

# Vitamin E Paradox in Alzheimer's Disease: It Does Not Prevent Loss of Cognition and May Even Be Detrimental

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**Abstract.** There is controversy as to whether vitamin E is beneficial in Alzheimer's disease (AD). In this study, we tested if vitamin E prevents oxidative stress and loss of cognition in AD. Fifty-seven AD patients were recruited and divided in two groups: placebo or treated with 800 IU of vitamin E per day for six months. Of these 57 patients, only 33 finished the study. We measured blood oxidized glutathione (GSSG) and used the following cognitive tests: Mini-Mental State Examination, Blessed-Dementia Scale, and Clock Drawing Test. Of those patients treated with vitamin E, we found two groups. In the first group, "respondents" to vitamin E, GSSG levels were lower after the treatment and scores on the cognitive tests were maintained. The second group, "non-respondents", consisted of patients in which vitamin E was not effective in preventing oxidative stress. In these patients, cognition decreased sharply, to levels even lower than those of patients taking placebo. Based on our findings, it appears that vitamin E lowers oxidative stress in some AD patients and maintains cognitive status, however, in those in which vitamin E does not prevent oxidative stress, it is detrimental in terms of cognition. Therefore, supplementation of AD patients with vitamin E cannot be recommended without determination of its antioxidant effect in each patient.

**Keywords:** Antioxidants, glutathione, neurodegeneration, oxidative stress, prevention

## INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease whose pathogenesis is still unclear. An excessive production and accumulation of amyloid- $\beta$  ( $A\beta$ ) in senile plaques may be a critical factor for the onset of the disease, although the mechanism by which  $A\beta$  causes damage and eventually neuronal death is still unknown [1].

Oxidative stress is an important factor in the pathogenesis of AD and it leads to excessive lipid peroxi-

dation [2–6]. An oxidation of nucleic acids, proteins, and DNA in brain tissue of AD patients has been well documented [7,8]. Moreover, Pope and colleagues [9] found low levels of antioxidants in the blood of patients with AD. Several studies have led to the conclusion that  $A\beta$  peptide could cause neuronal toxicity through oxidative stress [10].

Vitamin E is a powerful antioxidant and thus might cause beneficial effects and lower the progression of the disease. Perkins et al. [11] studied 4809 elderly persons and observed that serum levels of vitamin E were lower in these persons than in young ones. Moreover, the lowering of vitamin E when normalized for plasma levels of cholesterol was consistently correlated with memory loss.

A critical paper, published in 1997 [12], reported that vitamin E supplementation resulted in an improvement

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of the frailty of the patients but these authors did not observe a clear effect on cognition. Moreover, Tabet and colleagues [13] revised the data and concluded that there is not sufficient evidence to recommend treatment of AD with vitamin E.

On another line of thought, the idea of vitamin E as a completely safe molecule is changing. In fact, even if there is evidence that relatively high doses of vitamin C or E, up to approximately 0.5 g per day, do not cause toxicity [14], recent meta-analysis have concluded that supplementation with high doses of vitamin E may not be efficient in preventing chronic degenerative diseases, and in fact, they may increase mortality [15–17].

However, in all these studies the effect of vitamin E on redox status of the patients was never determined; this is a critical point. In fact, it was always assumed that patients administered vitamin E would improve their oxidative stress status. And this might not be the case, because of inter-individual variations of the effect of vitamin E. Thus the aim of this paper was to correlate the administration of vitamin E to AD patients with the progression of the disease and with the blood oxidative stress status as determined by well established and validated parameters like the glutathione redox ratio or plasma lipid peroxidation. We have found that vitamin E does not cause a reduction of glutathione redox ratio in all patients. In those in which it does not (non-respondent) vitamin E results in an even more pronounced loss of cognition than in patients treated with placebo. In patients in which vitamin E caused a reduction of oxidative stress, cognition was maintained in the six months of the duration of the study.

## MATERIALS AND METHODS

### *Patients*

This is a prospective, double blind, placebo-controlled study. Patients were recruited by an expert neurologist (MD Alonso) from the Department of Neurology of the Hospital Clínico Universitario, Valencia (Spain). A total of 57 patients with AD, diagnosed following the recommendations of NINCDS-ADRA, and 18 healthy controls of the same age and sex were enrolled. Informed consent was obtained from each patient or family member. The protocol was approved by Ethics Committee of the Hospital Clínico Universitario, Valencia and conforms to the Declaration of Helsinki [18]. At the time of enrollment, the patients

were not taking any other antioxidant medication and were only taking standard anti-cholinesterase drugs.

