
Plasma chain-breaking antioxidants in Alzheimer's disease, vascular dementia and Parkinson's disease

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Summary

We studied the plasma chain-breaking antioxidants α carotene, β carotene, lycopene, Vitamin A, Vitamin C, Vitamin E and a measure of total antioxidant capacity, TAC, in 79 patients with Alzheimer's disease (AD), 37 patients with vascular dementia (VaD), 18 patients with Parkinson's disease and dementia (PDem), and 58 matching controls, together with 41 patients with Parkinson's disease (PD) and 41 matching controls. Significant reductions in individual antioxidants were observed in all dementia groups. When compared to controls, the following were reduced: Vitamin A in AD ($p < 0.01$) and VaD ($p < 0.001$); Vitamin C in AD ($p < 0.001$), VaD ($p < 0.001$) and PDem ($p < 0.01$); Vitamin E

in AD ($p < 0.01$) and VaD ($p < 0.001$); β carotene in VaD ($p = 0.01$); lycopene in PDem ($p < 0.001$). Lycopene was also reduced in PDem compared to AD ($p < 0.001$) and VaD ($p < 0.001$). Antioxidant levels in PD were not depleted. No significant change in TAC was seen in any group. The reduction in plasma chain-breaking antioxidants in patients with dementia may reflect an increased free-radical activity, and a common role in cognitive impairment in these conditions. Increased free-radical activity in VaD and PDem could be associated with concomitant AD pathology. Individual antioxidant changes are not reflected in TAC.

Introduction

There is growing interest in the role of free-radical damage in neoplasia, vascular disease, neurodegenerative disease and ageing.¹ The brain is an ideal target for free-radical damage. It is composed of large quantities of lipids which make an excellent substrate for free-radical reactions. It has rich reserves of iron which catalyse such reactions, and has a high energy demand which is satisfied by a high metabolic turnover and oxidative reactions in brain-cell mitochondria. Electrons can leak from the mitochondrial respiratory chain, attach themselves to molecules and form free radicals. Therefore, the brain provides a site where substrate, free radicals and catalyst are brought together in close proximity.^{2,3} Evidence has accumulated that free radical injury may contribute to the pathogenesis of

Alzheimer's disease (AD) and Parkinson's disease (PD).⁴⁻⁶

Since a few free radicals are potentially sufficient to damage severely a large number of cells, there are counter-mechanisms to prevent and repair damage. Such mechanisms include antioxidants. One major antioxidant system is the exogenous chain-breaking antioxidants, which inhibit free-radical-mediated chain reactions, and include vitamin E (α -tocopherol), vitamin C, β -carotene and a number of other less important compounds. The most important lipid-phase antioxidant is α -tocopherol, which is found in lipid membranes and low-density lipoprotein (LDL) particles.

Plasma levels of chain-breaking antioxidants can be measured and, providing that the subject has

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adequate nutrition, should reflect oxidative activity in the body. However, the collective individual measurement of these antioxidants is costly and time-consuming. In addition, there appears to be synergism between antioxidants, and a summation of individual measurements may not accurately reflect the total antioxidant effect. A measure of total antioxidant capacity (TAC) may be a more efficient method of measuring antioxidant status of subjects, and several such assays are described.⁷

The aim of this study was to establish whether increased oxidation was evident as a reduction in individual plasma chain-breaking antioxidants and TAC in AD, VaD, PDem and PD. We also wished to establish, in these diseases, whether individual antioxidant changes were reflected in TAC.

