# Zinc and platelet membrane microviscosity in Alzheimer's disease

## The *in vivo* effect of zinc on platelet membranes and cognition

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*Objectives.* To investigate the effects of oral zinc supplementation on: (*i*) plasma zinc concentrations; (*ii*) platelet membrane microviscosity *in vivo*; and (*iii*) cognitive function of Alzheimer's disease (AD) patients.

*Design.* An open-labelled pilot study.

*Setting.* University of Stellenbosch Medical School and Stikland Hospital.

*Subjects.* Six volunteer AD patients.

*Outcome measures.* Plasma zinc levels, platelet membrane microviscosity and cognition (MMSE and ADAS-cog tests).

*Results.* Oral zinc supplementation (30 mg/day) did not increase plasma zinc levels significantly, but significantly increased platelet membrane microviscosity ($P = 0.02$; 6 patients). Four patients, who underwent 12 months of evaluation, showed modest cognitive improvement on psychometric testing (Mini-Mental State Examination and the cognitive portion of the Alzheimer's Disease Assessment scale scores).

*Conclusions.* While earlier literature promoted the use of zinc in AD patients, a recent study has contradicted this and implicated zinc in the aetiology of Alzheimer's disease. On the basis of the above results, it may be premature to single out zinc as a causal agent in AD.

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Recent years have led to the discovery of several genetic components implicated in the aetiology of Alzheimer's disease (AD).[1] Environmental factors, however, are believed to accelerate disease expression in genetically susceptible individuals, as is evidenced by the fact that in monozygotic twins, age of onset of AD may differ by as much as 15 years, or that the twins may even be discordant for the disease.[2] This contrasts with Huntington's disease, in which no discordancy has been reported.[2] The discovery of environmental factors responsible for AD in vulnerable individuals and protection against them could conceivably alter the date of onset, severity and course of the disease. A delay in the onset of AD by only 5 years would halve the prevalence of the illness, resulting in enormous cost-savings and preventing much human misery.[3]

Although AD is seen as a disease of the brain, there is mounting evidence that peripheral cells are also affected. In 1987 Zubenko *et al.*[4] discovered reduced platelet membrane microviscosity (reciprocal of membrane fluidity) in 55% of AD patients, as well as 8% of healthy control subjects. Because this abnormality in platelet membrane microviscosity occurred in only half of all AD patients, it could not be used as a diagnostic marker for AD. The observation that a small proportion of healthy control subjects as well as some healthy first-degree relatives had lowered platelet membrane microviscosity[5] has led to speculation about the susceptibility of these individuals to AD in later life. A long-term prospective study, however, would be required to establish whether this is indeed the case. Other authors have confirmed the presence of decreased platelet membrane microviscosity in AD patients.[6,7]

It has recently come to light that free radical-derived lipid peroxidation decreases platelet membrane microviscosity, and that high levels of ascorbic acid eliminate the membrane damage in the *in vitro* system.[8] Zinc also decreases lipid peroxidation *in vitro*.[9] Jeandel *et al.*[10] found that the blood concentrations of several free-radical scavengers that protect against free radical damage, including ascorbate and zinc, were significantly lower in AD patients than in control subjects.

Metal ions alter platelet membrane microviscosity by binding to charged headgroups of membrane phospholipids.[11] Van Rensburg *et al.*[12] demonstrated that aluminium ions decreased while zinc ions increased the microviscosity of platelet membranes *in vitro*.

A role for zinc in dementia is suggested by the fact that reduced levels of zinc are found in both plasma[10] and brain tissue of AD patients, especially in the hippocampus.[13] The highest concentration of zinc in the brain is found in the hippocampal mossy fibres,[14] localised in excitatory boutons, where zinc is co-released with glutamate during neuronal activity in the form of dense synaptic vesicles.[14] Westbrook and Mayer[15] suggested that zinc may regulate both excitatory and inhibitory synaptic transmission in the hippocampus and that zinc may therefore play an important role in long-term potentiation, i.e. in the processing of memory formation.

In 1992 a hypothesis was put forward that zinc deficiency in the hippocampus may contribute to the pathogenesis of neurofibrillary tangles and that this may be prevented by treatment with zinc compounds.[16] Recently, Bush *et al.*[17] contested this line of thinking by postulating that zinc actually contributed to AD by precipitating β-amyloid. This experiment essentially consisted of adding zinc to β-amyloid in a test tube and finding the latter aggregated.