We studied 18 age-matched controls and 57 AD patients (25 with mild, 26 with moderate, and 6 with severe dementia, determined with the Geriatric Dementia Scale), which were randomly assigned to receive vitamin E (800 IU per day), a standard dose normally used in Spain, or placebo for six months. Randomization was performed using a randomized list of numbers. Of the 57 AD patients, only 33 finished the study, i.e., took the medications for six months and returned to us after that period. Thus we obtained samples at the beginning of the study from 57 patients and 18 healthy controls. Of the patients who finished the study, 14 had been treated with placebo and 19 with vitamin E.

We checked that all patients had similar age and gender distribution in all groups. There were no significant differences in the results of cognition tests obtained between patients of each group at the beginning of the study.

Venous blood from each subject was obtained on the first day of the study and six months after. We measured blood total glutathione levels and oxidized glutathione (GSSG) and plasma malondialdehyde.

Cognitive state was assessed using the Mini-Mental State Examination (MMSE), Blessed-Dementia Scale, and Clock Drawing Test at the beginning and at the end of the study [19–21].

### *Measurement of oxidative stress parameters*

Total glutathione was measured using an HPLC method described by Reed et al. [22]. GSSG levels were measured by an HPLC method developed in our laboratory [23].

Plasma lipid peroxides were determined by measuring malondialdehyde (MDA), which is formed from such peroxides. MDA from the samples reacted with thiobarbituric acid (TBA) at 100°C to form a MDA-TBA adduct. The protein-free extract was separated by HPLC on a column of octadecyl silica gel to separate the MDA-TBA adduct from interfering chromogens. The adduct was eluted from the column with 50 mM phosphate buffer pH 6.8 in 50% methanol and quantified spectrophotometrically at 532 nm.

### *Statistical analysis*

We used the Student's T test to compare two means in parametric samples and the Mann-Whitney test for non-parametric samples. When we compared more than

Table 1  
Oxidative stress markers in AD patients

|                          | GSSG (nmol/ml) | GSSG/GSH %   |
|--------------------------|----------------|--------------|
| Control ( $n = 18$ )     | 23.75          | 2.04         |
| Patients ( $n = 57$ )    | 40.86          | 3.37         |
| Statistical significance | $P = 0.0017$   | $P = 0.0059$ |

two means, we used the ANOVA test for parametric samples and the Kruskal-Wallis test for non-parametric samples.

For single correlations we performed the Pearson Correlation for parametric samples and the Spearman test for non-parametric samples. Also, we used single linear regression models and multiple linear regression models for two or more variables, respectively.

## RESULTS

### *Determination of oxidative stress in patients treated with vitamin E*

Glutathione oxidation is an index of oxidative stress. We determined total glutathione oxidation at the beginning of the study and found that GSSG was increased in AD patients. These differences were statistically significant as shown in Table 1. Importantly, when we divided the group of patients into subgroups related to the degree of the cognitive loss, we found a significant increase in GSSG in those patients who had a moderate or severe loss of cognition, when compared with those patients with mild loss of cognition (Fig. 1A). Consequently, GSSG/GSH was also increased in AD patients (see Fig. 1B)

### *Effect of treatment with vitamin E on blood GSSG levels and on cognition in AD patients*

We determined the evolution of the cognitive capacity in AD patients using three tests: the clock drawing test, the Blessed test, and the MMSE. When we measured GSSG in blood of these patients, we found two populations: respondents ( $n = 9$ ) and non-respondents ( $n = 10$ ) to the treatment with vitamin E. The non-respondents did not show any significant decrease (a change of less than 10 nmol per mL of blood) in blood GSSG after six months of treatment with vitamin E whereas the respondents did; blood GSSG values at the start of the study were  $40 \pm 9$  nmol/mL and after six months on vitamin E, they were  $15 \pm 5$  nmol/mL,  $p < 0.05$ .