Methods

The study was approved by the local ethical committee. All subjects were recruited and assessed by the same physician, trained in the use of the scales and familiar with the diagnostic criteria. Written informed consent was obtained from subjects and carers prior to inclusion in the study. Patients were recruited from the Memory Clinic and Day Hospital, Belfast City Hospital, and from the Alzheimer's Disease Society. Dementia patients were assessed using a structured interview and examination that included the Folstein Mini Mental State Examination (MMSE),⁸ Geriatric Depression Scale,⁹ Barthel index of activities of daily living¹⁰ and the Hachinski Ischaemic Scale.¹¹ Dementia subjects were living in the community with a cognitively-intact carer who was responsible for preparation of meals. All participants/carers were specifically questioned about diet, which had to comprise three meals a day, one of which included meat and vegetables. A full and detailed nutritional assessment was not performed. A blood screen for systemic causes of dementia, including a full blood picture, liver, renal and thyroid function tests, a C-reactive protein, B12 and folate, and syphilitic serology was analysed and CT scan of brain performed. Diagnoses were made as probable AD and probable VaD according to the DSM IV,¹² NINCDS ADRDA,¹³ and AIREN¹⁴ criteria, respectively. Mixed and other forms of dementia except PDem were excluded. Patients included had mild to moderate dementia (MMSE > 10 and ≤ 24), were physically well, and living in the community. Control subjects were recruited from retirement clubs and from volunteers who have helped in other studies within the Department. These were cognitively intact and had no serious or unstable medical condition.

Patients with PD were recruited from out-patient

clinics (Neurology and Elderly Care) and Geriatric Day Hospital, Belfast City Hospital. PD and PDem patients with concomitant disease and/or atypical features were excluded. A diagnosis of PD was made in accordance with the UKPDSBB criteria¹⁵ and dementia was established using MMSE and DSM III R criteria.¹⁶ Since the PD group were significantly younger than those with dementia, additional younger controls were recruited from retirement clubs and from younger people who have previously assisted in departmental research.

Subjects were excluded from the study if haemoglobin or albumin levels were below normal ranges quoted in the Laboratory, Belfast City Hospital. In the case of haemoglobin, values lower than 12.5 g/dl for males and 11.5 g/dl for females were excluded and serum albumin < 35 g/l was also excluded. Other exclusion criteria included any serious concomitant disease, including diabetes mellitus, and significant gastrointestinal disease, and ingestion of vitamin supplements.

Three hundred and fifty subjects were screened for this study. Seventy-one were excluded on the grounds of low haemoglobin or albumin levels. The majority of those excluded were patients with dementia. A further 28 samples were unsuitable for analysis. Antioxidant assays were not performed on excluded subjects. A total of 251 subjects were included (23 of the dementia control group were suitable for controls in the PD control group).

Five ml of EDTA-anticoagulated blood was taken from each subject for antioxidant analysis. The blood was centrifuged immediately, plasma separated and divided into 0.5 ml aliquots. An equal measure (0.5 ml) of 10% metaphosphoric acid was added to one plasma aliquot, vortex-mixed and stored for ascorbic acid analysis. Plasma samples were frozen and stored at -70°C until immediately before analysis.

Subject types were mixed during sampling to reduce the effect of variations in sampling technique. Samples were stored and subsequently analysed in batches. To minimize bias and the effect of variation in laboratory analysis, each batch contained a mix of subject types, and technicians analysing the samples were unaware of diagnoses.

The following plasma antioxidants were measured and compared between subject groups: α -carotene, β -carotene, lycopene, vitamin A, vitamin C, vitamin E, vitamin E corrected for cholesterol, urate, bilirubin, total antioxidant capacity.

Retinol, α -tocopherol, α -carotene, β -carotene and lycopene were determined simultaneously using a HPLC method.¹⁷ Intra- and inter-batch CVs for all assays were < 8% and < 10%, respectively.

Plasma vitamin C measurement was made by a fluorimetric assay using a centrifugal analyser with

fluorescent attachment.¹⁸ Intra- and inter-batch CVs were 1.7% and 2.5%, respectively.

The TAC assay measures the inhibitory effect of antioxidants in plasma or other biological fluids when added to a reaction generating water-soluble peroxy radicals at a constant rate. The length of time for which the reaction is inhibited is measured and compared against inhibition produced by a standard preparation of trolox or ascorbic acid. TAC is then expressed as $\mu\text{mol/l}$ of trolox or ascorbate equivalents. On addition of plasma, the reaction is almost instantaneously suppressed. Various methods including chemiluminescence have been used to detect the reappearance of peroxy radicals once the antioxidants are exhausted. An enhanced chemiluminescent technique was used for TAC measurement in this study.¹⁹ The intra- and inter-batch coefficients of variation (CVs) for this assay were 3.6% and 6.5%, respectively.

