

Featured Article

# Serum concentrations of vitamin E and carotenoids are altered in Alzheimer's disease: A case-control study

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## Abstract

**Introduction:** Oxidative stress has been implicated in the pathogenesis of Alzheimer's disease (AD). We investigated associations between serum levels of lipophilic antioxidants and AD.

**Methods:** Serum concentrations of retinol, two forms of vitamin E ( $\alpha$ - and  $\gamma$ -tocopherol) and six carotenoids were quantified by high-performance liquid chromatography from patients with AD ( $n = 251$ ) and cognitively intact controls ( $n = 308$ ) and assessed by regression analyses.

**Results:** Serum levels of  $\alpha$ -tocopherol and all six carotenoids were significantly lower in patients with AD compared with cognitively intact controls ( $P < .001$ ). In contrast,  $\gamma$ -tocopherol was significantly higher in the serum of patients with AD (odds ratio = 1.17 [confidence intervals: 1.05–1.31]).

**Discussion:** Our findings implicate compromised serum antioxidant defenses in AD pathogenesis and differing biological roles for vitamin E isoforms. This highlights the need for improved understanding in the balanced upregulation of exogenous antioxidants related to dietary intake or supplement use in future nutritional intervention studies.

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## Keywords:

$\alpha$ -Carotene;  $\alpha$ -Tocopherol;  $\beta$ -Carotene;  $\beta$ -Cryptoxanthin;  $\gamma$ -Tocopherol; Lutein; Lycopene; Retinol; Zeaxanthin

## 1. Introduction

Alzheimer's disease (AD) is the most common dementia subtype accounting for approximately 75% of all cases [1]. Multiple neuropathological processes underlie the onset of cognitive decline leading to disease, a process likely influenced by a range of both modifiable and nonmodifiable risk factors [2].

Among the etiological processes proposed, there is increasing support for oxidative injury and inflammatory damage early in AD pathogenesis [3–5]. The brain is especially vulnerable to reactive oxygen species due to

neurons possessing relatively low levels of endogenous antioxidants to cope with their high metabolic activity. This antioxidant deficit results in oxidative damage to major cell components with elevated levels of inflammatory markers resulting in neuronal cell death [4].

Nutritional influences in AD may provide significant public health benefit, but the influence of dietary antioxidants on AD risk remains unclear. Plausible mechanisms by which nutritional factors such as serum carotenoids and vitamins may reduce cognitive decline have been proposed, such as antioxidative and/or anti-inflammatory processes, including those common to vascular and nonvascular diseases [6].

Despite numerous studies examining a variety of antioxidants, the most recent systematic review of serum antioxidant status in AD reported the overall quality of evidence to be low, due to insufficient control for potential confounders in studies with low power producing inconsistent

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findings, and also due to the relative absence of randomized control trials [7]. We sought to address these limitations through increased sample size with adjustment of effect estimates for potential confounding variables. We compared serum retinol,  $\alpha$ -tocopherol, and  $\gamma$ -tocopherol (referred to as antioxidant vitamins) as well as the six major carotenoids found in considerable concentrations in serum: lutein, zeaxanthin,  $\beta$ -cryptoxanthin,  $\alpha$ -carotene,  $\beta$ -carotene, and lycopene in patients with AD and cognitively intact controls. We sought to evaluate any associations between levels of exogenous antioxidants in sera of participants with AD risk.

## 2. Methods

### 2.1. Study population

This was a prevalent case-control study in which cases with AD were compared to cognitively intact controls. All recruitment and testing were performed by one investigator (M.A.W.) from August 2006 to 2008 and has been described elsewhere [8]. Methods reported were guided as far as possible by STROBE guidelines for case-control studies [9]. Cases and controls were not matched. The power calculation for sample size was based on a genetic outcome of interest, reported elsewhere [10]. Potential AD cases were identified as they appeared in the memory clinic in Belfast City Hospital, UK and Knockbracken Healthcare Park, UK or from records of previous attendees in the same clinics. AD was defined as per the diagnosis of a senior clinician using the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS ADRDA) criteria [11]. Exclusion criteria were being diagnosed with other types of dementia, including vascular or mixed dementia, applied to ensure as far as feasible the cases consisted of AD cases only. The carer was approached in person or by phone, an opportunity given for the carer and patient to ask questions and permission sought to contact them again. After at least 24 hours to read the information sheet, carers were contacted, and if they and the person with AD were willing to participate, the study visit was arranged.