While Mantyh *et al.*[18] showed that aluminium and iron, as well as zinc, in high concentrations were capable of aggregating β-amyloid, Bush *et al.*[17] also reported a negative effect of zinc on the cognition of a small sample (the number

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of patients and the dosage of zinc administered were not published) of AD patients within a few days of starting supplementation.[17] These findings did not concur with the results of our own work in progress at the time.

An open-labelled study was undertaken to investigate the *in vivo* effects of oral zinc supplementation on: (*i*) plasma zinc concentration; (*ii*) platelet membrane microviscosity; and (*iii*) cognitive function of AD patients over a 1-year period.

## Patients and methods

Six patients (4 men and 2 women; mean age 63.6 years, range 51 - 79) were diagnosed with AD according to the definition given by the National Institute of Neurological and Communicative Disorders and Stroke — Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)[19] work group and the criteria in the *DSM-III-R*,[20] as described previously.[7] The mean Mini-Mental State Examination (MMSE)[21] score for the patients before supplementation was 16 out of 30 (range 12 - 21). The study was approved by the Ethics Committee of the University of Stellenbosch.

The 6 AD patients were given oral zinc in the form of zinc chelated with methionine (15 mg twice daily). Plasma zinc levels and platelet membrane microviscosity were measured before, during and after the supplementation period. The patients acted as their own controls.

Plasma zinc levels were determined with a Varian Techtron Model 1200 atomic absorption spectrophotometer. Platelet membranes were isolated, and membrane microviscosity (reciprocal of membrane fluidity) was determined by the method of Zubenko *et al.*[4]

The cognitive functioning of 4 AD patients (2 men and 2 women; mean age 67.7 years, range 63 - 72; mean MMSE 18, range 14 - 21) was monitored over a 1-year period by the quarterly administration of a psychometric test battery which included the MMSE[21] and the cognitive portion of the Alzheimer's Disease Assessment Scale (ADAS-cog).[22] These patients were receiving oral zinc chelated with methionine (15 mg twice daily).

## Results

In spite of oral zinc supplementation, the resulting plasma zinc levels were variable, and even decreased in 2 instances (Table I). Normal plasma zinc values range between 14 and 34 µmol/l.

In all 6 patients, the platelet membrane microviscosity values increased to reach similar values within 1 - 3 months (Patients A - F; Fig. 1). For the group, the increase was statistically significant ($P = 0.02$; Wilcoxon rank test). Increased platelet membrane microviscosity values were sustained, provided zinc supplementation continued (results not shown).

Following the initiation of zinc supplementation, all 4 patients showed a modest temporary peak in psychometric performance at the 3-month mark, relative to their baseline test scores on the same battery. This cognitive improvement coincided with the caregivers' reports of improved day-to-day functioning and was also reflected in the MMSE scores (Fig. 2). For the MMSE, the highest score is 30, and the

**Table I. Effects of oral zinc supplementation on plasma zinc concentration (µmol/l) in 6 patients with Alzheimer's disease**

| Patient | Plasma zinc (µmol/l) | | |
|---|---|---|---|
| | Baseline | 1 month | 3 months |
| A | 11.5 | 14.4 | 15.4 |
| B | 14.5 | 15.2 | 18.8 |
| C | 13.0 | 20.2 | 15.4 |
| D | 12.6 | 13.6 | 13.8 |
| E | 14.3 | 11.4 | 13.6 |
| F | 20.6 | 17.5 | 18.0 |