Urate, cholesterol and bilirubin were measured using standard laboratory techniques on a Kodak Extrachem analyser.

As distributions of the resulting data were skewed, non-parametric methods of analysis were used. Kruskal-Wallis 1 Way Analysis of Variance (ANOVA) was used to describe the significant changes in variables. The Mann-Whitney Wilcoxon Rank Sum W Test was used in *post-hoc* analyses, and describes the significance of these changes between groups. This test was only performed on those variables showing significance in the ANOVA. The Mann-Whitney/Wilcoxon Rank Sum W Test was also used in the comparison of PD and respective controls. Significance was accepted at the 5% level.

Results

Dementia, PD and control populations are described in Table 1. The values for the variables studied are shown in Table 2 for dementia patients and controls. There were significant differences in plasma levels of the individual chain-breaking antioxidants β -carotene, lycopene, vitamins A, C, E, and also in serum cholesterol between dementia and control groups.

No significant difference in cholesterol-corrected vitamin E or TAC was detected (Table 2).

The statistical analysis for the comparisons between dementia groups and controls is shown in Table 3. Significant differences observed on comparing individual groups were as follows. β -Carotene was significantly reduced in VaD compared to controls ($p=0.01$) and to AD ($p=0.02$). Lycopene was significantly lower in PDem compared to controls ($p<0.001$), AD ($p<0.001$) and VaD ($p=0.001$). Vitamin A was significantly reduced in AD ($p<0.01$) and VaD ($p<0.001$) compared to controls. Vitamin C was significantly reduced in AD ($p<0.001$), VaD ($p<0.001$) and PDem ($p<0.01$) compared to controls. Vitamin E was significantly lower in AD ($p<0.01$) and VaD ($p<0.001$) compared to controls. Cholesterol was significantly higher in the control group compared to AD ($p=0.04$), VaD ($p<0.01$), and PDem ($p<0.01$).

The only difference between PD and controls was a significant reduction in bilirubin in PD (Table 4).

Discussion

This study demonstrates a significant depletion of specific chain-breaking antioxidants in the dementia groups studied. No significant changes in these antioxidants were demonstrated in PD. There was also no significant change in the total antioxidant measure TAC for any group in this study.

Vitamins A, C and E were depleted in AD compared to controls. Vitamins A, C and β -carotene were also significantly reduced in the VaD group when compared to controls. β -carotene was lower in VaD than in AD. Vitamin C and lycopene were depleted in PDem compared to controls. Lycopene was also significantly decreased in PDem compared to AD and VaD.

Changes in antioxidants have been observed in small studies of dementia subjects. Plasma vitamins A, C and E were reduced in a malnourished subgroup of AD patients.²⁰ In the present study, detailed assessment of nutritional status was not made, but an assessment was obtained from a history of

Table 1 Summary descriptions of dementia patients and controls

	<i>n</i>	Male	Female	Median age	Interquartile age range
Dementia controls	58	32	26	74	69–80
AD	79	49	30	79	72–83
VaD	37	19	18	79	69–85
PDem	18	10	8	72	67–84
PD controls	41	18	23	67	62–71
PD	41	16	25	67	62–74

AD, Alzheimer's disease; VaD, vascular dementia; PDem, Parkinson's disease with dementia; PD, Parkinson's disease alone.