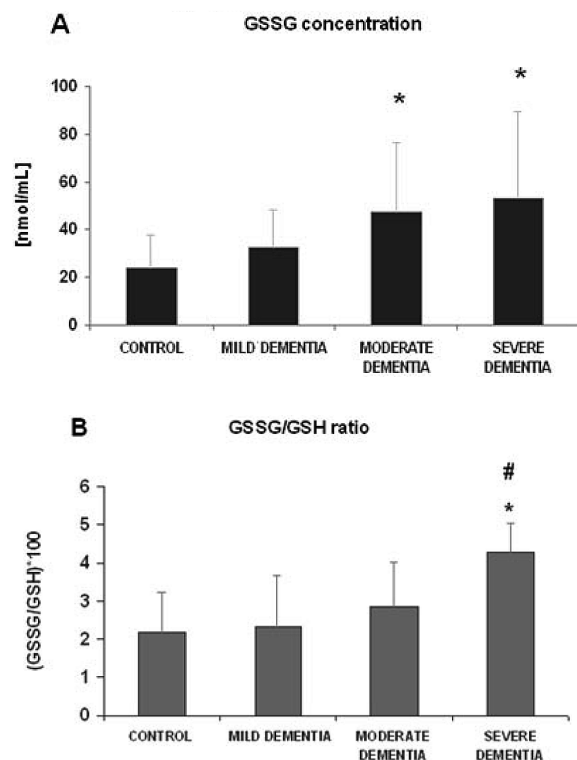


Fig. 1. Oxidative stress correlates with degree of dementia. Basal blood GSSG levels (nmol/mL, panel A) or GSSG/GSH (expressed as percent, panel B) of 57 patients with probable AD. Three groups were made: mild, moderate, and severe dementia (see methods). \* $p < 0.05$  vs. control; # $p < 0.05$  vs. mild dementia

When we measured the changes in cognition (using any of the three tests mentioned in Methods), we found that respondent patients (i.e., those who showed a lower blood GSSG level after treatment with vitamin E) were slightly better than the non-respondents. The non-respondent patients, particularly when studied with the MMSE, showed a marked fall in their cognition, whereas in the placebo, or in the respondent groups they did not show this loss in cognition (Fig. 2).

We observed a linear correlation between the difference of GSSG and the difference of MMSE score in the group treated with vitamin E, showing a major deterioration of cognitive status the patients with less diminution of GSSG levels (Fig. 3).

To confirm the effect of vitamin E treatment on oxidative stress in AD patients, we measured another parameter, lipid peroxidation measured as MDA. We found that patients at the start of the study had a MDA level of  $7.4 \pm 6.0$  nmol/mL ( $n = 33$ ). After six months of placebo, their MDA level was  $17.9 \pm 14.8$  nmol/mL ( $n = 14$ ), but after six months of vitamin E treatment

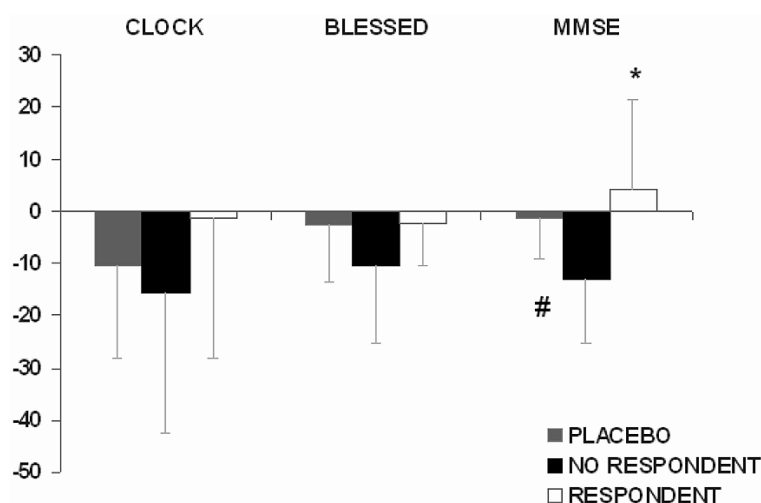


Fig. 2. Effect of treatment with vitamin E on the evolution cognitive status. Comparison between the differences in score (in percentages) of clock drawing test, Blessed test, and MMSE at the beginning and at the end of treatment with placebo or vitamin E for six months. Placebo ( $n = 14$ ), vitamin E (non-respondent,  $n = 10$ ), and vitamin E (respondent,  $n = 9$ ). \* $p < 0.05$  respondent vs. non respondent group. # $p < 0.05$  placebo vs. non respondent group.