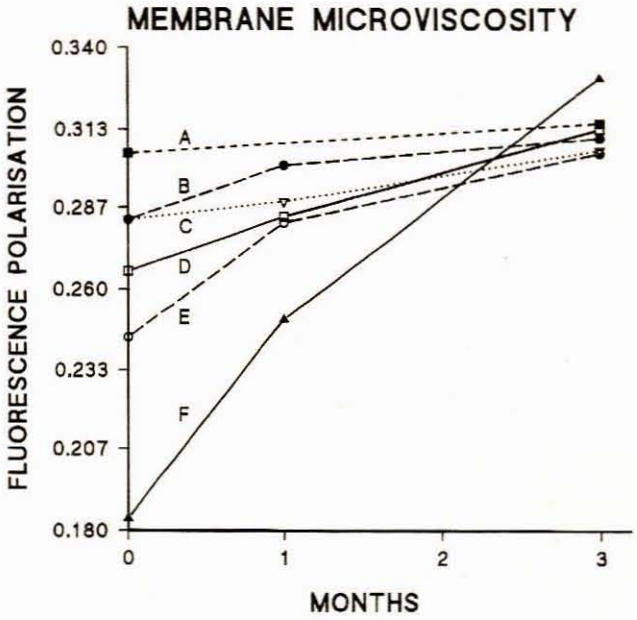


**Fig. 1. Platelet membrane microviscosity of 6 patients (A - F) receiving oral zinc supplementation (30 mg/day). In all patients, microviscosity increased over a period of 3 months to reach similar values. For the group, the increase was significant ($P = 0.2$). The patients were their own controls.**

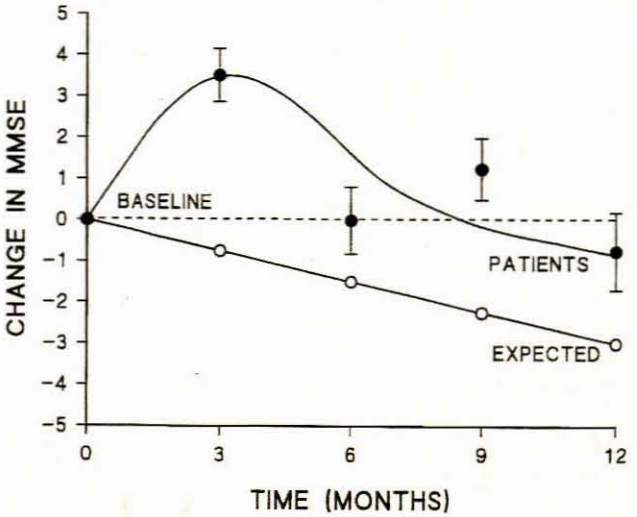


**Fig. 2. Change in cognitive performance (MMSE) of 4 AD patients receiving oral zinc supplementation for 12 months. Values represent mean ± SEM. For the MMSE, the expected decline is 2.4 - 3 points per year.**

expected decline for AD patients is some 3 points per year (range 2.4 - 4).[23] The MMSE decline for the 4 AD patients after 1 year of zinc supplementation was less than expected (Fig. 2).

For the ADAS-cog, the perfect score is 0, and in AD an increase of some 7 - 9 points per year is to be expected. Again, the decline in cognition of the 4 AD patients after 1 year of zinc supplementation was less than expected (Fig. 3).[23]

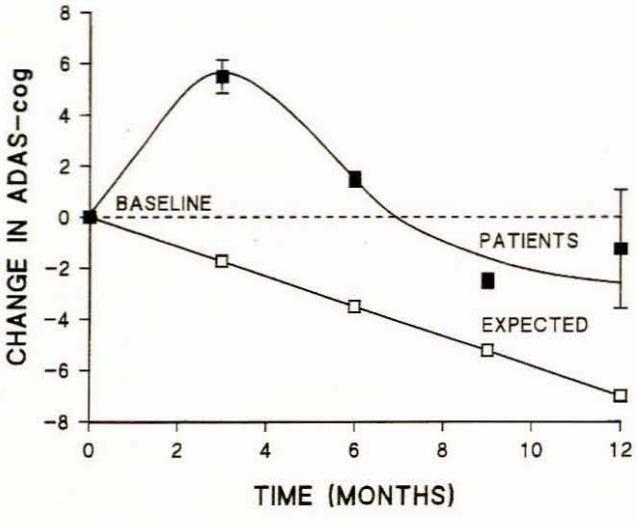


**Fig. 3. Change in cognitive performance (ADAS-cog) of 4 AD patients receiving oral zinc supplementation for 12 months. For the ADAS-cog, an increase of 7 - 9 points is expected. Values have been inverted to show decline in cognition, and represent mean ± SEM.**

## Discussion

Zinc supplementation unexpectedly did not result in higher plasma zinc levels in all patients. The variable plasma zinc levels found may have reflected either a defect in the absorption of zinc (unlikely, see below) or immediate redistribution of absorbed zinc into body stores.