Table 2 Antioxidants in dementia patients and controls

Antioxidant	Controls			AD			VaD			PDem			p
	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	
α -Carotene	0.04	0.02–0.07	0.04	0.02–0.07	0.03	0.02–0.07	0.02	0–0.04	0.05	0–0.04	0.02	0–0.04	0.05
β -Carotene	0.31	0.17–0.52	0.31	0.17–0.39	0.18	0.12–0.30	0.18	0.15–0.28	<0.05	0.15–0.28	0.18	0.15–0.28	<0.05
Lycopene	0.09	0–0.22	0.09	0–0.21	0.08	0–0.13	0	0–0	<0.001	0–0	0	0–0	<0.001
Vitamin A	1.77	1.34–2.31	1.41	1.09–1.86	1.20	0.88–1.52	1.47	1.15–1.99	<0.001	1.15–1.99	1.47	1.15–1.99	<0.001
Vitamin C	41	10–56	17	4–35	16	4–25	13	3–30	<0.001	3–30	13	3–30	<0.001
Vitamin E	25.6	21.5–32.1	23.5	18.0–26.3	20.8	15.7–25.2	22.8	17.2–30.4	<0.001	17.2–30.4	22.8	17.2–30.4	<0.001
Urate	0.30	0.23–0.37	0.31	0.24–0.37	0.26	0.21–0.33	0.24	0.23–0.33	0.01	0.23–0.33	0.24	0.23–0.33	0.01
Bilirubin	8	6–11	8	6–13	9	7–13	7	4–11	0.4	4–11	7	4–11	0.4
Cholesterol	5.65	5.0–6.30	5.20	4.7–6.0	4.8	4.05–5.90	4.85	4.25–5.60	<0.01	4.25–5.60	4.85	4.25–5.60	<0.01
Vitamin E/cholesterol	4.7	3.9–5.7	4.3	3.6–5.1	4.2	3.3–5.2	4.5	3.1–5.5	0.3	3.1–5.5	4.5	3.1–5.5	0.3
TAC	292	238–365	263	210–353	274	206–363	251	193–363	0.3	193–363	251	193–363	0.3

Units $\mu\text{mol/l}$ except cholesterol, urate and bilirubin, mmol/l ; vitamin E/cholesterol, $\mu\text{mol/l}$; vitamin E/cholesterol, $\mu\text{mol}/\text{mmol}$; and TAC, $\mu\text{mol/l}$ vitamin C equivalents. Results compared using Kruskal–Wallis 1-way ANOVA. Abbreviations as Table 1.

Table 3 Mann-Whitney Wilcoxon rank sum W test for comparison of antioxidants in dementia patients and controls

		AD	VaD	PDem
β -Carotene	C	0.4	0.01	0.06
	AD		0.02	0.11
	VaD			0.67
Lycopene	C	0.54	0.31	<0.001
	AD		0.13	<0.001
	VaD			<0.001
Vitamin A	C	<0.01	<0.001	0.14
	AD		0.07	0.67
	VaD			0.08
Vitamin C	C	<0.001	<0.001	<0.01
	AD		0.41	0.54
	VaD			0.99
Vitamin E	C	<0.01	<0.001	0.07
	AD		0.10	0.85
	VaD			0.53
Cholesterol	C	0.04	<0.01	0.01
	AD		0.13	0.17
	VaD			0.85

C, controls; AD, Alzheimer's disease; VaD, vascular dementia.

adequate food intake (patient/carer) and measurement of haemoglobin and albumin levels as previously described.²¹ Subjects were living in the community with a carer, and those with an inadequate diet or low haemoglobin or albumin were excluded. Dementia subjects in the present study had apparently normal nutritional status yet significant deficiencies of antioxidants were observed. In a previous small study AD, patients were found to be deficient in vitamins A and E and β -carotene, while VaD patients were deficient in vitamin E and β -carotene.²¹ In another study, plasma vitamin C and E were decreased in subjects with dementia, while plasma β -carotene was increased in VaD and an increase in TAC was observed in both dementias.²² The increase in TAC was said to represent a response to increased free radical activity. No reference was made to nutritional status in that study. It is suggested that oxidative modification of low-density lipoprotein is a prerequisite for atherosclerosis,²³ which in turn is a risk factor for VaD. Our findings of a reduced Vitamin A, C and E could be in keeping with such a hypothesis for atherosclerosis and resultant VaD.

