura T, Miyakawa T. Morphological changes of blood vessels in the brain with Alzheimer's disease. *Jpn J Psychiatr Neurol* 1991; **45**:661–5.
32. Karlhuber GM, Bauer HC, Eckl PM. Cytotoxic and genotoxic effects of 4-hydroxynonenal in cerebral endothelial cells. *Mutat Res* 1997; **381**:209–16.
33. Keller JN, Pang Z, Geddes JW, *et al*. Impairment of glucose and glutamate transport and induction of mitochondrial oxidative stress and dysfunction in synaptosomes by amyloid beta-peptide: role of the lipid peroxidation product 4-hydroxynonenal. *J Neurochem* 1997; **69**:273–84.
34. Kruman I, Bruce-Keller AJ, Bredesen D, Waeg G, Mattson MP. Evidence that 4-hydroxynonenal mediates oxidative stress-induced neuronal apoptosis. *J Neurosci* 1997; **17**:5089–100.
35. Mark RJ, Lovell MA, Markesbery WR, Uchida K, Mattson MP. A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid beta-peptide. *J Neurochem* 1997; **68**:255–64.
36. Page S, Fischer C, Baumgartner B, *et al*. 4-Hydroxynonenal prevents NF-κB activation and tumour necrosis factor expression by inhibiting IκB phosphorylation and subsequent proteolysis. *J Biol Chem* 1999; **274**:11611–18.
37. Seven A, Tasan E, Inci F, Hatemi H, Burcak G. Biochemical evaluation of oxidative stress in propylthiouracil treated hyperthyroid patients. Effects of vitamin C supplementation. *Clin Chem Lab Med* 1998; **36**:767–70.