The 6 AD patients who received oral zinc supplementation displayed a steady increase in platelet membrane microviscosity. This suggests that zinc plays an important role in maintaining the patency of the microviscosity of membranes in the body. This role could be structural,[24] in that zinc may bind to the charged headgroups of phospholipids in membranes,[25] or produce an alteration of the phospholipid composition of the membranes. Driscoll and Bettger[26] found that dietary zinc deficiency in rats caused a change in erythrocyte phospholipid composition. Zinc may also protect platelet membranes from decreased membrane microviscosity caused by lipid peroxidation,[8] through the inhibition of free radical reactions.[25]

The fact that oral zinc supplementation had a positive effect on platelet membrane microviscosity in the presence of variable plasma zinc levels, indicates that the latter does not reflect the zinc concentration present in these membranes.

Though preliminary, the results counter the claims made by Bush *et al.*[17] that zinc adversely affects the cognition of patients with AD. At the described dosages of zinc supplementation, all our patients were cognitively better off after 1 year than if they had not taken zinc. These results are in agreement with those obtained by Van Rhijn *et al.*,[27] who showed that 15 AD patients receiving dietary supplementation of zinc sulphate, sodium selenite and fatty acids over a 20-week period showed significantly improved performance on psychometric testing. The negative effect observed by Bush *et al.* in his AD patients within a few days of zinc supplementation (dosage not published) may have been due to aluminium, rather than zinc. As a general principle, an addition of one metal to the body leads to the redistribution of other metals. Zinc has been shown to cause the release of aluminium from membranes[12] and, as demonstrated previously, aluminium in this free form would have negative effects on several systems in the body, causing nuclear damage and alteration of neurotransmitter and enzyme functions.[28] Higher levels of zinc would compound this effect.

Furthermore, the very high levels of zinc found in the hippocampus are enclosed in protective membranous vesicles.[14] *In vitro* experiments would not account for this.

In 2 of the 6 patients receiving zinc supplementation, an increase in libido occurred, necessitating the prescription of cyproterone acetate. A rise in libido following zinc supplementation has previously been noted in the literature.[29]

## Conclusion

Zinc supplementation corrected the membrane microviscosity of platelets in a sample of 6 AD patients. A further ongoing study of 4 patients showed that zinc supplementation resulted in a modest temporary improvement in the cognition of all of these patients, as observed on psychometric testing. This is at variance with Bush *et al.*'s[17] report of rapid cognitive deterioration in AD patients receiving zinc supplementation. The latter effect may have been dose-related, thus altering the distribution of and upsetting the balance of other metals in the body, with negative consequences. We feel that it may be premature to single out zinc as a causal agent in AD.

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# Thyroid dysfunction in the elderly

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*Objectives.* To determine the prevalence of thyroid dysfunction in institutionalised elderly people in Cape Town and to assess the usefulness of an abnormal thyroid-stimulating hormone (TSH) concentration as a screening test in this group.

*Design.* Cross-sectional survey.

*Setting.* Four old-age homes in Cape Town.

*Subjects.* Old-age home residents aged 60 years and over.

*Outcome measures.* Serum concentrations of TSH, free thyroxine and free tri-iodothyronine.

*Results.* Serum TSH estimations were performed on 658 participants, and were abnormal in 103 (15.6%) — 41 (6.2%) being elevated (> 5.0 μU/ml) and 62 (9.4%) being low (< 0.4 μU/ml). There were 3 newly diagnosed cases of hyperthyroidism and 7 of hypothyroidism. Subclinical disease was diagnosed in 40 subjects. The overall prevalence of thyroid dysfunction in this population was 11.2%. In 22 (3.4%) this had previously been recognised, while in 50 (7.8%) the dysfunction was newly diagnosed by the current survey. The positive predictive value of a TSH concentration > 20 μU/ml in predicting hypothyroidism is 67%, while it will predict 100% of cases of subclinical hypothyroidism. A TSH concentration < 0.1 μU/ml will predict 23% of cases of hyperthyroidism, but 81% of cases of subclinical disease.

*Conclusions.* The prevalence of thyroid dysfunction in institutionalised elderly people in Cape Town is similar to that reported for elderly people in other centres. Thyroid dysfunction had not previously been recognised in approximately two-thirds of the subjects in this study. The serum TSH concentration is a reliable screening test for thyroid dysfunction in the elderly, but is less useful if used to identify biochemical thyroid disease. An elevated TSH concentration is a better predictor of thyroid dysfunction in the elderly than a depressed TSH concentration.

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Thyroid disease may manifest atypically in the elderly, leading to difficulties with clinical diagnosis.[1,2] There are well

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