The present study supports the hypothesis that excessive free-radical activity occurs in dementia, particularly in VaD and AD, and is manifest as a decrease in plasma chain-breaking antioxidants, especially vitamins A, C and E. Vitamin E was significantly reduced in AD and VaD, but when corrected for serum cholesterol, the reduction was not significant. This may have been a reflection of

Table 4 Antioxidants in Parkinson's disease without dementia (PD) and controls

Antioxidant	Controls		PD	
	Median	Interquartile range	Median	Interquartile range
α -Carotene	0.04	0.02–0.07	0.04	0.01–0.06
β -Carotene	0.25	0.13–0.49	0.26	0.16–0.44
Lycopene	0.1	0–0.26	0	0–0.29
Vitamin A	1.84	1.47–2.34	2.01	1.32–3.40
Vitamin C	44.0	13.0–61.0	38.0	16.0–56.0
Vitamin E	25.0	20.0–32.0	29.0	21.0–42.0
Urate	0.29	0.25–0.39	0.27	0.24–0.34
Bilirubin	9.0	6.0–11.0	7.0	5.0–9.0*
Cholesterol	5.5	5.0–6.3	5.8	4.7–6.7
Vitamin E/cholesterol	4.6	3.9–5.8	5.4	3.9–6.9
TAC	290.0	239.0–348.0	270.0	228.0–372.0

Units $\mu\text{mol/l}$ except urate, bilirubin, cholesterol, mmol/l ; vitamin E/cholesterol, $\mu\text{mol}/\text{mmol}$; TAC $\mu\text{mol/l}$ vitamin C equivalents. * $p=0.03$.

significantly increased cholesterol in the control group resulting in lower corrected vitamin E levels. The relatively high cholesterol in the control group was an unexpected and unexplained finding. One might have expected those subjects with vascular dementia to have a relatively increased cholesterol, and the AD group to have had increased cholesterol, through the effect of the over-representation of Apo $\epsilon 4$.²⁴

It is also possible that medication could have affected the results. Many patients and controls were on aspirin and treatments for stable ischaemic heart disease or hypertension, while PDem patients were on L-dopa preparations (five on selegiline). It could be expected that aspirin and L-dopa preparations in particular would have effects on antioxidant systems. This study was not of sufficient power to analyse for these differences.

Decreased levels of specific antioxidants were not reflected in the overall measure of antioxidant capacity TAC. However, TAC is largely influenced by urate¹⁹ which was not different between groups. These results indicate that TAC may not be a satisfactory indirect measure of free-radical activity. Recently, another method for the simultaneous determinant of plasma and erythrocyte antioxidant status has been described.²⁵ The test is based on the sensitivity to haemolysis of an erythrocyte suspension on the introduction of a radical initiator and the volume of plasma inhibiting 50% of the haemolysis. The reliability of this method has yet to be confirmed in pathological studies, and values are also influenced by plasma urate. There is the advantage of an additional measurement of lipid membrane sensitivity to free-radical attack which may act as a control for each plasma value, and this is an important measure

in itself as membranes are a key site for free-radical injury.

Studies of plasma chain-breaking antioxidants in Parkinson's disease have not shown any changes. Plasma concentrations of vitamins A and E in 27 elderly Parkinson's disease patients were no different from controls and while Vitamin C was observed to be significantly higher in Parkinson's disease, this was thought to be due to low levels in the control group.²⁶ Conversely, it was observed that vitamin C and E administration may slow disease progression in Parkinson's disease.⁵

Although there is much evidence for increased oxidation in the pathogenesis of Parkinson's disease,^{5,27,28} the present study demonstrated no deficiencies of plasma chain-breaking antioxidants in this disease. It is interesting that the PDem patients had significant decreases in plasma lycopene and vitamin C, but no significant changes in vitamins A and E compared to controls. It may also be quite significant that lycopene was reduced compared to VaD and AD. This may suggest some free-radical activity in PDem which is less intense than the other dementias and more intense than PD. Increased free radical activity in PDem may reflect the effect of concomitant AD pathology that is known to co-exist in this condition.