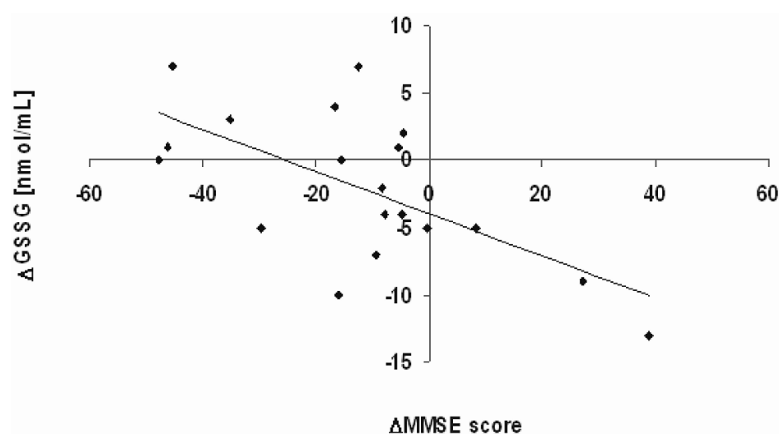


Fig. 3. Correlation between GSSG and MMSE. Correlation between changes in blood GSSG (GSSG at the end minus GSSG at the beginning) and the difference of MMSE score of AD patients treated with vitamin E ( $n = 19$ ).

they were  $9.9 \pm 9.1$  nmol/mL ( $n = 19$ ), i.e., significantly lower than placebo ( $p < 0.05$ ).

We reasoned that the improvement we found in patients respondent to treatment with vitamin E versus the absence of it in the non-respondent group could be due to concomitant diseases and especially to cerebral vascular damage associated with other risk factors such as dyslipidemia. To avoid this confounding factor, we omitted those patients with risk of cerebrovascular disease and repeated the study to relate changes in cognition with changes in antioxidant status associated with vitamin E. The results are in Fig. 4 which shows that the difference between the respondent and non-

respondent patients is even clearer when patients at risk of cerebrovascular disease are omitted.

Our results are in keeping with those of Isaac et al. [24] who, studying the effect of vitamin E on mild cognitive impairment (MCI), concluded that "there is no evidence of efficacy of vitamin E in the prevention or treatment of people with MCI. More research is needed to identify the role of vitamin E, if any, in the management of cognitive impairment."

Therefore, vitamin E acts as a double-edged sword improving or at least maintaining cognition in AD patients during the six months of treatment or markedly decreasing it depending on its effect on oxidative stress status in these patients.

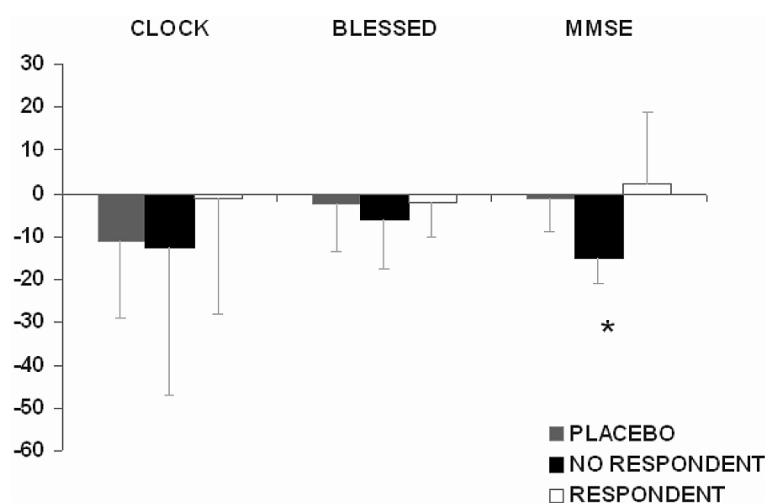


Fig. 4. Cognitive status evolution and treatment with vitamin E in AD without cerebrovascular disease. Comparison between the differences of score (in percentages) at the beginning and the end of study of clock drawing test, Blessed test, and MMSE between placebo ( $n = 14$ ), vitamin E (non-respondent,  $n = 6$ ), and vitamin E (respondent,  $n = 9$ ) excluding patients with risk of cerebrovascular disease evidenced by computerized axial tomography (CAT scan).  $*p = 0.02$ .