The recruitment strategies used in the enrollment of controls were designed to identify enough cognitively normal individuals in a practical manner. First, carers of patients attending any outpatient clinic in the study hospitals were approached. Second, a university press release invited participation in the study. Third, a series of talks given to AD patient-support groups in the region led to further volunteers coming forward. Fourth, controls asked their friends and relatives to participate. Exclusion criteria for controls included age under 65 years, to mirror if not formally match cases' ages, a Mini-Mental State Examination (MMSE) score of below 26 of 30 to try to exclude undiagnosed AD cases from being controls, or any history of neurological dis-

ease or dementia. After the study visit, there was no follow-up for cases or controls.

Ethics and clinical governance approval was obtained before commencement of the study which adhered to the tenets of the Declaration of Helsinki. On enrollment, all study participants underwent an assessment that involved drawing a blood sample, measuring blood pressure, and performing a MMSE. The final component of the assessment involved the completion of questionnaires via interviews with the subject, as well as their carer when appropriate.

### 2.2. Detection of serum dietary antioxidants

Serum samples were coded and stored at  $-80^{\circ}\text{C}$  for extraction and batch analysis in a blinded fashion. Serum concentrations of retinol,  $\alpha$ -tocopherol,  $\gamma$ -tocopherol, and six carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein, lycopene, zeaxanthin) were determined by high-performance liquid chromatography (HPLC) with diode array detection [12].

Chromatograms were analyzed using ChromQuest 4.2 software (Thermo Fisher Scientific, MA, USA). The mobile phase consisted of 97% methanol/3% tetrahydrofuran with solvents degassed using an in-line degasser before the HPLC pump. The flow rate was 1 mL/minute and the column was maintained at  $31^{\circ}\text{C}$ . The HPLC components were all ThermoSeparation Products allowing for the simultaneous detection of three wavelengths of magnitude 325, 292, and 450 nm.

All analytes were quantified using external standards (Sigma-Aldrich, Poole, Dorset, UK and Chemos GmbH, Regensburg, Germany) with validation against the National Institute of Standards and Technology standard reference material 968d for fat-soluble vitamins, carotenoids, and cholesterol in human serum. In-house quality control samples were also included in every run. The inter-assay and intra-assay coefficients of variation for vitamin A, E, and the carotenoid assay were both  $<15\%$  [12].

### 2.3. Other variables

DNA extracted from the blood sample drawn enabled *APOE* genotyping of participants by "Sequenom iPLEX assay." A family history of AD and all comorbid health conditions were documented as present or absent as determined by self-report or consultation of medical notes. Smoking history was measured as a cumulative dose in pack-years.

### 2.4. Statistical analysis

Summary statistics for continuous variables and frequencies and relative frequencies by group were calculated. Independent t-tests (for continuous variables) or chi-square tests (for categorical variables) were used to compare participant characteristics between cases and controls. Pearson's correlation coefficients were performed to identify

associations between cognitive indices with age and other continuous variables.

Logistic regression models with dementia status (AD or control) as the outcome and levels of serum levels of vitamins A and E and the carotenoids entered as continuous explanatory variables were used to calculate odds ratios (ORs) for AD and 95% confidence interval (95% CI), per unit increase in the serum levels of vitamins A and E and the carotenoids. These ORs were calculated before and after adjustment for confounders. In the adjusted analysis, variables were eligible for inclusion in the final logistic regression model if a significant association was found ( $P < .05$ ) on univariate analysis or if there was sufficient prior plausibility for their association with AD. To determine the final model, a backward selection procedure was conducted and only variables that were significantly associated with the dependent variable (AD) were retained. Following this procedure, the final model, used to calculate adjusted ORs, contained age, number of *APOE*  $\epsilon 4$  alleles, systolic blood pressure (SBP), smoking pack-years (calculated as the product of total years and average cigarettes per day), and educational attainment (recorded as leaving school before age 14). All statistical analyses were performed using IBM SPSS statistics version 23 (IBM Corp., Armonk, NY).