Oxidation may not be the sole mechanism producing neurodegeneration in the heterogeneous disorder of AD. However, it has been suggested as a possible mechanism for some of the 20 identified risk factors.²⁹ Enzyme antioxidants, studied in post-mortem AD brain homogenates^{30–32} and in transgenic mice,^{33–35} provide further evidence of increased oxidation. Several studies have also observed that both the deposition and the cytotoxicity of β -amyloid

protein (BAP) seem to be mediated by free radicals.^{36–38} It may be that dementia is a sequelae of cerebral insults from a variety of pathologies in those individuals with incompetent antioxidant mechanisms. Oxidative chain reactions may be precipitated by BAP or other pathological events and the inability to inhibit these reactions efficiently may allow more extensive secondary neuronal destruction which manifests as cognitive impairment. Further research is required to clarify the area of neuronal protection and repair mechanisms and the role of antioxidants in these processes.

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References

- Halliwel B. Free radicals, antioxidants, and human disease: curiosity, cause or consequence. *Lancet* 1994; **344**:721–4.
- Stadtman ER. Protein oxidation and aging. *Science* 1992; **257**(5074):1220–4.
- Lohr JB. Oxygen radicals and neuropsychiatric illness. Some speculations. *Arch Gen Psychiat* 1991; **48**:1097–106.
- Halliwel B. Oxidants and the central nervous system: some fundamental questions. Is oxidant damage relevant to Parkinson's disease, Alzheimer's disease, traumatic injury or stroke? *Acta Neurologica Scandinavica* 1989; **12**(Suppl.):23–33.
- Fahn S, Cohen G. The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. *Ann Neurol* 1992; **32**:804–12.
- Jenner P. Oxidative damage in neurodegenerative disease. *Lancet* 1994; **344**:796–8.
- Wayner DD, Burton GW, Ingold KU, Barclay LR, Locke SJ. The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxy radical-trapping antioxidant activity of human blood plasma. *Biochim Biophys Acta* 1987; **924**:408–19.
- Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *J Psychiat Res* 1975; **12**:189–98.
- Yesavage JA. Geriatric Depression Scale. *Psychopharmacol Bull* 1988; **24**:709–11.
- Mahoney FI, Barthel DW. Functional evaluation: Barthel Index. *Maryland State Med J* 1965; **14**:61–5.
- Hachinski VC, Lassen NA, Marshall J. Multi-infarct dementia. A cause of mental deterioration in the elderly. *Lancet* 1974; **272**:207–10.
- American Psychiatric Association Task Force on Nomenclature and Statistics. *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. Washington DC, American Psychiatric Association, 1994.
- 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.
- Roman GC, Tatemichi TK, Erkinjuntti T, Cummings JL, Masdeu JC, Garcia JH, Amaducci L, Orgogozo JM, Brun A, Hofman A, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology* 1993; **43**:250–60.
- Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J Neurol, Neurosurg Psychiat* 1988; **51**:745–52.
- American Psychiatric Association Task Force on Nomenclature and Statistics. *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edn revised. Washington DC, American Psychiatric Association, 1987.
- Thurnham DI, Smith E, Flora PS. Concurrent liquid-chromatographic assay of retinol, alpha-tocopherol, beta-carotene, alpha-carotene, lycopene, and beta-cryptoxanthin in plasma, with tocopherol acetate as internal standard. *Clin Chem* 1988; **34**:377–81.
- Vuilleumier JP, Keck E. Fluorimetric assay of vitamin C in biological materials using a centrifugal analyser with a fluorescent attachment. *J Micronutrient Anal* 1989; **5**:25–34.
- Whitehead TP, Thorpe GHG, Maxwell SRJ. Enhanced chemiluminescent assay for antioxidant capacity in biological fluids. *Analytica Chim Acta* 1992; **266**:265–77.
- 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.
- Zaman Z, Roche S, Fielden P, Frost PG, Niriella DC, Cayley AC. Plasma concentrations of vitamins A and E and carotenoids in Alzheimer's disease. *Age Ageing* 1992; **21**:91–4.
- Sinclair AJ, Johnstone J, Warner C, Bayer AJ. Altered plasma antioxidant status in subjects with Alzheimer's Disease and Vascular Dementia. *Age Ageing* 1996; **25**(suppl 2):3.
- Steinberg D, Parathasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989; **320**:915–24.
- Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis* 1988; **8**:1–21.
- Abella A, Messaoudi C, Laurent D, Marot D, Chalas J, Breux J, Claise C, Lindenbaum A. A method for simultaneous determination of plasma and erythrocyte antioxidant status. Evaluation of the antioxidant activity of vitamin E in healthy volunteers. *Br J Clin Pharmacol* 1996; **42**:737–41.
- King D, Playfer JR, Roberts NB. Concentrations of vitamins A, C and E in elderly patients with Parkinson's disease. *Postgrad Med J* 1992; **68**:634–7.
- Dexter DT, Holley AE, Flitter WD, Slater TF, Wells FR, Daniel SE, Lees AJ, Jenner P, Marsden CD. Increased levels of lipid hydroperoxides in the parkinsonian substantia nigra: an HPLC and ESR. *Movement Disorders* 1994; **9**:92–7.