## DISCUSSION

### Oxidative stress in AD

The fact that AD patients suffer from oxidative stress has been well recognized, particularly from the pioneer work of George Perry and Mark Smith [3,6]. In this paper, we show that systemic oxidative stress occurs in AD patients and that this correlates with the cognitive status of these patients.

Glutathione redox ratio (GSSG/GSH) is an excellent means of determining the redox status of the patients. In our research group, we have used it to determine oxidative stress in a number of situations, both physiological and pathological, like exercise [25–27], AIDS [28], diabetes [29], obstructive sleep apnea [30], or aging [31–33]. The main caution to be taken is determination of oxidized glutathione. This metabolite is very easy to determine except for the fact that when in the presence of physiological concentrations of the reduced form, i.e., GSH, auto-oxidation of the thiol group in glutathione occurs and since the ratio GSSG/GSH is about 0.01, oxidation of glutathione leads to major changes in the measured level of GSSG. Great care must be taken to prevent such an auto-oxidation. We developed a method to determine the glutathione redox ratio with accuracy. Using our methodology [23], we have been able to prove that there is systemic oxidative stress in AD patients and, very importantly, this correlates well with the cognitive status of these patients. The risk of vascular disease does not affect this conclusion.

### Heterogeneity of the antioxidant response to vitamin E treatment

We have found that not all patients respond equally to antioxidant treatment. In fact, we noticed that there were two groups of patients: “respondents” and “non-respondents”. In those respondents, vitamin E treatment resulted in a reduction of the GSSG levels. But there was a group of patients that did not respond to vitamin E treatment in terms of reduction of the glutathione redox pair (non-respondents). Quite surprisingly, the non-respondent group of patients did not improve their cognitive functions when treated with vitamin E. In fact, in some of the cases, vitamin E treatment was even worse than placebo. An important conclusion that may be drawn from these facts is that any supplementation study with oral antioxidants (vitamins or otherwise) should be followed by determination of oxidative stress parameters, for instance glutathione or lipid peroxidation. Our results, with the limitations of the rather small number of patients, indicate (with statistical significance) that giving vitamin E to AD patients may be harmful, especially if the oxidative stress status of the patients is not carefully monitored. The fact that one treats a patient with an antioxidant vitamin does not necessarily mean that it is going to result in an improvement in the antioxidant status of the patient.

### Usefulness of vitamin E in the treatment of AD

Our initial guiding hypothesis was that since there is oxidative stress in AD, treatment with conventional

antioxidant vitamins might be useful to prevent some of the loss in cognition that occurs in the disease. We chose vitamin E because Sano and colleagues [12] showed that it ameliorates the general well-being of AD patients even though clear effects were not seen in cognition. Our results show that in respondent patients, i.e., in those in whom vitamin E caused a reduction of the glutathione redox pair, the changes in cognition were similar to those of placebo. In non-respondent patients, vitamin E seemed to be detrimental. Although a clear conclusion about the mechanism of this effect cannot be drawn at this time, one can speculate that it could be due to the fact that vitamin E might behave as pro-oxidant in some cases [34–36].

#### *Upregulation of endogenous antioxidant defenses in AD*

The question that remains open is the following: if AD causes oxidative stress, what can we do to effectively protect cells against oxidative stress and modify the rate of cognitive loss in these patients? Based on previous experiments in our laboratory, we have reached the conclusion that it is far better to improve endogenous antioxidant defenses, such as superoxide dismutase or glutathione peroxidase, than to give exogenous antioxidants, such as vitamins C or E [37,38]. In our opinion, estrogens act in this way. We have shown that the increased longevity in females as compared to males in many species, including humans, can be attributed to the fact that estradiol upregulates longevity-related genes [39–41]. Phytoestrogens such as genisteine act in a very similar way [42,43]. Thus, the possibility of treating AD patients with estrogens or, more likely, phytoestrogens such as those in *Ginkgo biloba* or other pharmaceutical preparations remains open. At this stage, we can state that this is a far better option to improve endogenous antioxidant status than giving antioxidant vitamins themselves.

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