A sensitivity analysis was performed in cases only to investigate the associations between cognitive indices of disease severity (MMSE) and serum antioxidants. Multiple linear regression was used with serum antioxidants as the outcome and MMSE as an exploratory variable along with age, number of *APOE*  $\epsilon 4$  risk alleles, SBP, smoking status, and educational attainment.

### 3. Results

Demographic and clinical characteristics of the study population ( $n = 559$ ) are presented in Table 1 for both cases

( $n = 251$ ) and controls ( $n = 308$ ). AD patients were more likely to be older than controls (80.2 vs. 76.5 years), with at least one *APOE*  $\epsilon 4$  allele (69% vs. 25%). There were no significant differences in gender between cases and controls, with males constituting 36% of cases and 39% of controls. Cases with AD had a significantly lower MMSE score (18.0 vs. 28.8) and SBP (134 mm Hg vs. 144 mm Hg) compared to control subjects. Markedly higher proportions of patients with AD had left school at 14 years of age (58% vs. 45%) compared to cognitively intact controls. Furthermore, on average, patients with AD had accumulated twice as many smoking pack-years as participants in the control group (17.7 vs. 10.0 pack-years;  $P < .01$ ). The AD patients and the control subjects had similar disease burden beyond AD diagnosis. A significantly greater proportion of AD patients were more likely to be taking aspirin/clopidogrel (48% vs. 39%;  $P = .02$ ) than controls.

Univariate analyses of serum levels of vitamin A, E, and serum carotenoids were available for 251 AD cases and 308 controls. Table 2 shows the associations between AD and serum levels of the dietary antioxidants analyzed. AD patients had significantly lower serum levels of retinol ( $P = .02$ ),  $\alpha$ -tocopherol, lutein, zeaxanthin,  $\beta$ -cryptoxanthin,  $\alpha$ -carotene,  $\beta$ -carotene, and lycopene ( $P < .001$ ) compared to controls. By contrast, the mean serum level of the vitamin E compound,  $\gamma$ -tocopherol, was significantly higher among AD cases compared to cognitively intact controls ( $P < .001$ ). Of interest, a 1  $\mu\text{mol/L}$  increase in  $\gamma$ -tocopherol resulted in a 19% increase in risk associated with AD (OR per  $\mu\text{mol/L}$  increase, 1.19 [CI: 1.10–1.30]), whereas all other antioxidant vitamins and carotenoids analyzed were negatively correlated with AD. With the exception of retinol (OR per  $\mu\text{mol/L}$  increase, 0.68 [CI: 0.44–1.07]), all associations remained significant after adjustment for age, *APOE*  $\epsilon 4$  SBP, smoking status, and educational attainment (Table 2). Higher levels of  $\gamma$ -tocopherol remained a significant predictor of increased AD risk in the adjusted analyses (OR per  $\mu\text{mol/L}$  increase,

Table 1  
Summary statistics of subject characteristics

Characteristic	All ( $N = 559$ )	Control ( $n = 308$ )	Cases ( $n = 251$ )	<i>P</i> -value
Mean age, years (SD)	78.1 (7.4)	76.5 (6.7)	80.2 (7.7)	<.001
Male, <i>n</i> (%)	216 (38)	125 (39)	91 (36)	.22
Mean MMSE (SD)	24.4 (6.8)	28.8 (1.2)	18.0 (6.5)	<.001
Presence of $\epsilon 4$ allele, <i>n</i> (%)	240 (42)	71 (25)	169 (69)	<.001
Mean systolic blood pressure, mm Hg (SD)	139 (18)	144 (18)	134 (18)	<.001
Education—left school at 14 years, <i>n</i> (%)	274 (48)	138 (45)	136 (58)	<.01
Never smoked, <i>n</i> (%)	321 (59)	191 (62)	130 (54)	.12
Diabetes mellitus, <i>n</i> (%)	59 (10)	37 (12)	22 (9)	.17
Hypertension, <i>n</i> (%)	221 (39)	131 (43)	90 (38)	.14
Cardiovascular disease, <i>n</i> (%)	129 (23)	76 (25)	53 (22)	.27
Cerebrovascular disease, <i>n</i> (%)	70 (12)	38 (12)	32 (13)	.42
Hypercholesterolemia, <i>n</i> (%)	216 (38)	124 (41)	92 (39)	.37
Aspirin/clopidogrel, <i>n</i> (%)*	229 (40)	116 (39)	113 (48)	.02

Abbreviations: SD, standard deviation; MMSE, Mini-Mental State Examination.