28. Kish SJ, Morito C, Hornykiewicz O. Glutathione peroxidase activity in Parkinson's disease brain. *Neurosci Lett* 1985; **58**:343–6.
29. Henderson AS, Easteal S, Jorm AF, Mackinnon AJ, Korten AE, Christensen H, Croft L, Jacomb PA. Apolipoprotein E allele epsilon 4, dementia, and cognitive decline in a population sample. *Lancet* 1995; **25** **346**:1387–90.
30. Ceballos I, Javoy-Agid F, Delacourte A, Defossez A, Lafon M, Hirsch E, Nicole A, Sinet PM, Agid Y. Neuronal localization of copper-zinc superoxide dismutase protein and mRNA within the human hippocampus from control and Alzheimer's disease brains. *Free Radical Res Commun* 1991; **12–13**:571–80.
31. Richardson JS. Free radicals in the genesis of Alzheimer's disease. *Ann NY Acad Sci* 1993; **695**:73–6.
32. Chen L, Richardson JS, Caldwell JE, Ang LC. Regional brain activity of free radical defense enzymes in autopsy samples from patients with Alzheimer's disease and from nondemented controls. *Int J Neurosci* 1994; **75**:83–9.
33. Ceballos I, Nicole A, Briand P, Grimber G, Delacourte A, Flament S, Blouin JL, Thevenin M, Kamoun P, Sinet PME. Expression of human Cu-Zn superoxide dismutase gene in transgenic mice: model for gene dosage effect in Down syndrome. *Free Radical Res Commun* 1991; **12–13**:581–9.
34. Ceballos-Picot I, Nicole A, Sinet PM. Cellular clones and transgenic mice overexpressing copper-zinc superoxide dismutase: models for the study of free radical metabolism and aging. *EXS* 1992; **62**:89–98.
35. Ceballos-Picot I. Transgenic mice overexpressing copper-zinc superoxide dismutase: a model for the study of radical mechanisms and aging. *Comptes Rendus des Seances de la Societe de Biologie et de Ses Filiales* 1993; **187**:308–23.
36. Smith MA, Richey PL, Taneda S, Kutty RK, Sayre LM, Monnier VM, Perry G. Advanced Maillard reaction end products, free radicals, and protein oxidation in Alzheimer's disease. *Ann NY Acad Sci* 1994; **738**: 447–54.
37. Vitek MP, Bhattacharya K, Glendening JM, Stopa E, Vlassara H, Bucala R, Manogue K, Cerami A. Advanced glycation end products contribute to amyloidosis in Alzheimer disease. *Proc Nat Acad Sci USA* 1994; **91**: 4766–70.
38. Harris ME, Hensley K, Butterfield DA, Leedle RA, Carney JM. Direct evidence of oxidative injury produced by the Alzheimer's beta-amyloid peptide(1–40) in cultured hippocampal neurons. *Exp Neurol* 1995; **131**:193–202.