\*Medications taken with a frequency >5%.

Table 2  
Serum antioxidant associations in subjects with Alzheimer's disease and controls

Antioxidant	All (N = 559)	Controls (n = 308)	Cases (n = 251)	Unadjusted odds ratio (CI)	Adjusted odds ratio (CI)*
	Mean (SD)	Mean (SD)	Mean (SD)		
Retinol (μmol/L)	1.51 (0.66)	1.57 (0.78)	1.44 (0.47)	0.65 (0.45–0.93)	0.68 (0.44–1.07)
γ-Tocopherol (μmol/L)	4.49 (2.18)	4.14 (1.99)	4.92 (2.32)	1.19 (1.10–1.30)	1.17 (1.05–1.31)
α-Tocopherol (μmol/L)	17.7 (8.5)	19.1 (10.2)	16.0 (5.3)	0.92 (0.90–0.95)	0.92 (0.88–0.96)
Lutein (mmol/L)	48.5 (28.5)	53.3 (30.2)	42.5 (25.0)	0.98 (0.98–0.99)	0.98 (0.97–0.99)
Zeaxanthin (mmol/L)	12.2 (6.99)	13.7 (7.65)	10.3 (5.54)	0.90 (0.87–0.93)	0.92 (0.88–0.96)
β-Cryptoxanthin (mmol/L)	15.2 (12.9)	17.8 (14.3)	11.9 (10.0)	0.95 (0.94–0.97)	0.96 (0.93–0.98)
α-Carotene (mmol/L)	23.6 (19.1)	26.9 (20.9)	19.6 (15.6)	0.97 (0.96–0.99)	0.98 (0.96–0.99)
β-Carotene (mmol/L)	123 (94.1)	139 (105)	103 (74.9)	0.99 (0.99–0.99)	0.99 (0.99–0.99)
Lycopene (μmol/L)	0.44 (0.54)	0.54 (0.60)	0.32 (0.43)	0.29 (0.17–0.49)	0.32 (0.16–0.65)

Abbreviations: SD, standard deviation; CI, confidence interval.

\*Multiple logistic regression analysis adjusted for age (years), number of *APOE* ε4 alleles, smoking (pack-years), systolic blood pressure, education (leaving school before 14 years).

1.17 [CI: 1.05–1.31]). The effect estimates in the adjusted analyses were unchanged for α-tocopherol, lutein, and β-carotene, identifying significant negative associations with ORs of 0.92, 0.98, and 0.99, respectively, equating to an 8%, 2%, and 1% reduction in risk of AD per unit increase in serum concentration.

The inverse associations observed between serum levels of zeaxanthin, β-cryptoxanthin, α-carotene, and lycopene and AD risk were attenuated but remained significant in the adjusted analyses. Lower serum levels of zeaxanthin (OR per mmol/L increase, 0.92 [CI: 0.88–0.96]), β-cryptoxanthin (OR per mmol/L, 0.96 [CI: 0.93–0.98]), α-carotene (OR per mmol/L increase, 0.98 [CI: 0.96–0.99]), and lycopene (OR per μmol/L increase, 0.32 [CI: 0.16–0.65]) were observed in those with AD.

In a sensitivity analysis of cases only to assess antioxidant associations with AD severity, only serum lutein was associated with MMSE score (serum lutein increased by 0.765 nmol/L [CI: 0.098–1.433; *P* = .03] per unit increase in MMSE score; Table 3).

Table 3  
Sensitivity analysis of serum antioxidant levels by MMSE score in Alzheimer's disease cases only

Antioxidant	Adjusted estimate*	95% confidence intervals	<i>P</i> -value
Retinol (μmol/L)	−0.001	−0.014, 0.012	.93
γ-Tocopherol (μmol/L)	0.019	−0.041, 0.079	.53
α-Tocopherol (μmol/L)	0.037	−0.089, 0.163	.56
Lutein (mmol/L)	0.765	0.098, 1.433	.03
Zeaxanthin (mmol/L)	0.102	−0.043, 0.246	.17
β-Cryptoxanthin (mmol/L)	0.101	−0.165, 0.367	.45
α-Carotene (mmol/L)	0.083	−0.354, 0.520	.71
β-Carotene (mmol/L)	1.492	−0.532, 3.516	.15
Lycopene (μmol/L)	0.001	−0.008, 0.009	.92

Abbreviation: MMSE, Mini-Mental State Examination.

\*The average increase in each serum antioxidant level per unit increase in MMSE score. Adjusted for age (years), number of *APOE* ε4 alleles, smoking (pack-years), systolic blood pressure, education (leaving school before 14 years).

#### 4. Discussion

This is one of the largest case-control studies to compare serum levels of dietary antioxidants between subjects with AD and cognitively intact controls. Our study has shown that patients with AD have significantly lower serum levels of retinol, α-tocopherol, and the six carotenoids measured. Moreover, our findings support the previous evidence on the potential role of vitamins and carotenoids as antioxidants within AD etiology and may reflect a reduction in defenses against oxidative damage among patients.

Before considering the potential impact of our findings for future research in the area, the study had a number of limitations for consideration. First, there may be residual confounding factors not measured in our sample that influence serum antioxidant status but which have not been controlled for in our data. Unfortunately, the most obvious omissions are measurements of energy intake and supplement use among participants. Taking these both in turn, undernutrition/low energy intake could be determined via anthropometric measures such as BMI. In addition, food diaries or 24-hour recalls could estimate energy intake and supplement use but may be of limited value due to recall bias in those with AD. Additional information on dietary intake would also have proven beneficial in ascertaining whether reduced dietary intake of carotenoids and vitamins occurred as a result of cognitive decline or whether increased antioxidant turnover due to elevated levels of oxidative stress manifests in the compromised antioxidant status of AD patients. Similarly, without sufficient knowledge regarding supplement use among participants, it is difficult to draw strong conclusions from the relationships observed. Without a reliable measurement of these factors, our findings related to serum micronutrient deficiency could potentially be confounded.

Second, the causal and temporal relationships between the deficiency in serum antioxidants and AD cannot be determined due to the cross-sectional nature of our study. There is evidence to support the hypothesis that lower levels of



plasma antioxidants may manifest as a consequence of AD [13]. However, current research suggests these findings may have been influenced by reverse causation bias. The evidence from a meta-analysis by Da Silva et al. discounts the contribution of energy malnutrition to the pro-oxidative state suggesting oxidative stress and subsequent depletion of serum antioxidants is an early event in AD that occurs in the presymptomatic stage of MCI [14]. Within the confines of our cross-sectional study, the sensitivity analysis we performed in cases only supports the findings of Da Silva and colleagues insofar as only serum lutein was significantly depleted with increasing disease severity, as determined by MMSE score (Table 3). Further support by experimental studies in transgenic mice found markers of oxidative stress that preceded the appearance of the hallmarks of the disease [15]. Because of these inconsistencies, a large, prospective study would help clarify the precise interrelationship and nature of this association.

Finally, other methodological issues may further complicate the interpretation of our data. The opportunistic approach of clinic-based sampling offered the best chance of recruiting a sufficient number of subjects for the study to be adequately powered, while avoiding systematic bias, although this approach may have inhibited subject demographics and limited the generalizability of conclusions. The results of any association study are vulnerable to ascertainment bias. For example, those with poor dietary intake may have been overrepresented if they were more likely to be having difficulties with activities of daily living and therefore seek medical help. Future studies should aim to adopt a truly random approach to recruitment, using population census records for identifying controls and a comprehensive AD patient database to sample cases. Equally, recall bias may have led to underestimates of the prevalence of confounding factors among cases, despite a carer being present and medical notes consulted as needed.

Although AD cases were significantly older than cognitively intact controls, no significant change in the effect estimates was observed when these were adjusted for age. The standard deviation for the mean MMSE score in AD patients was 5 times greater than that of the control group, indicative of the wide range of AD severity categorized by the MMSE included in our study.

The main classes of exogenous antioxidants include polyphenols, vitamins, and carotenoids. The carotenoid class can be further subdivided into carotenes and xanthophylls. The subclass of carotenes consists of  $\alpha$ -carotene,  $\beta$ -carotene, and lycopene, whereas the xanthophylls include lutein, zeaxanthin, and  $\beta$ -cryptoxanthin. Lycopene is a naturally occurring red pigment found in tomatoes and is the most predominant carotenoid, accounting for more than half of total carotenoid in human serum [16]. Because of its unsaturated chemical structure, lycopene has the most powerful antioxidant properties among all serum carotenoids with an oxygen quenching ability twice that of  $\beta$ -carotene and 10 times higher than  $\alpha$ -tocopherol [17].

Consequently, lycopene demonstrated the largest effect size observed, by far, of all the antioxidants considered within our study.

Two isomers of the xanthophyll subclass, lutein and zeaxanthin, are particularly common in dark green leafy vegetables and corn and are present in high concentrations within the brain and retina where they exert neuroprotective and antioxidant effects. In addition, they have been reported to enhance gap junction communication in neurons [18]. Another study by Min and colleagues reported high serum concentrations of lutein and zeaxanthin associated with reduced risk of AD-related mortality in adults [19]. Our study provides further support of the protective effect of serum lutein and zeaxanthin against oxidative damage, given the subtle reductions in risk observed in cognitively intact controls of 2% and 8%, respectively, for each mmol/L unit increase.

There has been limited research investigating the mechanisms that underlie the inverse associations between serum antioxidant levels and AD risk [4,5]. However, given the antioxidant deficiency observed among AD patients, the evidence suggests these micronutrients are involved in the mediation of oxidative stress leading to neurodegeneration. The biological plausibility may be explained in part by the protective effects antioxidants exert against damage from reactive oxygen species. However, in the absence of evidence of a causal relationship between low serum antioxidant levels and increased AD risk, we must exert caution in our interpretation as we cannot exclude reverse causality.

Vitamin E is one of the principal vitamins with antioxidative properties and is a collective term used for eight naturally occurring compounds consisting of four tocopherols and four tocotrienols. The isoforms,  $\alpha$ -tocopherol and  $\gamma$ -tocopherol, are the most abundant in both diet and tissues. Typically, an American diet may provide twice as much  $\gamma$  as  $\alpha$ -tocopherol, due to different compositions of dietary fats [20]. As such, the higher concentration of  $\gamma$ -tocopherol observed in the AD group could result from differential dietary intake or as a consequence of disturbed tocopherol metabolism and antioxidant capacity in patients with AD [20–22].

Evidence suggests that  $\gamma$ -tocopherol is more effective than  $\alpha$ -tocopherol in reducing oxidative damage, scavenging free radicals, and exhibiting anti-inflammatory effects [23–25]. This evidence may provide biological plausibility for the opposing associations of both isoforms with AD risk observed in our study. An antagonistic interaction between both isoforms was previously reported where elevated levels of  $\alpha$ -tocopherol were associated with reduced  $\gamma$ -tocopherol functionality in serum [26].  $\gamma$ -Tocopherol levels in the serum of AD patients may vary depending on the severity of the serum depletion of  $\alpha$ -tocopherol and its subsequent bioavailability which highlights a significant concern of trials investigating vitamin E supplementation in AD using  $\alpha$ -tocopherol alone [27].

In addition, the opposing associations we observed for  $\alpha$ - and  $\gamma$ -tocopherol may highlight a potential limitation of previous vitamin E trials, as proposed by La Fata et al. in a recent systematic review of vitamin E trials in AD [28]. They identified reporting inconsistencies regarding the specification of the form of vitamin E administered across intervention studies and postulated on the potential impact this may have on trial outcomes. Two studies by Dysken et al. [27] and Sano et al. [29] administered the same isoform of vitamin E, namely that of  $\alpha$ -tocopherol, and subsequently measured serum levels of the isoform across all subjects. The congruency in both the vitamin E form administered and outcome measures recorded enables direct comparisons of both studies. Conversely, the isoforms used in two additional RCTs of vitamin E in AD were not specified [30,31]. Our data indicate opposing effects of vitamin E isoforms in AD, highlighting the necessity for a more standardized approach to trials in relation to the isoform of vitamin E administered to more accurately determine the true effect it may exert on the onset and progression of AD from mild to severe forms [32]. Our results may offer some understanding of the null results reported from previous trials whereby opting to supplement the wrong isoform in an unbalanced monotherapy overlooked the antioxidant effects of other forms of tocopherol as well as the synergistic effect with other dietary antioxidants in ROS removal [33]. We suggest these admissions should form important learning points in the restructuring of future trials of dietary antioxidant supplementation.

An alternative approach involving the supplementation of a specific combination of antioxidants and micronutrients known to be deficient in AD patients has been explored in two RCTs using a medical nutrition drink [34,35]. The multicounty trials demonstrated significant improvements in memory domain scores in drug-naïve patients with mild AD. These findings mark a critical step toward achieving more meaningful improvements in neurological domains in shifting from a target-based to a more balanced approach, in addressing antioxidant deficiencies.

A balanced approach to supplementation is further supported by the outcomes of The Cashe County Trial which examined the degree to which vitamin supplements, namely vitamins E and C, was associated with occurrence of AD [36]. The authors, Zandi et al., found the use of vitamin E and C supplements in combination to be associated with reduced incidence of AD thus providing further support for the synergistic benefit of a combined supplementation approach. Reviewing these findings in the context of our own results highlights the importance of an informed choice of micronutrients for future clinical trials so that synergetic effects are maximized and the antagonist effects of specific vitamin E isoforms are elucidated.

The major strengths of our study were the large number of subjects, the range of severity of AD cases included, and the number of potentially confounding factors considered. Our study focused solely on AD to the exclusion of other dementia subtypes, such as vascular dementia. In addition, micronutrient serum measurement is an objective determinant independent of the dietary intake estimates remembered over a period.

Our findings are of major public health importance as the search for modifiable risk factors takes precedence in the absence of preventative or curative treatments. Identifying individuals at increased future risk of developing AD, and detecting adverse risk at the earliest stages, may be most effective in delaying disease progression and preserving function within the aging brain.

However, there is still insufficient evidence regarding a causal relationship between low antioxidants level and development of AD. The replication of our findings in a more robust study design could allow for the introduction of an inexpensive nutritional intervention that could be implemented via dietary or supplementary approaches. Future research should consider a wider spectrum of antioxidants or indeed the various forms of vitamin E through expanded analyses to include hydrophilic antioxidants and other subclasses such as flavonoids, to further improve our understanding in this area.

In conclusion, this is one of the largest case-control studies to demonstrate lower serum antioxidant levels among patients with AD compared to cognitively intact controls. The opposing effects with AD risk demonstrated by the two isoforms of vitamin E highlight the complexity of the associations between antioxidants and disease and the need to consider issues like isoform, dose, dietary intake, and possible interactions when designing clinical trials. Moreover, it provides arguments for the remodeling of previous approaches to address antioxidant imbalance in support of possible interactions between various dietary antioxidants. Future large-scale, longitudinal studies and clinical trials that consider changes in antioxidant status with declining cognitive function are required. Given the potential implications of nutritional status in AD etiology, the therapeutic potential of nutrition warrants further investigation and may prove beneficial in AD management.

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## RESEARCH IN CONTEXT

1. Systematic review: We reviewed existing literature investigating associations between serum dietary antioxidants and Alzheimer's disease (AD). Despite numerous studies, findings reported have been inconsistent partly through smaller sample sizes in a limited number of antioxidants. We measured serum antioxidants in 559 samples from a prevalent case-control study that compared Alzheimer's disease to cognitively intact controls.
2. Interpretation: We report lower serum levels of retinol,  $\alpha$ -tocopherol, and six carotenoids in participants with AD, which all remained significant after adjustment for recognized AD risk factors, with the exception of retinol. In contrast,  $\gamma$ -tocopherol levels were significantly higher in those with AD. Our results are consistent with hypotheses supportive of a neuroprotective role of dietary antioxidants.
3. Future directions: Our data provide support for serum antioxidants in AD. Additional analyses that expand on the subclasses and isoforms of dietary antioxidants reported are required to better inform nutritional interventions as a potential therapeutic strategy for AD.

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